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ELECTROPHYSIOLOGICAL METHODS IN BIOLOGICAL RESEARCH

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ELEKTROFYSIOLOGICKÉ METODY V BIOLOGICKÉM VÝZKUMU

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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

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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

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

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 ($t + 273°C$)

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:

$$V = V_0 + 2{\cdot}303 \frac{RT}{nF} \log a \qquad (2)$$

and for $n = 1$, $t = 20°C$

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

or for $n = 1$, $t = 25°C$

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

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.

The concentration cell

If two identical metal electrodes are immersed in a solution of the salt of the same metal, the potential arising at both phase boundaries will be the same so that no potential difference can be registered. If, however, the concentration of the solution is higher at one electrode than at the other, a potential difference E_c arises between them, according to equation (1)

$$E_c = V_1 - V_2 = V_0 + \frac{RT}{n\mathrm{F}} \ln c_1 - V_0 + \frac{RT}{n\mathrm{F}} \ln c_2$$
$$E_c = \frac{RT}{n\mathrm{F}} \ln \frac{c_1}{c_2} \tag{3}$$

depending mainly on the relative concentrations of the corresponding cations or, more exactly, on their relative activities

$$E_c = \frac{RT}{n\mathrm{F}} \ln \frac{c_1 f_1}{c_2 f_2} \tag{4}$$

The electrode immersed in the more dilute solution is negative.

Liquid junction potential

Different concentrations at the electrodes may be obtained by separating the two solutions by a porous membrane, an agar bridge or by other means, slowing down equilibration of the concentration gradients but permitting free ion exchange.

If the mobilities of cations and anions in the solution differ, the more mobile ions will diffuse more rapidly into the dilute solution than the less mobile ones. In this way a certain separation of more and less mobile ions occurs at the junction with a corresponding potential difference, the so called diffusion potential or liquid junction potential. Electrostatic forces, of course, prevent any analytically detectable separation of ions with opposite charges.

The size of the diffusion potential E_d at the junction between two concentrations of the same electrolyte is given by the equation

$$E_d = \frac{RT}{n\mathrm{F}} \frac{u - v}{u + v} \ln \frac{c_1}{c_2} \tag{5}$$

where u is the mobility of the cation and v the mobility of the anion. The mobilities of the biologically most important anions and cations are given in tab. 1. If u is larger than v, the dilute solution (c_2) is positive, since the concentrated solution (c_1) passes the positive charges to the dilute solution more rapidly than the negative ones. The electromotive force of a concentration cell is either increased or decreased by the liquid junction potential. Thus, for

TABLE 1

Mobilities of some physiologically important ions at 18°C
(10^{-4} cm². Volt^{-1} . sec^{-1})

Cations	H^+ 32·7	K^+ 6·7	Na^+ 4·5	NH_4^+ 6·7	$\frac{1}{2}Mg^{++}$ 4·7	$\frac{1}{2}Ca^{++}$ 5·3
Anions	OH^- 18·0	Cl^- 6·8	HCO_3^- 4·6	$\frac{1}{2}SO_4^{--}$ 7·1		

instance, a potential difference is produced between two hydrogen electrodes (platinum covered with platinum black and partly immersed in a solution containing H ions, partly in an hydrogen atmosphere) immersed in 0·1 N and 0·01 N-HCl, connected by a liquid junction. This potential consists of:

a) a concentration potential

$$E_c = \frac{RT}{nF} \ln \frac{c_1}{c_2}$$

which at 20°C and the given concentration of both solutions (10 : 1) is equal to $E_c = 0{\cdot}058$ V (the more dilute solution negative)

b) a diffusion potential

$$E_d = \frac{RT}{nF} \frac{u - v}{u + v} \ln \frac{c_1}{c_2}$$

which after substitution of the corresponding mobilities ($u = 32{\cdot}7 \cdot 10^{-4}$, $v = 6{\cdot}76 \cdot 10^{-4}$ cm² . sec^{-1} . V^{-1}) at 20°C is equal to

$$E_d = 0{\cdot}058 \frac{25{\cdot}9}{39{\cdot}5} = 0{\cdot}038 \text{ V}$$

(the more dilute solution positive).

Since in the given case the polarity of the diffusion potential is the opposite to that of the concentration potential, the resulting potential will be determined by the difference between the two,

$$E = E_c - E_d = 0{\cdot}020 \text{ V}$$

(the more dilute solution is negative).

If, however, calomel electrodes are immersed in these solutions, a different result is obtained. The diffusion potential does not change and (assuming equal activity of H^+ and Cl^- ions) the size of the concentration potential also remains constant. Its polarity, however, is reversed: with calomel electrodes which are in equilibrium with the anions of the system the more dilute solution

is positive. As a result of this the polarity of the concentration and diffusion potentials is the same and the resultant potential difference is

$$E = E_c + E_d = 0{\cdot}096 \text{ V}.$$

Practically, of course, diffusion potentials rather than concentration potentials are measured in biological experiments. That is so because we prefer to use nonpolarisable Ag-AgCl or calomel electrodes for the determination of potential differences. Their electrode vessels are filled with a solution of exactly defined concentration and composition. No potential difference is produced between such two equal electrodes, but diffusion potentials can arise at the junction between the electrode solution and tissue fluid or different solutions applied to the tissue.

Diffusion potentials also occur between two solutions of equal concentration but different composition. If, in the simpler case, we have two equally concentrated solutions with the same anion but different cations (e. g. 0·1n NaCl in the electrode vessel and 0·1n KCl at the treated part of the nerve) then the size of the diffusion potential is given by the equation

$$E_d = \frac{RT}{F} \ln \frac{l_{Na} + l_{Cl}}{l_K + l_{Cl}} \tag{6}$$

or in general

$$E_d = \frac{RT}{F} \ln \frac{l_{k_1} + l_a}{l_{k_2} + l_a} \tag{7}$$

where l_{Na}, l_K, l_{Cl} are the limiting equivalent conductances ($cm^2 . \Omega^{-1}$) of the corresponding ions (the mobilities mutiplied by Faraday constant) at 18°C. After substituting we obtain:

$$E_d = 0{\cdot}058 \log \frac{43{\cdot}3 + 65{\cdot}3}{64{\cdot}5 + 65{\cdot}3} = 0{\cdot}058 \log 0{\cdot}836$$

$$E_d = 0{\cdot}058 \,.\, (0{\cdot}922 - 1) \doteq - 0{\cdot}005 \text{ V}$$

(NaCl solution positive).

A more complex relationship occurs with two solutions of different concentrations with a common anion and different cations. The following applies for the diffusion potential

$$E_d = \frac{(l_{k_1}c_1 - l_a c_1) - (l_{k_2}c_2 - l_a c_2)}{(l_{k_1}c_1 + l_a c_1) - (l_{k_2}c_2 + l_a c_2)} 0{\cdot}058 \log \frac{l_{k_1}c_1 + l_a c_1}{l_{k_2}c_2 + l_a c_2} \tag{8}$$

where l_{k_1}, l_{k_2} and l_a are the limiting equivalent conductances c_1 and c_2 the concentration of ions on either side of the junction. Thus, for instance, a capillary electrode filled with 3M-KCl gives the following diffusion potential against 0,1M-NaCl:

$$E_d = \frac{43{\cdot}3 \,.\, 0{\cdot}1 - 65{\cdot}3 \,.\, 0{\cdot}1 - 64{\cdot}5 \,.\, 3{\cdot}0 + 65{\cdot}3 \,.\, 3{\cdot}0}{43{\cdot}3 \,.\, 0{\cdot}1 + 65{\cdot}3 \,.\, 0{\cdot}1 - 64{\cdot}5 \,.\, 3{\cdot}0 - 65{\cdot}3 \,.\, 3{\cdot}0} \; 0{\cdot}058 \,.$$

$$. \log \frac{43{\cdot}3 \,.\, 0{\cdot}1 + 65{\cdot}3 \,.\, 0{\cdot}1}{64{\cdot}5 \,.\, 3{\cdot}0 + 65{\cdot}3 \,.\, 3{\cdot}0}$$

$$E_d = \frac{0{\cdot}2}{-400{\cdot}2} \, 0{\cdot}058 \log \frac{10{\cdot}8}{384{\cdot}4}$$

$$E_d \doteq (-\,0{\cdot}0005) \,.\, 0{\cdot}058 \,(-\,1{\cdot}57) = 0{\cdot}00004 \text{ V}$$

(NaCl solution negative).

The low value of the diffusion potential in this case is due to the high KCl concentration, the anions and cations of which have nearly the same mobility. This considerably decreases the numerator and increases the denominator of the fraction in the equation for E_d.

If, instead of KCl, 3M-NaCl is used in the same electrode vessel, the diffusion potential against 0·1M-NaCl is given by equation (5)

$$E_d = \frac{43{\cdot}3 - 65{\cdot}3}{43{\cdot}3 + 65{\cdot}3} \, 0{\cdot}058 \log \frac{3{\cdot}0}{0{\cdot}1}$$

$$E_d = \frac{-\,22{\cdot}0}{108{\cdot}6} \, 0{\cdot}058 \,.\, 1{\cdot}477 \doteq 0{\cdot}0174 \text{ V}$$

(the more dilute solution negative).

When the microelectrode filled with 3M-KCl is moved from 0·1M-NaCl into 0·1M-KCl (this approximately corresponds to passing from extracellular into intracellular space), the diffusion potential changes only slightly from 0·04 mV to 0·53 mV (microelectrode positive).

The diffusion potential of the microelectrode filled with 0·1M-NaCl would under the same circumstances change by 4·4 mV (the microelectrode positive) and that of a microelectrode filled with 3M-NaCl by 0·35 mV (microelectrode more positive):

$$E_d = \frac{64{\cdot}5 \,.\, 0{\cdot}1 - 65{\cdot}3 \,.\, 0{\cdot}1 - 43{\cdot}3 \,.\, 3{\cdot}0 + 65{\cdot}3 \,.\, 3{\cdot}0}{64{\cdot}5 \,.\, 0{\cdot}1 + 65{\cdot}3 \,.\, 0{\cdot}1 - 43{\cdot}3 \,.\, 3{\cdot}0 - 65{\cdot}3 \,.\, 3{\cdot}0} \,.$$

$$. \, 0{\cdot}058 \log \frac{64{\cdot}5 \,.\, 0{\cdot}1 + 65{\cdot}3 \,.\, 0{\cdot}1}{43{\cdot}3 \,.\, 3{\cdot}0 + 65{\cdot}3 \,.\, 3{\cdot}0}$$

$$E_d = \frac{-\,0{\cdot}1 + 66{\cdot}0}{13{\cdot}0 - 325{\cdot}8} \, 0{\cdot}058 \log \frac{13}{325{\cdot}8} = (-\,0{\cdot}21) \,.\, 0{\cdot}058 \,(-\log 25{\cdot}1)$$

$$E_d = (-\,0{\cdot}21) \,.\, 0{\cdot}058 \,(-\,1{\cdot}40) = 0{\cdot}0170 \text{ V}$$

(the more dilute solution negative).

As can be seen, the diffusion potential is directly proportional to the difference in mobilities of the cations and anions of the solution used for filling

the electrodes. If different ions are present at the junction, the diffusion potential is determined chiefly by the more concentrated ones. In order to reduce the diffusion potentials occurring between the electrode and the test solution or tissue, 3M-KCl is used for filling the microelectrodes or saturated KCl for filling very stable reference electrodes (e. g. for measuring *p*H). Agar bridges with 3M-KCl are used for connecting solutions of different concentrations and for reducing the liquid junction potential. When working with nonpolarisable macro-electrodes (with Ag-AgCl or calomel electrodes), diffusion of KCl from the electrode vessel might act on tissue which reacts very sensitively to even small increases in potassium ion cencentration in the extracellular space. Such electrodes are preferably filled with solutions similar in composition to extracellular fluid (physiological saline, Ringer solution). Even in such cases, however, large diffusion potentials are usually not produced as long as the composition of the extracellular fluid remains within physiological limits.

Membrane potential

A diffusion potential occurs at any junction of two solutions of different composition or concentration. Somewhat different conditions prevail if such solutions are separated by a collodion or protein membrane. If the latter's pores are large, only the corresponding diffusion potential is produced. In membranes with smaller pores, the difference in permeability to cations and anions gradually increases. This is due to the fact that protein molecules forming the membrane behave as acids at a normal *p*H and are therefore charged negatively. For that reason they repel negatively charged anions, which are unable to pass through the pores of the membrane. It is sufficient, however, to decrease the *p*H of the solution and the amphoteric proteins change their negative charge for a positive one, and the membrane ceases to be permeable for cations and becomes permeable for anions. Membranes selectively permeable to anions even at normal *p*H can be produced by impregnating collodion membranes with basic dyes or alkaloids.

The potential arising between two concentrations of the same solution separated by a collodion membrane which is nearly impermeable to anions can be calculated from the equation for the diffusion potential

$$E_d = \frac{u - v}{u + v} 0{\cdot}058 \log \frac{c_1}{c_2}$$

in which $v = 0$ so that

$$E_m = \frac{u}{u} 0{\cdot}058 \log \frac{c_1}{c_2} = + 0{\cdot}058 \log \frac{c_1}{c_2} \qquad (9)$$

For a membrane selectively permeable to anions in a similar way $u = 0$ and

$$E_m = \frac{-v}{v} 0{\cdot}058 \log \frac{c_1}{c_2} = -0{\cdot}058 \log \frac{c_1}{c_2} \tag{10}$$

In both cases the sign of the potential will be that of the more dilute solution.

Thus, for instance, in a cell with a negative membrane

$$\text{Hg.Hg}_2\text{Cl}_2\text{.KCl (sat).0·1M-KCl.membrane.0·01M-KCl.KCl (sat).Hg}_2\text{Cl}_2\text{.Hg,}$$

in which we can neglect the diffusion potentials produced between the saturated KCl in the calomel electrodes and the KCl solutions on both sides of the membrane, the potential will be near the value 58 mV predicted by equation (9).

If Ag-AgCl electrodes were directly immersed in the solution on both sides of the membrane, we should measure in the cell

$$\text{Ag-AgCl(solid).0·1M-KCl.membrane.0·01M-KCl.AgCl(solid).Ag}$$

not only the membrane potential, but also the concentration potential corresponding to different concentrations of Cl ions at each electrode. This potential, the size of which is determined by equation (3) has the same polarity as the membrane potential (the more dilute solution is positive), so that the resultant potential will approximate 116 mV.

If the collodion membrane separates the same concentrations of two salts with different cations and a common anion, then the potential across the membrane will mainly depend upon relative mobilities of the cations in the membrane. If they remain the same as in aqueous solutions, the membrane potential E_m would be determined by equation (7), in which $l_a = 0$.

$$E_m = 0{\cdot}058 \log \frac{l_{k_1}}{l_{k_2}}$$

This, for instance for a 0·1M-NaCl and 0·1M-KCl solutions would result in an increase in the diffusion potential from 5 mV to

$$E_m = 0{\cdot}058 \log \frac{64{\cdot}5}{43{\cdot}3} = 0{\cdot}058 \log 1{\cdot}49 = 0{\cdot}010 \text{ V}$$

The potentials actually recorded, however, are much greater. This indicates that the mobility of one kind of cations is decreased more than that of another kind. Thus, while in water the proportion of mobilities of K^+ and Na^+ is 1·49, this same proportion in a collodion membrane is 7·15. Conditions are even more complex on membranes surrounded by solutions of different ionic composition and having a different and variable permeability for anions and cations of the system. Such conditions prevail on biological membranes of cells and tissues. Since biological membranes are part of the living system of the cell, their characteristics are determined not only by passive physico-chemical

processes but also by metabolic activity. So-called active transport of ions (the ability of living tissue to transfer a certain kind of ions against electrochemical gradients) is particularly important for electrogenesis. These problems will be discussed in more detail later.

B. The membrane theory of bioelectric phenomena

At the beginning of this century the first attempts were made to give a quantitative explanation of demarcation potential — potential difference between the intact surface of a muscle or nerve fibre and a cut surface — using physicochemical laws and equations derived by Nernst. Bernstein (1902) following the ideas of du Bois-Reymond (1849), suggested that the source of this potential is not the injured site but the undisturbed surface of the tissue. The potential difference is produced on a hypothetical membrane between the external medium and the inside of the fibre. The electrode placed onto the injured site is actually in contact with the inside of the fibre and thus with the inner side of the membrane. The electrical potential is due to a tendency of potassium ions, accumulated within the fibre, to leave the latter through the membrane, which is selectively permeable to K^+. Quantitatively the relation between the potential and the potassium concentration in the external medium (K_o) and the internal medium (K_i) is given by Nernst's relation.

Overton (1902) made another important discovery nearly at the same time. He found that the muscle loses its irritability in solutions devoid of Na ions, and put forward the hypothesis according to which muscular activity is accompanied by an exchange of intracellular K^+ for extracellular Na^+. Despite the fact that much study has been devoted to these problems, only technical progress of the last 20 years (microelectrode and micro-injection techniques, radioactive tracers, chemical micro-analysis etc.) and the choice of suitable experimental objects, especially the giant fibres of the squid and *Loligo* have made further advance possible.

The first experiments with intracellular electrodes yielded unexpected results. According to Bernstein's membrane theory the action potential was considered to be an expression of membrane depolarisation. It was shown experimentally, however, that more than depolarisation occurs during the action potential, since the polarity of the membrane is not destroyed but reversed. It was therefore necessary to supplement and alter the original hypotheses. It is mainly to Hodgkin, Huxley and Katz that we owe the elaboration of the modern membrane theory (Hodgkin 1951, 1958).

The axoplasma of nerve or muscle fibres contains K^+ as the main cation. Organic acids, chiefly amino acids, are the main anions. The surface of the

fibres is formed by a membrane 50- 100 Å thick (according to data obtained by electron microscopy) with a specific transverse resistance 1000 Ω . cm² in the squid (Cole, Hodgkin 1939) and a capacitance of about 1 μF/cm² (Curtis, Cole 1938). This membrane forms a diffusion barrier between the interior and exterior. If it is practically impermeable to proteins, amino acids and Na-ions, a Donnan equilibrium is formed across it. Less Cl will be present on the side of the protein anions. For the majority of excitable tissues the following ratio was found:

$$\frac{[K_i]}{[K_o]} \doteq \frac{[Cl_o]}{[Cl_i]} = 20 \quad \text{to} \quad 50$$

On the basis of this distribution, the equilibrium potential produced on a membrane selectively permeable to K^+ can be calculated from the Nernst relation

$$E_K = 0{\cdot}058 \log \frac{[K_i]}{[K_o]} = 0{\cdot}070 \div 0{\cdot}099 \text{ V} \tag{1}$$

the inside of the cell being negative.

This potential was actually measured with a microelectrode introduced into the inside of a nerve or muscle fibre. Its value changes according to equation (1) with changing external potassium concentrations. When decreasing K_o below the physiological range, however, the membrane potential does not increase beyond a certain level.

Sodium has a completely different distribution. It is the main extracellular cation and occurs in only small amounts intracellularly (usually $Na_i : Na_o = 1 : 10$). Such a distribution can be explained by an active metabolic process removing Na^+ from the cell against a concentration gradient. The distribution of Na^+ corresponds to an equilibrium potential

$$E_{Na} = 0{\cdot}058 \log \frac{[Na_i]}{[Na_o]} = - 0{\cdot}058 \text{ V} \tag{2}$$

The negative sign shows that the inside of the cell in this case is positive. This potential cannot be normally observed because of the low permeability of the resting membrane for sodium. Different conditions, however, occur when a nerve impulse passes. The permeability of the active membrane for Na^+ increases about 500 times so that it is now several times larger than that for K^+. This results in the production of a new equilibrium corresponding to E_{Na} with a sign opposite to that of the resting membrane potential. For that reason the action potential is higher than the membrane potential. It is actually the sum of E_K and E_{Na}. This assumption was confirmed by experiments showing that the amplitude of the action potential depends on the extracellular concentration of Na^+ roughly according to equation (2).

The voltage clamp method permitted a more detailed analysis of ionic currents in the membrane (Hodgkin, Huxley, Katz 1952). This method utilizes sudden displacement of the membrane potential by an imposed potential

difference put between a microelectrode introduced longitudinally into the inside of the axon and the external solution. If the displaced membrane potential is held at a fixed value by a feed-back amplifier, changes in membrane conductivity can be studied by recording the current flowing through the membrane under these conditions. It was shown that depolarization of the membrane by 15 to 100 mV at first evokes an inward current, flowing into the axon, although the electric pulse resulted in increased interior positivity and should therefore initiate an outwardly directed current. Within 0·5 to 1 msec. this inward current is changed to an outward current, which attains a constant value, pursuing a sigmoid curve. This second component corresponds to a potassium current carried by K^+ efflux from the depolarised fibre while the first component might be explained only by the assumption that the depolarised membrane changes its permeability to sodium. This explanation is supported by the fact that equilibration of the electrical (depolarisation by $E_K + E_{Na}$) or chemical ($Na_i = Na_o$) sodium gradient between the interior and exterior of the axon causes the initial current component to disappear.

The use of the same depolarisation in a normal Ringer solution and immediately afterwards in Ringer solution in which 90% of sodium has been replaced by choline makes it possible to obtain a summation curve $I_{Na} + + I_K$ in the first case, a curve for I_K alone in the second case and by subtraction the isolated I_{Na} curve. The sodium current rapidly reaches a maximum but then immediately begins to drop towards zero despite the continued depolarisation.

On the basis of these data it is now possible to describe the events occuring in a nerve fibre during a nervous impulse. Currents flowing through the interior of the axon in one direction and through the external fluid in the reverse direction form local circuits between the resting and active regions of the nerve fibre. These currents displace the resting potential of the membrane just ahead of the impulse. When a certain degree of depolarisation is reached (20 mV), the permeability of the membrane for Na^+ increases. Due to the entry of Na^+, depolarisation is enhanced until, at the peak of the action potential, an equilibrium potential for sodium is attained. This prevents further increase of Na^+ influx and the sodium permeability of the membrane begins to decline. At the same time permeability for K^+ rises. Increased K^+ efflux returns the membrane potential to the original value and accelerates the decrease in sodium permeability. The raised potassium permeability persists for a further few msec. For the period corresponding to the refractory period, the mechanism increasing sodium permeability is blocked. Only when the membrane is again capable of increasing its permeability for Na^+ can a new action potential be conducted. During impulse conduction the axoplasm has gained an insignificant amount of Na^+ and has lost about the same amount of K^+.

The movements of potassium and sodium during impulse activity can be expressed quantitatively on the basis of theoretical considerations and direct measurements. If we assume that sodium enters the inside of the axon only until depolarisation of the membrane occurs, then according to Keynes and Lewis (1951), the minimum necessary amount of Na can be calculated from the capacitance of the membrane ($C = 1{\cdot}5\ \mu F/cm^2$ for *Loligo*) and the amplitude of the action potential ($V = 100$ mV). The necessary quantitity of electricity is thus

$$C \,.\, V = 1{\cdot}5\ \mu F/cm^2 \,.\, 100\ mV = 150\ m\mu coulomb./cm^2$$

which may be transferred to the interior of the axon by $\frac{C \,.\, V}{F}$ gramequivalents of Na^+. This is $\frac{150 \,.\, 10^{-9}}{96 \,.\, 500}$ gramequivalents/cm^2/imp. $= 1{\cdot}5 \,.\, 10^{-12}$ grameq./ /cm^2/imp. Experimentally (using accumulation of Na^{24} in the axon, K^{42} efflux from a previously saturated axon and activation analysis) a sodium influx of $3{\cdot}8 \,.\, 10^{-12}$ grameq./cm^2/imp. was found, i. e. about 2—3 times more than the theoretical assumption. This can be explained if increased Na influx outlasts the crest of the action potential. The same quantity of electricity is necessary for repolarisation of the membrane. This is now supplied by a K^+ current. Experimentally values of $3{\cdot}6 \,.\, 10^{-12}$ grameq./cm^2/imp. were found.

Despite general agreement between theoretically assumed and experimentally determined values of membrane and action potentials, there are some cases in which the calculated and determined values differ considerably. Thus, for instance, the resting membrane potential does not follow equation (1) if K_o falls below 3·5 meq./l. This is due to the fact that the selective permeability of the membrane for K and Na is only relative. Goldman's equation takes relative permeabilities into account

$$E_m = 58 \log \frac{P_K [K_i] + P_{Na} [Na_i] + P_{Cl} [Cl_o]}{P_K [K_o] + P_{Na} [Na_o] + P_{Cl} [Cl_i]} \qquad (3)$$

For the resting membrane $P_K : P_{Na} : P_{Cl} = 1 : 0{\cdot}04 : 0{\cdot}45$.
For the active membrane $P_K : P_{Na} : P_{Cl} = 1 : 20 : 0{\cdot}45$.

It follows from equation (3) that after removal of potassium from the external medium the value E_m is determined by further members of the denominator, especially by the value $P_{Na} [Na_o]$. It appears that an estimation of the relative permeabilities $P_K : P_{Na}$ ought rather to be 1 : 0·01 for the resting and 1 : 30 for the active membrane.

Another cause of the discrepancy between theoretical and measured values is the unstable state of isolated excitable structures, which lose K^+ and gain Na^+ apparently due to injury during dissection. Under such conditions,

resting membrane potentials of only about 50 or 60 mV were recorded instead of 90 mV. Equations (1), (2) and (3) characterise steady state systems in which the efflux (m_o) of a certain ion is compensated by an equally strong influx (m_i). They can therefore only be applied if $\frac{m_i}{m_o} = 1$.

The relation between influx and outflux, internal and external concentrations and the steady state potential for a certain ion is given by the equation derived by Ussing (1949):

$$\frac{m_i}{m_o} = \frac{c_o}{c_i} e^{\frac{EF}{RT}} \tag{4}$$

or after converting to logarithms

$$\ln \frac{m_i}{m_o} = \ln \frac{c_o}{c_i} + EF/RT \tag{5}$$

If we substitute for E the membrane potential calculated according to equation (1)

$$E = \frac{RT}{F} \ln \frac{c_i}{c_o}$$

we obtain

$$\ln \frac{m_i}{m_o} = 0$$

If, however, the value of E in equation (5) differs from the theoretical value, $\frac{m_i}{m_o}$ is not equal to 1. Thus in the nerve fibre of *Loligo* $\frac{m_i}{m_o} = 0{\cdot}35$ was obtained for $E = 62$ mV, $K_i = 300$ mM. Experimentally, using labelled potassium, it was found in the same fibre

$m_i = 10{\cdot}6 \cdot 10^{-12}$ mol. cm^{-2} . sec^{-1} and

$m_o = 27{\cdot}5 \cdot 10^{-12}$ mol . cm^{-2} . sec^{-1} and thus

$\frac{m_i}{m_o} = 0{\cdot}39$, which agrees well with the calculated ratio.

The recovery mechanism maintaining a stable internal medium in nerve and muscle fibres by removing entering Na^+ and reabsorbing outflowing K^+, particularly during and after intensive impulse activity, is a necessary supplement to passive ionic movements. During the action potential ions move along existing concentration gradients, which provide the immediate source of energy for conduction. The recovery process, on the other hand, requires movements of ions against electrochemical gradients, making participation of metabolic energy necessary. A number of poisons affecting cellular metabolism (2,4-dinitrophenol, NaCN, Na_3N) retard the excretion of Na^{24} from stimulated

nerve previously loaded with Na^{24}. The membrane and action potentials do not change. Chemical analysis has shown a close relationship between the mechanism excreting sodium, the so called sodium pump, and high energy phosphate compounds (ATP, arginine phosphate, creatine phosphate etc.). The movement of K^+ across the membrane probably depends on the action of the sodium pump not only passively (removal of one cation from inside the cell must be followed by entry of another in order to fulfil the conditions of electroneutrality in the internal fluid and of osmotic balance between internal and external fluid) but also actively. This is demonstrated by the fact (Hodgkin and Keynes 1955) that removal of K from the external medium decreases the Na-outflux. According to some theories the membrane contains a carrier that combines with Na^+ on the inner side of the membrane to form a neutral molecule X. This passes through the membrane and at its outer surface Na^+ is released and K^+ accepted, thus changing the carrier into a neutral molecule Y, which diffuses back through the membrane to release K^+ inside the cell. Recent results in this field, however, are still full of contradictions so that, at present, it is scarcely possible to give a satisfactory explanation of active transport.

It remains, of course, an open question how far results obtained with giant fibres of the squid may be applied to other excitable structures, especially nerve cells of the mammalian brain. It has been possible in recent years to demonstrate convincingly that the fundamental characteristics of the membrane of the neurone body and that of the giant fibre are similar. This was made possible by the use of intracellular recording of potentials in motoneurones of the spinal cord (Brock et al., 1952; see also Eccles J. C. 1957) and other nerve cells (pyramidal cells of the cerebral cortex — Phillips 1956 a, b, Renshaw cells — Frank and Fuortes 1956, sympathetic ganglion cells — Eccles R. M. 1955).

According to Eccles (1957), the average capacitance of the motoneurone membrane is 3×10^{-9} F, its potential — 70 mV and its resistance is 800 KΩ. The membrane potential corresponds to the equilibrium potential of chloride ions, but is somewhat higher than would correspond to the electrochemical equilibrium for K^+ (— 90 mV). Depolarisation of the membrane by 10—30 mV evokes a selfregenerative process of increased sodium permeability. Thus the equilibrium potential for Na^+ is approchaed and a spike attaining 80 to 110 mV produced. Membrane potential changes are initiated at the synapses — points of contact between the axon of one neurone and the cell body or dendrites of the other. The synaptic change in membrane potential is not due to direct electrical action of presynaptic activity. This follows chiefly from the fact that the postsynaptic potential starts at a time when the presynaptic spike has nearly dissappeared. It has been demonstrated that a number of synapses cannot be stimulated electrically (Grundfest 1958). It seems that as a result of the arrival of an impulse a transmitter substance is released which

diffuses through the synaptic space and characteristically affects the properties of the subsynaptic membrane (Eccles 1957).

Synaptic changes of a membrane potential are of two kinds: excitatory (EPSP-excitatory postsynaptic potential) and inhibitory (IPSP-inhibitory postsynaptic potential). While the EPSP is similar, to a certain extent, to local changes in a nerve fibre and is characterised by depolarisation of the membrane, the IPSP manifests itself by hyperpolarisation of the membrane or by an increase in the latter's resistance to depolarising effects. The EPSP is due to increased membrane permeability to all ions, the IPSP, on the other hand, to increased membrane permeability to K^+ and Cl^- with the low Na^+ permeability preserved.

Since a number of synapses may be active on one neurone, the resultant reaction is determined by complex mutual interaction. The contrary changes in membrane potential produced by IPSP and EPSP summate algebraically and thus may cancel each other under certain conditions. Excitatory synaptic effects sufficient to evoke a spike become ineffective if they coincide in time with inhibitory effects — inhibition occurs.

The membrane theory thus gives a satisfactory explanation of processes occuring in excitable tissues and makes even possible their quantitative description. Other hypotheses have been proposed, however, to explain the nerve impulse by changes occuring in the whole axoplasma, especially in its protein structures. The founder of this theory (so called alteration theory) was L. Hermann (1879), its modern followers are Nasonov and Aleksandrov (1940), Segal (1956), Heilbrunn (1956), Ungar (1957) and Troshin (1956).

II

Electrophysiological apparatus and technique

In contrast to many other biological disciplines, electrophysiology requires a fairly good knowledge of several branches of physics, particularly electricity (electrotechniques and electronics), both theoretical and practical.

This first chapter is in no way an exhaustive account, and is only intended to inform the reader about those parts of physics and electronics that are important for electrophysiology.

Instructions for the design and construction of apparatus, although important didactically, are not given here. They are found to a sufficient extent in the literature.

The following literature may be recommended to electrophysiologists: Any good textbook of physics for university students, books giving instructions for construction and calculations of the most important circuits and their component parts, such as the book by Terman (1943) translated and extended by Smirenin (1950), containing more than 1000 references. The following are important references for constructing amplifiers: Bajda and Semenkovich (1953), Valley and Wallman (1948), Haapanen (1952), Haapanen, Hyde and Skoglund (1952), Kozhevnikov (1956), Kaminir (1956), Nüsslein (1952), Korn and Korn (1955), Johnston (1947), Grundfest (1950). The theory and practice of electrophysiological techniques is described in detail in the work of Whitfield (1959) and Donaldson (1958).

As already stated, the fundamental methods of electrophysiological research are the following: stimulation of tissues and registration of potential differences between different points in tissue, cells or fibres. Time changes of those potential differences, occurring usually in response to electric stimulation, but also, as the result of natural stimuli, or metabolic processes or even without any evident stimulus, are recorded. In general, the experimental system — electrical stimulation — tissue — recording of potential changes — is the most common to day, but by no means the only one possible. Technically simpler and older methods of stimulation (electrical or other) and observation of mechanical (or other non-electrical) responses of tissues or animals are still

important. Beginners are advised to follow the well tested path from the more simple to the more complex, especially if their practical experience with electrotechnical apparatus is limited.

Stimulation technique

The purpose of stimulation is generally to produce a change in vital tissue processes, usually production of excitation (an impulse) in excitable tissue. Usually one of the important requirements is the possibility of using a quantitatively variable stimulus. The size of the stimulus is defined either absolutely, so that results obtained from completely different experiments may be compared, or relatively, so that only results obtained under the same conditions with the same apparatus and in the same tissue can be compared. If only electrical stimulation is taken into account, the following points must be considered in order to evaluate the possibility of quantitative determination of stimulus parameters: 1) which physical parameter (whether voltage, current etc.) determines the extent of the effect of the stimulus on the tissue, 2) how does this value, which is usually only measured outside the tissue, change within the tissue.

The physical effect of the stimulus on the tissue

Ions and molecules with a large dipole moment are the only components of living matter subject to the action of electricity. The change caused by an electrical stimulus of medium duration would then be a change in ion concentration at a certain point (as the result of different ion mobilities) or a change in structure due to changed orientation of linked dipoles.

In both cases the action of an electrical stimulus is determined not only by duration, but also by the intensity of the field produced by the stimulus in the tissue.

In this section only the action of the electrical stimulus on ionic movements will be considered. This evidently is more important and simpler. It must be kept in mind, however, that difficulties encountered when defining the stimulus effect on ionic movements in tissues are even greater for dipole movements.

The production of an impulse in an isolated nerve fibre lying on metal electrodes in a non-conductive medium (paraffin oil) will be considered first (Fig. 1).

From a purely physical aspect the field intensity may be defined (and this is better for clarity's sake) as the potential difference per unit length $E =$

$= \frac{V}{s}$. This means that the intensity of the field will be the greater the larger the potential difference between the electrodes and the smaller the distance between them. This is true only if the medium is homogeneous and the electrodes are large. In non-homogeneous, geometrically complex media, with electrodes of small dimensions, the intensity of the field must be defined according to the

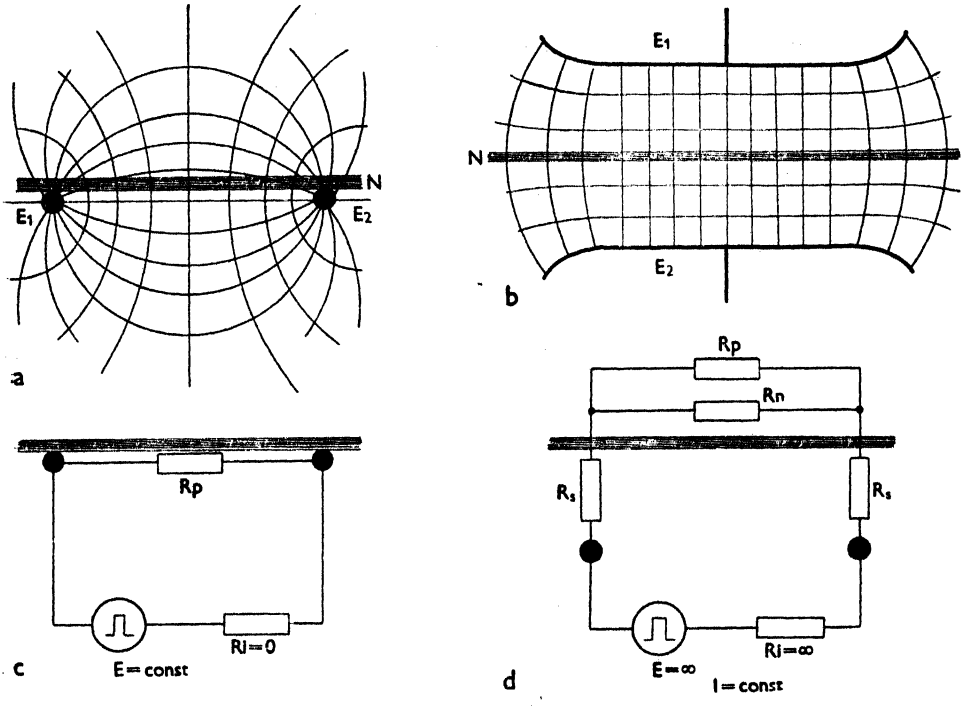

Fig. 1. Electrical stimulation of a nerve. 1a,b: electric field between stimulating electrodes of small dimensions (a) and between large flat electrodes (b). N-nerve. E_1, E_2 — electrodes. c: stimulation with constant voltage. d: stimulation with constant current.

equation $E = \frac{dv}{ds}$, which states that the field intensity is given by the relative increase (differential) in potential between two points infinitely close to each other.

Our objective is 1) to find such an arrangement of electrodes and fibre that the field intensity at the stimulated point is least dependent on small changes in the geometric arrangement (especially on changing the position of the nerve fibre with respect to the electrodes), 2) to have an intensity of the field at the stimulated point that is least dependent on tissue and medium resistance and 3) to define exactly the point at which the impulse originates and to keep this point constant.

The first condition is fulfilled most satisfactorily with large plane electrodes situated as far as possible from one another and parallel to the fibre, which is situated half way between the electrodes (Fig. 1b). This arrangement, however, complies poorly with the third condition, since the field intensity will be nearly constant over a relatively large area of the fibre and thus the point of origin of the impulse will be more or less left to chance. The third condition is fulfilled the more exactly the smaller the electrodes (or the smaller thedifferent electrode), the closer the electrodes are to each other and, most important, the closer they are to the nerve fibre (or the closer the different electrode is to the fibre) (Fig. 1a). The second condition can be complied with only in two special cases:

a) The variable leakage resistance (R_p) in parallel with the stimulated element (which need not have a constant resistance) is connected directly to the electrodes. The independent field intensity can be fulfilled by using a supply with a zero internal resistance (R_i), or, as it is usually called, a constant voltage output (Fig. 1c).

b) The variable leakage resistances (R_s) are connected in series with the stimulated element. The resistance of the latter (R_n) or that including further parasitic resitance (R_p) connected in parallel, is constant (Fig. 1d). The above condition can then be fulfilled using a high electromotive force with a high internal resistance, so that the current does not depend upon the external resistance. This is usually called constant current output.

Even this very rough analysis shows that the three above conditions cannot be fulfilled at the same time. Under certain circumstances it is possible to ensure approximately that the actual effective stimulus is proportional to the stimulus applied at the electrodes. It is, however, quite impossible to ensure identity of the effective stimulus for different tissues, not even with the same apparatus and equipment, and it cannot be excluded, even with the same kind of preparation, that in different experiments the actually effective stimuli are very different from the applied stimuli of the same characteristics. These difficulties may be overcome to a great extent using intracellular stimulation.

The physical characteristics of the stimulus

As explained above, the effectiveness of a stimulus is given by transfer of ions and changes in their concentration and hence it is determined not only by the intensity of the field but also by its time course, which may be, however, infinitely variable. If only periodic stimuli are considered (single stimuli being a special case) the harmonic stimulus is determined by three physical parameters: the amplitude (of voltage or current), the frequency and the

duration (or number of periods). The so called rectangular pulse is determined by four parameters: the amplitude, the width of the pulse, the repetition frequency and the duration of the pulse train (or the number of pulses). The amplitude can, of course, be either positive or negative. If these stimuli have a D. C. component, a further parameter is added. More complex stimuli are determined by a correspondingly larger number of parameters so that it is often impossible to characterise them exactly.

In electrophysiological experiments it is often important to isolate one variable factor while maintaining the other factors constant, so that complex relationships between stimulus and response may be understood. If different stimuli are considered from this aspect, the most essential question is how far it is possible to change only one parameter, i. e. how far a change in one parameter results in an alteration of another parameter or of other functions determining the effectiveness of the stimulus.

With a harmonic stimulus, a change in amplitude also changes the slope of the stimulus, i. e. the rate at which the voltage increases and decreases at the electrodes. A change in frequency also means a change in the slope of the stimulus. In addition, the effect of the stimulus may depend to a large extent on the phase of the wave at which the stimulus is switched on or off.

Rectangular pulse (ideal). The slope of front and trailing edges is infinite and thus not dependent upon the amplitude and frequency. Even though the slope cannot be infinite in reality, it is very large, nevertheless, so that its change with the amplitude is of little significance and with changing frequency the slope usually changes hardly. Consequently rectangular stimuli are now preferred to harmonic or more complex or undefinable stimuli (e. g. damped oscillations of an induction coil are not used any more).

It must further be pointed out that, under certain conditions, unpredicted effects may occur when a stimulus is applied, which are not considered when interpreting the results, and may lead to incorrect conclusions. These may be heat effects, electrolysis of the medium, entry of the electrode metals into the tissue (particularly copper, but other metals as well), and polarisation of the electrodes.

It must further be borne in mind that the stimulus may also act at a point other than that assumed, e. g. at the earthing plate, at different clamps etc. (see p. 148). In general the danger is the greater the greater the slope of the front and trailing edges of the pulse and the greater the amplitude of the stimulus.

Finally it must be remembered that the shape of the stimulus at the excitable elements themselves may considerably differ from the shape which can be registered at the stimulating electrodes. Distortion may be smaller when using a low resistance output. In general it is the larger the closer the shape of the stimulus approaches the ideal rectangular pulse and the shorter its duration.

The main requirements for a stimulator

Practical requirements for a good stimulator are thus as follows: a well defined shape of the stimulus, independent of other stimulus parameters (amplitude, duration etc.); a low internal impedance; easy, rapid and sufficiently fine control of individual stimulus parameters over a wide range. In addition to the above fundamental requirements there are others, that are of prime importance when recording responses: insulation (resistive and capacitive) of the stimulator output from earth and also from other parts of the apparatus, the possibility of synchronising the stimulus with the time base of the oscilloscope, with the time marker, with another stimulator etc., as will be discussed later on (see p. 129 and 132).

Different kinds of stimulators possess these features to different extents, and in choosing a stimulator for a definite purpose, the important stimulus parameters must be taken into consideration. Different kinds of stimuli will be described below, together with their electrophysiologically important characteristics.

The stimulator thus produces at the electrodes a potential difference with an exactly defined shape. The potential may either be determined in absolute units or may be automatically adapted to tissue resistance between the electrodes in such a way that the current passing between them is exactly defined.

Kinds of stimuli

Some physical aspects of the stimulus parameters will be considered now together with their relation to the electric circuits of the stimulator and the tissue.

Periodic voltage — Harmonic voltage

The most simple type is the harmonic or sine-wave stimulus. Here the potential e on the electrodes is defined by equation

$$e = E_c \sin \omega t \quad \text{where} \quad \omega = \frac{2\pi}{T} = 2\pi f$$

E_0 is the maximum potential value, ω is the angular frequency, T the period (repetition interval), f the frequency (number of periods per sec.).

The current passing between the electrodes is defined by equation

$$i = I_0 \sin (\omega t + \varphi) = I_0 \sin \omega(t + t_0)$$

where I_0 is the maximal current, φ is the phase shift of potential and current, t_0 is the time shift of potential and current. The following relation is valid between constants φ and t_0

$$\varphi = \omega t_0 = 2\pi f t_0 = 2\pi \frac{t_0}{T}$$

It is evident, chiefly from the last equation, that φ defines the relative and t_0 the absolute shift of potential and current.

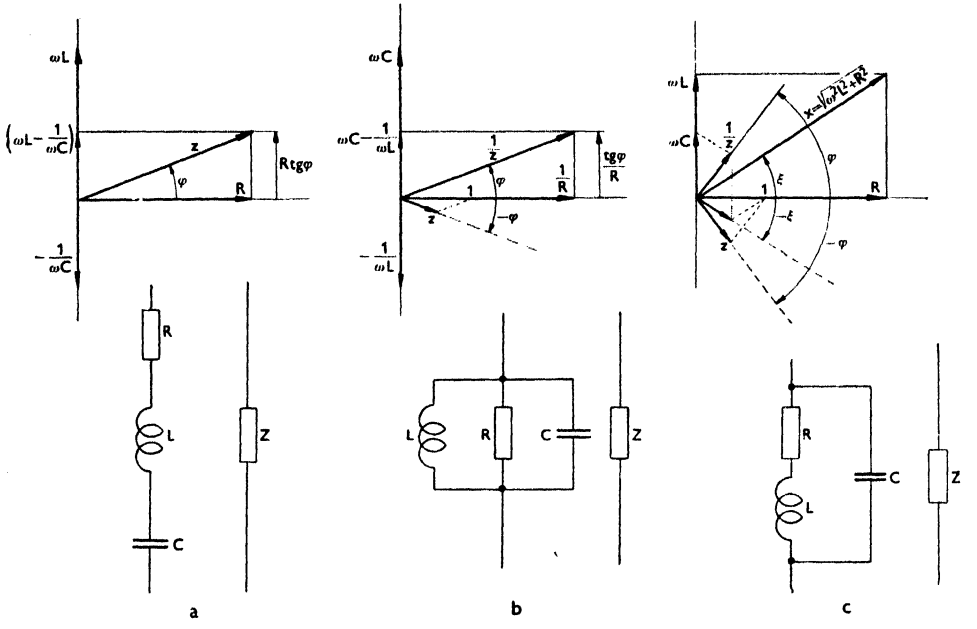

Fig. 2. Graphical determination of impedance in complicated circuits. Bottom: diagram of the circuit. Top: the corresponding vector diagram. The inverse values of some vectors necessary for further calculation are constructed in such a way that a parallel line is drawn to the given vector passing through point (+1;0). The point of intersection of this line with the side of the negative angle of the original vector is the end point of the required inverse value.

The potential and current are generally not in phase (i. e. they are shifted) because during the passage of current through some conductors (coils, condensers, electrolytes) new potentials arise which are added to the original potential *e*. During passage through a condenser the current leads the voltage by 90°, or voltage lags behind the current by 90°. When passing through an ideal coil, on the other hand, the current lags behind the voltage by 90°. In a resistance the current and potential remain in phase. In more complex circuits containing resistances, capacitances and inductances the current and potential may be shifted from +90° to −90° according to which component

is more important. Their relative significance is best analysed using a vector diagram as shown in Fig. 2. The ohmic resistance does not depend on f; the coil and condenser form resistances dependent on the frequency, so called reactances; inductive reactance $Z_L = \omega L$, capacitive reactance $Z_C = \frac{1}{\omega C}$; L is the inductance of the coil in henrys (H), C = capacity of the condenser in farads (F). In networks containing the components R, L and C in series, the total impedance of the circuit Z and the phase shift are

$$Z = \sqrt{R^2 + \left(\omega L - \frac{1}{\omega C}\right)^2} \quad \text{and} \quad \tan\varphi = \frac{\omega L - \frac{1}{\omega C}}{R}$$

For R, L and C in parallel the admittance is

$$\frac{1}{Z} = \sqrt{\frac{1}{R^2} + \left(\frac{1}{\omega L} - \omega C\right)^2} \quad \text{and} \quad \tan\varphi = \frac{-\frac{1}{\omega L} + \omega C}{\frac{1}{R}} = R\left(\omega C - \frac{1}{\omega L}\right)$$

One of the more complex cases (resistance and inductance in series, capacitance in parallel) is shown and analysed in Fig. 2c. Other complex examples may be solved in an analogous way. Further details must be sought in electrotechnical textbooks.

Let us now consider the time relation between current and voltage. If the circuit only contains an ohmic resistance, Ohm's law is valid:

$$e = R \,.\, i$$

For complex circuits more complicated relationships exist.

The power of A. C. and r. m. s. current and voltage values

The instantaneous power of a sinusoid current is

$$n = e.\, i. = E_0 I_0 \sin\omega t \sin(\omega t + \varphi)$$

It is evident that during one period T, n is zero four times (twice when $t = 0$, twice when $\omega t + \varphi = 0$) and attains extreme values also four times. These values, however, differ. The average power of the sinusoid current is then:

$$N = \int_0^{2\pi} ei \, \mathrm{d}t$$

$$N = \frac{1}{2} E_0 I_0 \cos\varphi$$

In order to obtain formal agreement of this equation with the analogous equation for the power of D. C. current (which is $N = EI$ or $N = \frac{E^2}{R}$ or $N = I^2R$) we introduce

$$E_{eff} = \frac{E_0}{\sqrt{2}} \quad \text{and} \quad I_{eff} = \frac{I_0}{\sqrt{2}} \text{ and obtain}$$

$$N = E_{eff} I_{eff} \cos \varphi = \frac{E_{eff}^2}{Z} \cos \varphi = I_{eff}^2 Z \cos \varphi$$

E_{eff} and I_{eff} are the r. m. s. (root mean square) values of current and voltage and after substituting for $\sqrt{2}$

$$E_{eff} = 0{\cdot}707 E_0 \quad \text{and} \quad E_0 = 1{\cdot}414 E_{eff} \quad \text{and similarly for } I_{eff} \text{ and } I_0.$$

Mean current. As shown, the r. m. s. values are defined on the basis of power. Values of alternating current (or voltage) may, however, be defined also in another way as integrated current (or voltage) corresponding to the area limited by the time axis and the curve of the current (or voltage).

$$I_m = 2I_0 \int_0^{\pi} \sin \omega t \, \mathrm{d}t = \frac{2}{\pi} I_0$$

$$E_m = 2E_0 \int_0^{\pi} \sin \omega t \, \mathrm{d}t = \frac{2}{\pi} E_0$$

These values are termed mean values and thus

$$E_m = 0{\cdot}636 E_0 \quad \text{and}$$

$$E_0 = 1{\cdot}572 E_m.$$

In electronics and physics E_0 and I_0 are usually used. In engineering and usual calculations r. m. s. values are used. Mean values are applied only rarely, especially when determining the quantity of electricity (e. g. in integrators). In this case $Q = I_m \, . \, t$ (if $t = nT$).

The sensitivity of different measuring apparatus must also be considered.

Measuring A. C. voltage and current

a) Apparatus with a rotating coil measure the D. C. component.
b) If a rectifier is added, the apparatus measures mean current values, but as an A. C. apparatus they are usually calibrated in r. m. s. values. They thus show r. m. s. values although their deviation is due to mean current (they automatically recalculate mean current to r. m. s. current). If currents and potentials of non sinusoid shape are measured, such instruments do not

show correct values. They can then be only used for measuring mean currents, which must, however, be calculated from the figures on the scale according to

$$I_m = 0{\cdot}900 I_{eff}$$

where I_m is the actual mean current and I_{eff} the value shown by the apparatus.

c) Dynamic apparatus always measure exact r. m. s. values (and if connected as wattmeters, the power).

d) Electrostatic apparatus show the r. m. s. voltage (only in some special connections do they show other, e. g. maximal, values).

f) Electronic voltmeters show either mean or maximal values according to how they are connected.

g) Oscilloscopes show the whole time course of the current or voltage.

h) Coulombmeters and ampere-hour meters show the amount of electricity that has passed

$$Q = \int_0^t i dt$$

i) Watt-hour meters show the amount of energy passing through the circuit.

Nonharmonic voltage

Periodic nonharmonic voltage.

This can always be transformed to a sum of harmonic potentials, the frequencies of which are a multiple of the basic frequency (so called second, third etc. harmonics), the phases of which are generally shifted. This analysis into a sum of higher harmonic components is termed harmonic (also Fourier) analysis. This may be performed mathematically, graphically, mechanically or electrically (using so called harmonic analysers) (Smirenin 1950).

While the harmonic potential remains harmonic in all circuits not having any nonlinear elements*) and only its phase changes, the shape of the non-

*) Nonlinear elements change their characteristics with the size of the voltage or current. For instance, ohmic resistance independent of the temperature, the capacitance of a condenser and inductance of an air cored coil are all linear elements since their values are constant and independent of the voltage and current. The resistance of a bulb increases on heating (and thus with the current); inductance of an iron cored coil first increases with the current and then (after saturation) decreases; the resistance between electrodes of a valve depends on the voltage across these and other electrodes. The resistance of a rectifier (diode, germanium diode, selenium rectifier) depends on the voltage and current. Thus valves, rectifiers, bulbs, ironcored coils and transformers are examples of nonlinear elements.

harmonic potential is preserved only in circuits containing no other elements but ohmic resistances.

Inductance and capacitance produce changes in the shape of the stimulus by changing the amplitude of its components (especially of higher harmonics) and by affecting their phase relations. These changes may be used to adjust the shape of the pulse for which purpose nonlinear elements are often used. They also cause serious difficulties, however, because the shape, particularly of sharp (rectangular or triangular) pulses, is distorted. In fundamentally the same way these components act on individual stimuli and hence their influence will be analysed systematically.

Aperiodic voltage; Voltage step

A D.C. voltage step (positive or negative). Fig. 3.

The voltage across the electrodes rises suddenly to a constant value E (Fig. 3a). Obviously this ideal time course can only be approximated. Depending upon how close an approximation is required, the appropriate apparatus is chosen. Let us consider an ideal voltage generator (Fig. 3b) composed of a galvanic cell E, a switch S and a resistance in series R_i (this is really the internal resistance of the source). To the generator a capacitance C_p (the capacitance of the output and the electrodes) and a resistance R_i is connected in parallel. If S is switched on, the current begins to flow with an initial value of $i_0 = \frac{E}{R_i}$ which, on charging C_p, decreases asymptotically to a value

$$i_\infty = \frac{E}{R_i + R_e}$$

The charge of C_p increases with time until its voltage attains a value

$$e_\infty = E \frac{R_e}{R_i + R_e}$$

The instantaneous voltage on it is

$$e = e_\infty \left(1 - e^{-\frac{t}{\tau}}\right) \quad \text{(Fig. 3-e)}$$

where e is the base of natural logarithms ($e \doteq 2{\cdot}7$); $\tau = R_i C_p$ is the so called time constant. This is the time during which C_p is charged to a voltage of $0{\cdot}63 e_\infty$, i. e. approximately $\frac{2}{3}$ of the maximum voltage. The time constant is an important characteristic of the circuit. During 2τ, C_p is charged to $\frac{9}{10}\, e_\infty$, during 3τ to $0{\cdot}96 e_\infty$ etc. It may, therefore, be generally assumed that the maximum value is attained after 2 to 4τ (with an error of 10—1%).

It can be seen that the "parasite capacity" C_p prolongs the rising time of the front of the D. C. pulse and this prolongation depends not only on C_p but also on the resistance R_i.

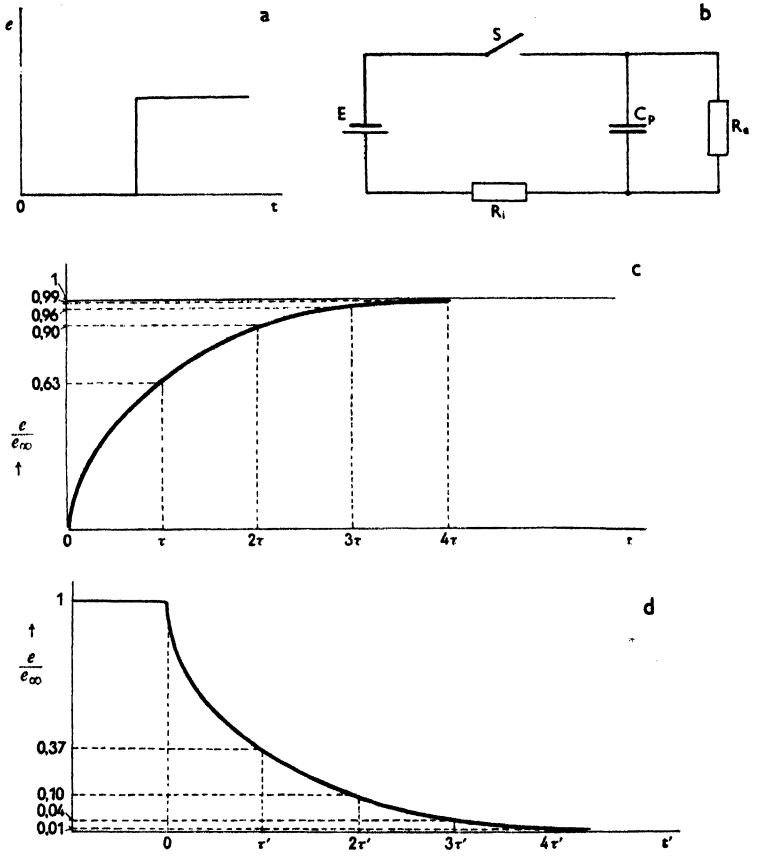

Fig. 3. Distortion of the voltage step by parasitic capacitance C_p. a) ideal step, b) equivalent circuit diagram. Distortion when switching the switch S on (c) and off (d).

Rectangular pulses

All that was said in the preceding paragraph is also valid for the front edge of a rectangular pulse. Its trailing edge will also be distorted by the parasitic capacitance C_p. If the key S is opened in time ∞ (i. e. practically in time $t > 2\tau$) the voltage at R_e does not drop to zero immediately but falls as C_p is discharged across R_e (Fig. 3d). The voltage across R_e is:

$$e = e_\infty e^{-\frac{t'}{\tau'}}$$

where t' is the time from the moment when S is opened and $\tau' = R_e C_p$ is the time constant of the trailing edge of the pulse. The shape of a rectangular pulse with the width t_p is shown in Fig. 4 for a) $t_p \gg \tau$, b) $t_p \approx \tau'$ and $c/\tau > t_p$. (In all cases $\tau' \doteq 3\tau$.) It is evident that in the first case the parasite capacitance only distorts the shape of the pulse but its amplitude is nearly independent of small changes in t_p. Such a shape as shown in Fig. 4a, may still be used in

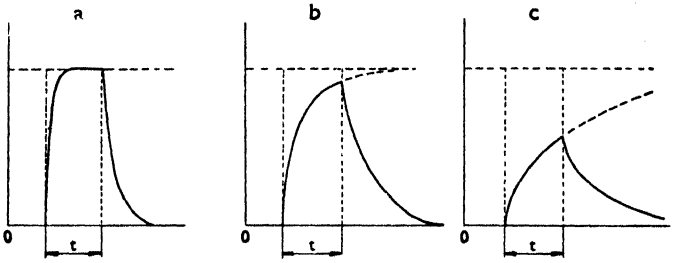

Fig. 4. Distortion of the pulse by $RC = \tau$, $R'C' = \tau'$

a) $t \gg \tau$; $\tau' = 3\tau$, b) $t = 2\tau$; $\tau' = 3\tau$, c) $t < \tau$; $\tau' = 3\tau$.

For details see text.

stimulators for the shortest pulses available. The conventional condition is approximately $t_p > 4\tau$ ($\tau > \tau'$), or $t_p > 4\tau'$ (if $\tau < \tau'$).

The impulse shown in Fig. 4b, on the other hand, will be undesirable in a good stimulator, since for instance, a slight change in the length of the pulse (or the time constant RC) also results in a considerable change in amplitude. The shape of the pulse in Fig. 4c scarcely resembles the required rectangular shape.

Damped oscillations

So far only a circuit containing resistance and capacitance has been considered. The course of the voltage as was found in this case is called aperiodic. A similar course would also be seen with resistance and inductance, but this case hardly ever occurs in electrophysiology. If however, inductances are connected in the circuit in addition to resistance and capacitance, much more complex relations are generally obtained. If the resistance (or conductance) predominates, the shape of the distorted pulse is again aperiodic. If the effect of reactances is higher, damped oscillations appear with the voltage step. If the resistance is neglected at a first approximation, the oscillation frequency is

$$f = \frac{1}{2\pi\sqrt{LC}} \quad \text{or} \quad \omega = \frac{1}{\sqrt{LC}} \quad \text{i. e.} \quad T = 2\pi\sqrt{LC}\,.$$

The effect of resistance is to decrease the oscillation frequency somewhat and chiefly to damp the oscillations*). Since parameters may differ when switching the current on and off, the oscillations at the front and trailing edge of the pulse may differ considerably. As an example oscillations occurring in the secondary coil of a Du Bois-Reymond inductorium are shown in Fig. 5.

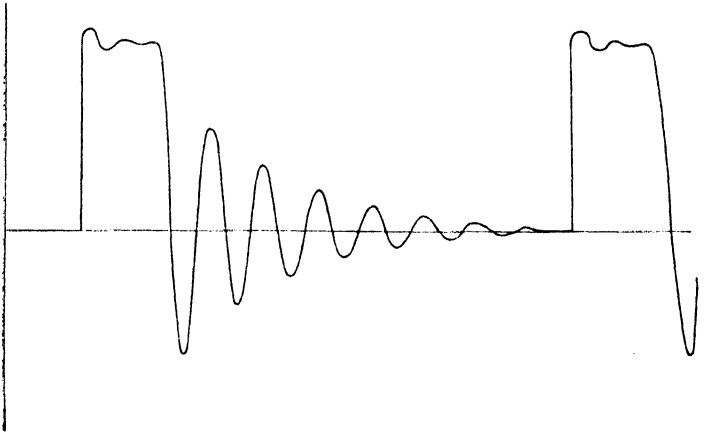

Fig. 5. Damped oscillations of the du Bois-Reymond inductorium.

If a transformer is used in the stimulator output it is usually necessary to eliminate oscillation phenomena. Oscillations can be reduced (Fig. 6):

1) By damping resistance: An appropriate resistance is connected in parallel to the coil so that the voltage course becomes aperiodic. This, however, prolongs the front and trailing edge of the wave so that only specially designed transformers ought to be used having small capacitance between the turns, layers of turns and coils on a common core. If the frequency of the oscillations before connecting the aperiodic resistance is sufficiently high, the prolongation of the front and trailing edge caused by damping is not significant. The amplitude is, however, decreased considerably.

2) By damping with a valve, diode or germanium rectifier connected as the damping resistor in case 1 (Fig. 6b). The use of a germanium diode is particularly simple, cheap and effective. With it the second half of the wave is strongly damped while the first half remains practically unchanged. Thus

*) In a parallel circuit (Fig. 2) critical damping is attained if $R = 2\sqrt{\frac{L}{C}}$; if the by-pass resistance R is greater, the circuit oscillates, if it is smaller, the distortion of the edges is too large. Since we usually do not know the inductance L and especially the parasitic capacitance C of the transformer with sufficient accuracy, it is best to find the damping resistance by trial and error.

most of the oscillation energy is transformed to heat and, the third half-wave is only $\frac{1}{10}$ of the first. It is important that the slope of the front and trailing edge is much steeper than when damping with resistances.

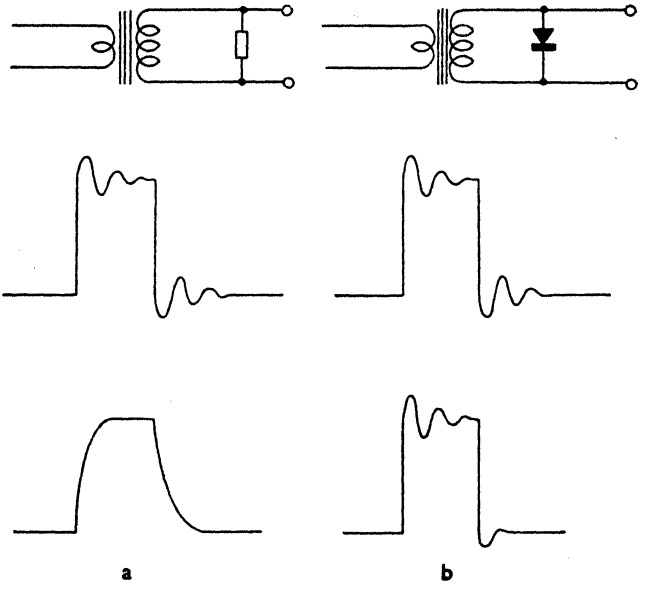

Fig. 6. Damping of undesirable oscillations in the transformer by resistance (a) or a germanium diode (b). Top: circuit diagram. Middle: pulse shape before damping. Bottom: after connecting a resistance or diode.

"Sawtooth" oscillations

Sawtooth waves are of two types: with a steep front or a steep trailing edge. What has been said above also holds true for the steep side of this wave form. It is usually not difficult to lead the gradual part through the circuit with considerably less distortion than the steep side.

Triangular pulses are distorted mainly at their peaks where aperiodic flattening or oscillations due to the same causes as in rectangular impulses occur.

Repetitive stimuli

Multiple stimuli, especially paired rectangular pulses. These are used more and more frequently. In addition to what has already been stated concerning single rectangular impulses, the intervals between them must also be considered. A low time constant must be maintained, particularly if the first impulse is much higher than the second.

Stimulators

1. Mechanical stimulators

Mechanical stimulators are rarely used at present although the best of them may be more satisfactory than the average electronic apparatus. In addition to simplicity of electrical circuit, their main advantage lies in the fact that their parameters do not change in time, are not influenced by the instability, interference and hum of the mains supply and, last but not at least, that perfect resistive and capacitative insulation of their output voltage from earth and other parts of the apparatus particularly from the amplifier, can be attained. Their main disadvantage is that they cannot be used universally and that sometimes it is impossible or difficult to change important parameters of the stimuli.

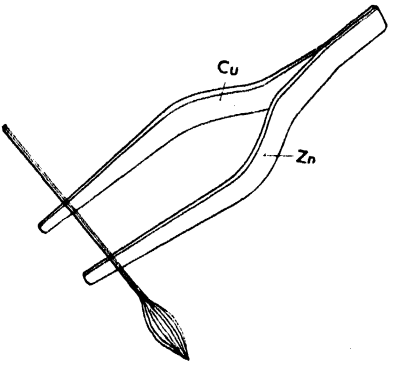

Fig. 7. Stimulation of a nerve-muscle preparation with a galvanic forceps.

1) Galvanic forceps (Fig. 7). Two arms, one of zinc, the other of copper, are soldered together with tin solder. Actually these are two electrodes of the original Volta cell which are short circuited. The stimulated tissue is the electrolyte. During dissection, the galvanic forceps is applied on nerves in order to determine which branch leads to which muscle.

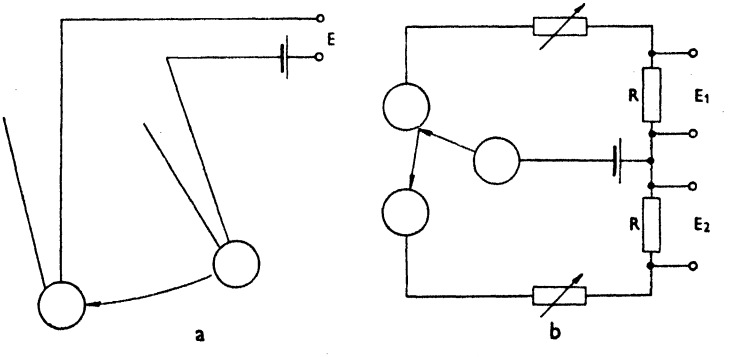

Fig. 8. Ebbecke's rheotome. a) simple, b) diagram of more complex rheotome for stimulating with a pair of pulses of different amplitudes.

2) Ebbecke's rheotom (Fig. 8). Steel spheres are suspended as pendula. When the spheres come into contact, a short rectangular pulse of a very good

shape is produced. The duration of the pulse, however, is constant and depends almost entirely upon the diameter and material of the spheres. Ebbecke's spheres may also be used for stimulating with two pulses or with a series of pulses at one or more pairs of electrodes (Fig. 8b).

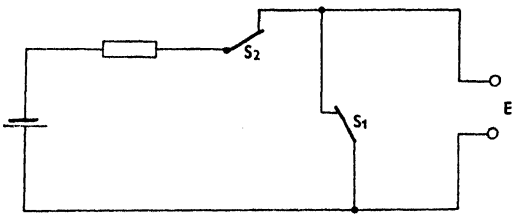

Fig. 9. Stimulator with two break (N. C.) contacts.

3) A magnetic stimulator produces an impulse by a change in the magnetic flux in a coil.

4) Stimulators with movable contacts. There are usually quite unsatisfactory, since contacts bounce, especially when switched on, and thus several short pulses followed by a long one and finally again by several short ones are obtained. Only the arrangement shown in Fig. 9 is still used sometimes. Electrodes are short-circuited by switch S_1 at rest. When this switch is opened a voltage appears at the electrodes. This is interrupted by switch S_2 which disconnects the source. The switches usually are switched off with a pendulum. The pulse width depends upon the distance of the switches, their position with respect to the equilibrium position and the amplitude of the pendulum oscillations. Calculations, especially for longer pulses, are fairly complex. Short pulses cannot be regulated very exactly.

5) The inductorium of Du Bois-Reymond is only of historical interest, although now and again it is still used. The shape of the stimulus is badly defined (Fig. 5), and much depends on the coil distance and on the external circuit. The continuously changing contact areas of the interrupter also make stimulus shape irreproducible. In addition the inductorium is a source of strong electromagnetic disturbances affecting both the recording instruments and the preparation itself (apolar and unipolar stimulation).

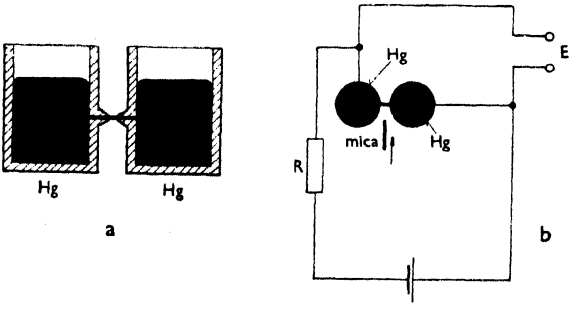

Fig. 10. Mercury stimulator according to Petráň. a) vessels connected by a column of mercury; b) circuit diagram.

6) The mercury stimulator according to Petráň (1955) is really a contact stimulator. It uses the principle applied by Peters (1859) for recording second signals in astronomical clocks. Bouncing spring contacts are replaced, however,

by mercury contacts. The vessel (Fig. 10) is filled with mercury until it flows out through the side opening near the bottom. If two such vessels are placed opposite to each other with the openings exactly facing each other, the mercury ceases to flow and surface tension forms a connection between the two vessels (Fig. 10a). If this connection is interrupted by inserting a piece of paper or mica between the contacts, a rectangular pulse is obtained without any oscillations, since the mercury menisci are joined firmly by surface tension. Thus these two vessels form a perfect mechanical contact which, when connected as shown in Fig. 10b gives good rectangular pulses lasting from $0{\cdot}2 \cdot 10^{-3}$ sec. to 10^{-2} sec. and longer.

The interrupter must be prepared very carefully from mica 0·02 to 0·1 mm thick. A sheet of triangular shape is mounted on a rotating arm exactly perpendicular to the connection of the mercury contacts. The arm is rotated by a small motor or fixed to a pendulum. The impulse frequency is determined by the number of revolutions of the motor (for single impulses a pendulum is more suitable), the duration of the impulse is regulated by the speed at which the arm moves and by the width of the mica sheet interrupting the mercury column. This effective width can be easily adjusted when using a triangular sheet by raising or lowering the vessels. The apparatus can give several impulses with exact time relations when several interrupters are connected to one axis or the number of mercury vessels is increased. Synchronisation with the sweep of the oscillograph is achieved by either using an auxiliary mechanical contact or a magnet and coil. A disadvantage is that mecury may spill in fine drops. It is therefore best to enclose the whole apparatus in a transparent cover.

Transformers

Transformers are often used in electrophysiological instrumentation, especially in connection with stimulators, both electromechanical and electronic, e. g. as mixing, derivative and isolation transformers. They are also used for isolation of stimuli from earth, for separating off the A. C. component or for simultaneous stimulation at several pairs of electrodes which must not be connected directly etc. In all these cases the lowest and highest frequencies transferred by the transfomer must be lower and higher than the frequencies*) used for stimulation. Lower frequencies are distorted by insufficient inductance (the transformer acts as a differentiating coil), higher frequencies by stray

*) This applies not only to the basic frequency, but also to all higher harmonics necessary for maintaining the given shape of the stimulus.

capacitance (capacitances between turns and coils). In order to emphasize a certain frequency a resonant circuit*) or a tuned transformer is used.

For close coupling between the coils (the mutual inductance M is only slightly smaller than the geometric mean of inductances of the primary coil L_1 and the secondary coil L_2) only one coil is tuned. With loose coupling ($M \ll \ll \sqrt{L_1 L_2}$) often both coils are tuned and thus not only the transferred fre-

*) The networks shown in Fig. 2 are essential for explaining resonant circuits. We know (Fig. 2a) that

$$Z = \sqrt{R^2 + \left(\omega L - \frac{1}{\omega C}\right)^2}$$

It is evident that there is a certain frequency ω_0 for which $\omega L - \frac{1}{\omega C} = 0$ and thus $Z_0 = R$. This value Z_0 is evidently the least of all Z in a given circuit. Thus for $Z_0 = Z_{minim.}$ the resonance frequency

$$\omega_0 L = \frac{1}{\omega_0 C}$$

or

$$\omega_0 = \frac{1}{\sqrt{LC}}; \quad f_0 = \frac{1}{2\pi\sqrt{LC}}; \quad T = 2\pi\sqrt{LC}$$

i. e. Thomson's equation.

This connection (R, C, L in series) is termed a *series resonant circuit.*

It follows from: $R = 0$ that

$Z = 0$

the ideal series resonant circuit (with an ideal coil with zero resistance) has a *zero impedance* for the resonance frequency.

In an analogous way for a network shown in Fig. 2c we have

$$\frac{1}{Z} = \sqrt{\left(\frac{R}{R^2 + \omega^2 L^2}\right)^2 + \left(\frac{\omega L}{R^2 + \omega^2 L^2} - \omega C\right)^2}$$

Here too there is evidently a resonance frequency for which $\frac{1}{Z}$ is minimal. This occurs if $\frac{\omega_0 L}{R^2 + \omega_0^2 + L^2} = \omega_0 C$

hence

$$\omega_0 = \frac{1}{\sqrt{LC}} \sqrt{1 - \frac{R^2 C}{L}}$$

Usually, however $R \ll \omega \, . \, L$ and thus $\omega_0 \approx \frac{1}{\sqrt{LC}}$ for $R = 0$ in the preceding equations $\frac{1}{Z} = 0$ and therefore $Z = \infty$. For the resonance frequency (ω_0) an ideal *parallel resonant circuit* has an *infinite impedance*. It is important to bear in mind that for a resonance frequency the parallel as well as the series resonance circuits represent pure resistances since $\tan \varphi = 0$.

quency can be controlled but also the transferred band width. For calculations and further details see Terman (1943).

The inductance of coils is icreased by the use of laminated iron cores. As losses due to eddy currents rise with the frequency the lamination is the finer the higher the transferred frequency. For the same reason powdered-iron cores or special alloys (e. g. permalloy) or ferrites are used.

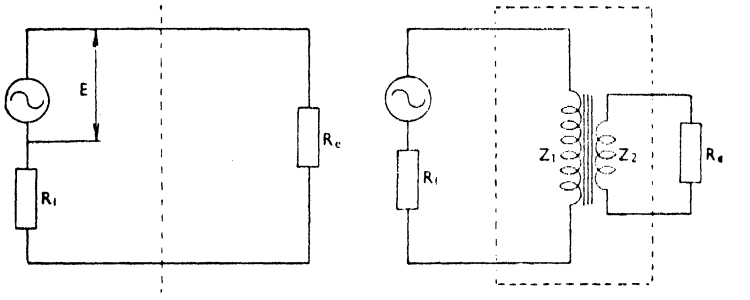

Fig. 11. Matching of the source resistance (R_i) to the load resistance (R_e) using a transformer.

Transformers are also often used for matching the load impedance to the source impedance. It can easily be demonstrated that a given power supply with impedance R_i will have its maximum possible output when the impedance of the load R_e is equal to R_i ($R_e = R_i$). This will be explained in an example (Fig. 11a)*). The resistance of the source $R_i = 100\ \Omega$ and its e. m. f. $E = 100$ V.

If a load having an impedance $R_e = 1\ \Omega$ is connected to this source the power will be

$$N = 1\,\mathrm{W}\ \left(I = \frac{100\ \mathrm{V}}{101\ \Omega} \doteq 1\ \mathrm{A}\,;\ N = I^2R\right)$$

for $R_e =$ 10 Ω $N =$ 8 W
for $R_e =$ 100 Ω $N =$ 25 W
for $R_e =$ 1000 Ω $N =$ 8 W
for $R_e =$ 10000 Ω $N =$ 1 W

If $R_e \neq R_i$ and if A. C. current is used, the load impedance can be adjusted to

*) The abridged proof is as follows

$$N = RI^2 \qquad I = \frac{E}{R_i + R_e} \qquad N = \frac{R_e}{(R_i + R_e)^2} \cdot E^2$$

for $N =$ max the differential quotient $\frac{\mathrm{d}N}{\mathrm{d}R_e}$ must be zero.

$$\frac{\mathrm{d}N}{\mathrm{d}R_e} = E^2\,\frac{(R_i + R_e)^2 - 2R_e(R_i + R_e)}{(R_i + R_e)^4} = 0$$

and thus $R_e = R_i$.

the source impedance using a matching transformer (Fig. 10b). If z_1 is the number of turns on the primary coil, z_2 the number of turns on the secondary coil, V_1, V_2 the primary and secondary voltages, I_1 and I_2 the primary and secondary currents and $p = \frac{z_1}{z_2}$ the turns ratio, then the following relation is approximately valid:

$$\frac{z_1}{z_2} = p = \frac{V_1}{V_2} = \frac{I_2}{I_1}$$

By division we obtain:

$$\frac{\frac{V_1}{I_1}}{\frac{V_2}{I_2}} = p^2 .$$

As $\frac{V_1}{I_1} = Z_1$ is the impedance of the primary coil and $\frac{V_2}{I_2} = Z_2$ the impedance of the secondary coil, $\frac{Z_1}{Z_2} = p^2 = \frac{z_1^2}{z_2^2}$. The values in the figure must be chosen in such a way that $Z_1 = R_i$; $Z_2 = R_e$ and the primary to secondary turn ratio

$$p = \frac{z_1}{z_2} = \sqrt{\frac{R_i}{R_e}}$$

With complex circuits containing resistances, capacitances and inductances even more perfect adjustment may be obtained by matching not only the source and load impedance but also the phase angles of the primary and the secondary current so that they are of equal value, but of opposite sign.

2. Electronic stimulators

These, according to the shape of the stimulus, are divided into, 1) generators of harmonic oscillations, 2) generators of rectangular pulses, 3) generators of special wave forms. It must be pointed out right at the beginning that any problem may usually be solved by different types of apparatus and that the problem must be thoroughly analysed at first in order that the most suitable approach for the required purpose be chosen. Criteria important for this choice will be given for all examples mentioned.

In general the same purpose may be achieved in two fundamentally different ways: by using a simple single purpose apparatus which produces impulses of a given frequency, duration and amplitude, or by using a universal apparatus consisting of several parts, the first of which may initiate oscillations of a given frequency, the second may change them to rectangular pulses, the following adjust their duration and the last one gives them the required size.

Although this second way may seem needlessly complex, it is much more suitable in practice since 1) it permits the variation of any parameter independently of all others, 2) it permits the use of the apparatus for various purposes according to immediate requirements, 3) developing and producing individual standardized parts to be combined as necessary is more economical, 4) failures and disturbances are more easily found and repaired in such apparatus.

In electronics, one of the most important criteria for impulse generators is the pulse width and the slope of its leading edge. This is not very important for our purposes, since it is relatively easy to attain values that are quite suitable for electrophysiology. On the contrary, however, requirements concerning the flatness of the top of pulses or the slope of the trailing edge are sometimes stricter than in electronics. The individual functional elements and the single purpose apparatus will be dealt with systematically below. In the next part the connection of these elements into complete apparatus will be described (see page 86). It is assumed that the reader is acquainted with the fundamental electrical laws and the principles of vacuum-tubes (diodes, triodes, pentodes) and germanium rectifiers (see also p. 94).

1. Generators of harmonic oscillations

a) High frequency oscillators

High frequency oscillators are amplifiers whose grid or anode impedance or both are frequency dependent (they have parallel resonant circuits, see p. 48), so that amplification depends on frequency and is maximal for the resonance frequency. In addition there is a feedback circuit between the anode and the grid by which a fraction of the output is returned to the input. The energy fed back should be only slightly larger than losses in the circuit. The phase or polarity of the feedback is also important. For a positive (regenerative) feedback (which increases the amplification), the voltage transferred from the anode to the grid must be reversed in phase, i. e. if the anode voltage decreases, the grid voltage must be increased by the feedback and vice versa.*)

*) In other words: if a positive or negative voltage change appears accidentally or is induced across the input, the absolute value of this change is increased by the positive feedback. A negative (degenerative) feedback, on the contrary, results in decreased changes in input voltage because the output is fed back in such a phase as to oppose the input (i. e. plate voltage is returned to the grid of the same valve unchanged in phase). It may of course happen that the feedback is negative for a certain frequency and positive for another. Negative feedback is used for improving the frequency-response characteristics of low frequency amplifiers. It may then happen that such an amplifier becomes an oscillator at a high frequency because the negative feedback has changed to a positive one for this high frequency.

The oscillator works as follows: a small change in voltage is amplified, and returned to the grid by the positive feedback. This continues until the feedback is no longer capable of compensating for the decrease in amplification due to the shift of the grid voltage to the curved part of the tube charac-

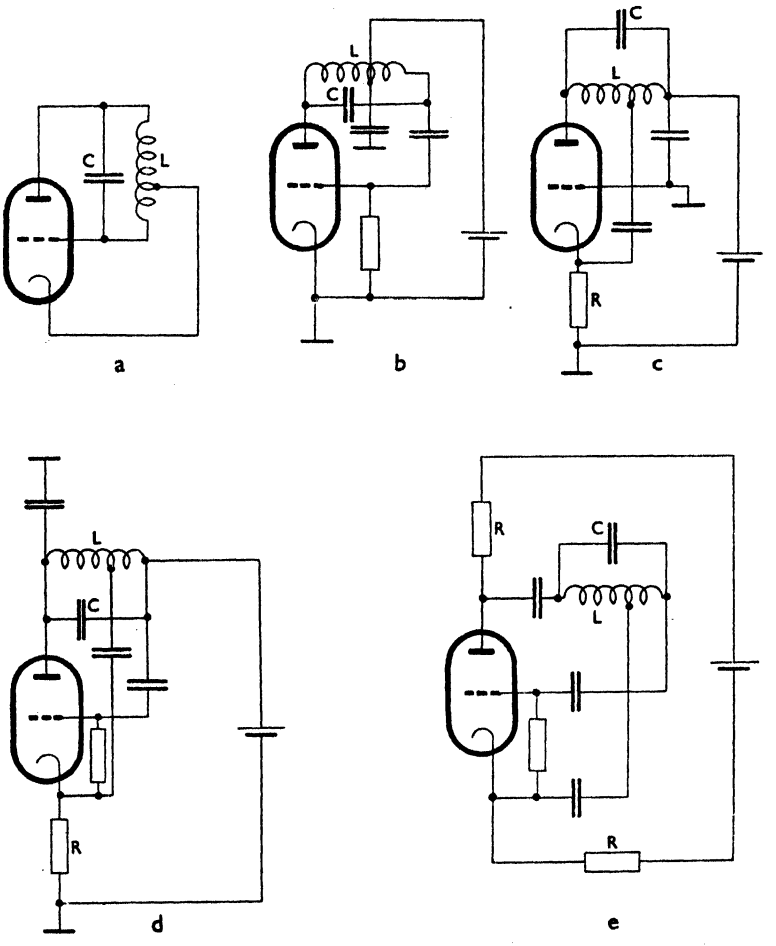

Fig. 12. Hartley's oscillator. a) basic diagram; b—e possible variations; b) with an earthed cathode; c) with an earthed grid; d) with an earthed anode; e) with an oscillation circuit not connected galvanically with any electrode. In place of R a choke is more suitable.

teristic. The plate and the grid voltages (their absolute value) now begin to fall. The rate of decrease is regulated by the resonant circuit. The grid and the plate voltages are again increased by the positive feedback etc. It is evident that the frequency of the oscillator depends on the resonance frequency ω_0 of the frequency control unit and on the amount of the feedback. The smaller

the feedback, the more exactly the frequency of the oscillator approaches the resonance frequency ω_0 and the sinusoidal wave form.

When constructing an apparatus a certain circuit must be chosen (for details see Smirenin 1950, Terman 1943). Only one of the most usual and most reliable circuits, Hartley oscillator, will be described here (Fig. 12). The resonant circuit is connected between the plate and the grid (Fig. 12a). The feedback voltage is obtained by connecting the tap of the coil to the cathode (the coil thus acts as an autotransformer). This connection is also termed a three-point circuit since the coil is connected to the valve at three points. One of these points must be earthed, either directly or across a condenser. Several circuit variants may be used, e. g. the coil may have the D. C. voltage of the plate, cathode or grid. This is best seen in Figures 12 b-c. Similar combinations are possible for all other types of oscillators. A Hartley oscillator may be constructed with conventional valves and air- or iron-cored coils for frequencies of several kc/sec. to several tens or hundreds of Mc/sec.

b) Low frequency (audio frequency) generators

α) LC oscillators are analogous to the high frequency generator described above. Hartley's circuit is usually not employed. Instead a circuit with separate oscillation and feedback coils on the same laminated iron core is used. The oscillators are very sensitive to the amount of the feedback. It is difficult to construct them in such a way that their frequency can be regulated continuously and they are therefore used only as a source of fixed frequencies. The frequency selector simultaneously switches on the oscillation condensers (fixed) and corresponding resistors controlling the amount on the feedback. They are little used, since large coils are expensive.

β) Two-point oscillators. For detail see Terman (1943), Smirenin (1950). Their description is beyond the scope of this book.

γ) Beat-frequency oscillators were the most exact laboratory oscillators up to recent times. Low frequency is produced as the frequency difference between two high frequencies (about 100 Kc/sec) one of which changes over a small range. Thus the frequency difference is changed e. g. from 0 to 16 000 c/sec (Philips GM 2307 oscillator).

δ) They have been replaced by much simpler and more reliable RC generators. Fundamentally these are amplifiers with a feedback having variable phase shift. There is a certain frequency for which the phase shift is just 180 degrees. Because of this frequency dependent feedback the amplifier works as an oscillator with a frequency for which the amplification is maximal, i. e. a frequency for which the phase shift is 180 degrees. The circuit shown in Fig. 13a

is suitable as a fixed frequency source, that shown in Fig. 13b as a variable frequency oscillator controlled by variable resistors and capacitors.

Such an oscillator may also be used as a filter, if the feedback is adjusted so that the oscillator just ceases oscillating. If now any low and distorted volt-

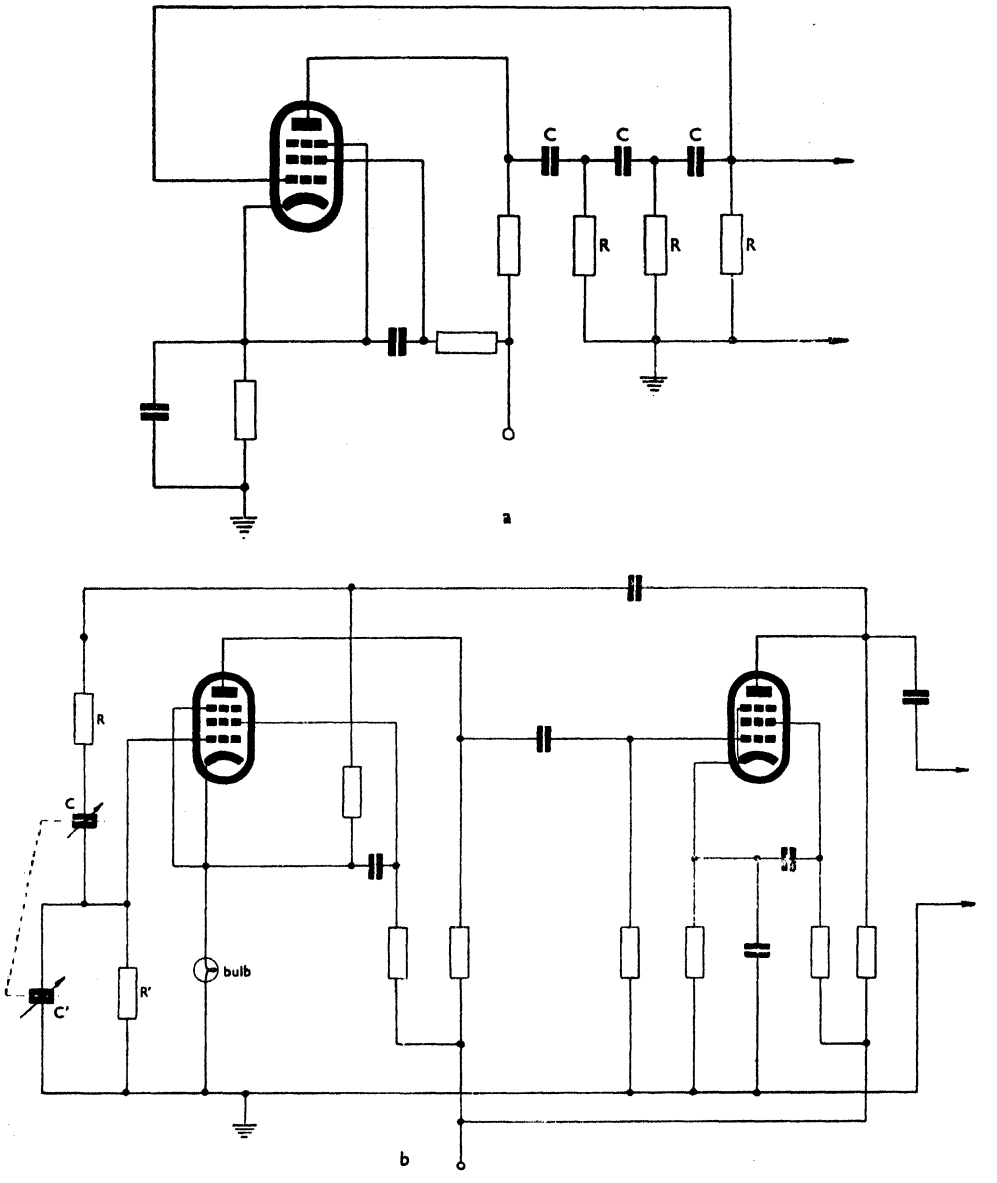

Fig. 13. *RC* oscillators. a) with *RC* phase inverter in the feedback from anode to grid; b) tuned by either a change in *C* or *R* forming Wien's bridge. The bulb serves to stabilise the amplitude.

age of a frequency to which the ocillator is tuned is inserted into the grid circuit, a sinusoidal voltage of the same frequency is obtained at the output.

Using a somewhat higher exciting voltage, this "filter" may oscillate with its fundamental frequency even if a higher harmonic or a subharmonic frequency is used at the input.*) It can thus also be used as a frequency multiplier or divider with the advantage that it is often possible to obtain a nearly sinusoidal voltage by using a single stage apparatus.

Generators of nonharmonic oscillations

These produce a voltage having a shape that differs considerably from the sine wave. In other words, their oscillations have many higher harmonic components.

For physiology, a rectangular pulse is most important. Its main parameters are the amplitude E, the repetition interval T or the repetition frequency $f = \frac{1}{T}$, the pulse width τ and the quiescent interval $T-\tau$, the ratio of the pulse width to the repetition interval, so called duty cycle $\frac{\tau}{T} = \tau f$. Another important waveform are the sawtooth waves (ascending and descending) where in addition to E, T and f, the duration (absolute and even more relative) of the steep edge of the wave is essential (usually the shortest possible is required). It must be borne in mind that the rising part of the sawtooth wave is never linear with time but always curved. The voltage-rise curve is mostly exponential but it may also be the middle, almost straight, portion of a sine wave (passing through zero) etc.

The majority of oscillators that are described here function according to one of the following principles: a) they have a very large positive feedback which results in an unusually rapid rise and fall of voltage. Flattening of the pulse top then occurs in the curved part of the valve characteristic. These include multivibrators, blocking oscillators and transitrons. b) An important characteristic of a discharge in gases is made use of, i. e. that the ignition voltage is usually higher than the extinction voltage.

The majority of oscillators can be driven with an external A. C. voltage close to the fundamental frequency of the oscillator or to harmonic or subharmonic frequencies. The majority of oscillators can be changed to shaping circuits, i. e. they can be blocked so that they do not oscillate unless an external signal is applied. They then produce only a single wave.

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*) If the frequency of the filter is F, the n-th harmonic frequency is $f_n = nF$ and the n-th subharmonic (submultiple) one $f_s = \frac{1}{n} F$.

Multivibrators

A multivibrator is a two-stage resistance-coupled amplifier with a tight feedback. The output of the second tube is returned to the grid of the first, and the plate of the first tube is connected to the grid of the second. Both couplings may be capacitative (astable multivibrator), resistive (bistable multivibrator) or mixed.

Astable (free-running) multivibrators

The circuit is usually more or less symmetrical, the valves and the corresponding capacitors and resistors being equal. The apparatus works as follows (Fig. 14): The positive voltage appearing at the grid of the first valve is amplified in this valve and reversed in phase 180 degrees. In the second valve it is further amplified and again reversed in phase. Thus the plate voltage from valve E_2, which is fed back across C_{g_1} to grid E_1, is added to the original signal voltage, returning along the same path through the feedback. As a result, the plate voltage in E_1 rapidly drops and that in E_2 rapidly increases, so that the first valve is rapidly "opened" to the highest possible plate current (limited by the load resistance) and the second is closed so that practically no current flows through it. The current in E_1 can rise no longer nor can it continue to fall in E_2 and despite the feedback, the valves can no longer amplify. Under these conditions the grid E_1 has a maximum positive voltage (i. e. approximately zero), E_2 a maximum negative voltage (about -10 to -50 V). The condenser C_{g_1} is discharged across R_{g_2} with a time constant $R_{g_2}C_{g_2}$. When the voltage across C_{g_1} has fallen (the voltage of E_2 has risen) sufficiently for a plate current to flow through valve E_2, the plate voltage at anode E_2 decreases somewhat (by the voltage drop caused by the plate current flowing through R_{g_2}). This decrease is carried by the feedback condenser C_{g_1} to the grid of E_1 through which the maximal possible current has been flowing. This current is somewhat decreased and thus the plate voltage of E_1 rises to a certain extent. This increase is transferred across C_{g_2} to grid E_2 and, consequently, the rate of current increase in E_2 is accelerated. Thus a decrease in plate voltage of E_2, and a corresponding plate current decrease in E_1 is rapidly built up. In this way the current is interrupted in E_1 and attains maximal values in E_2 during a very short time. This state lasts until the large voltage across C_{g_2} is discharged through R_{g_1} to such an extent that a plate current begins to flow in E_1. This results again in a rapid "reverting" of the multivibrator and conditions as described at the beginning are again restored. From this description the time curves of voltage at the individual electrodes may be derived (Fig. 14c): The time of reverting is exceptionally short in comparison with repetition

interval T, so that for the large majority of the operation cycle either a maximal or zero current flows through one or the other valve. Thus the voltage at the anodes is constant — either minimum (about 10 V) or maximum (the full

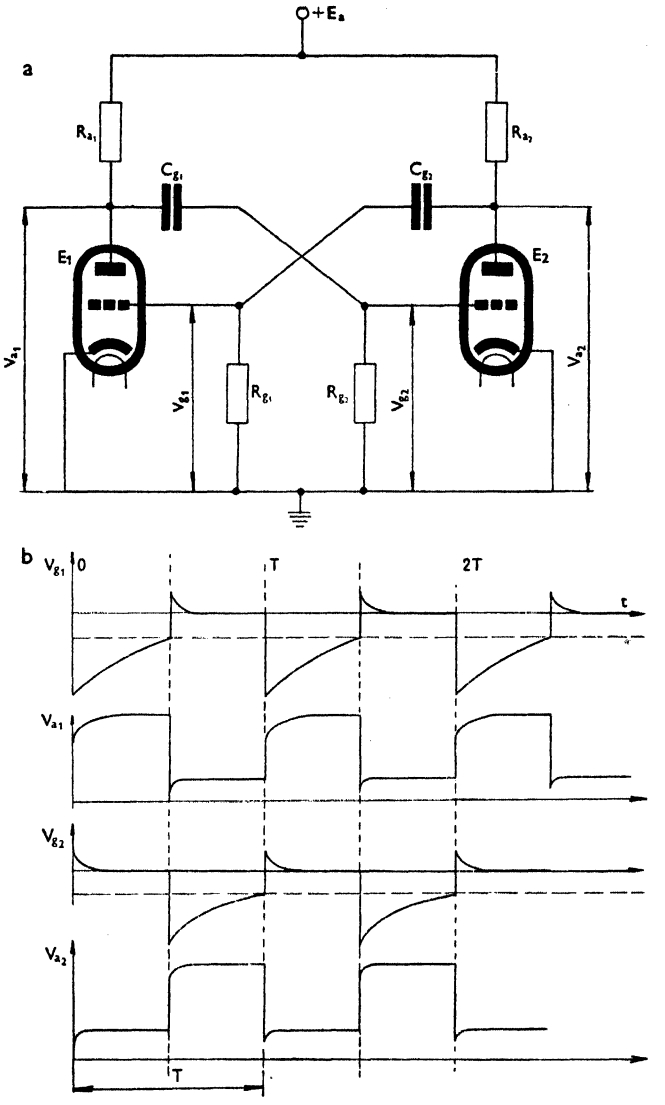

Fig. 14. Multivibrator. a) circuit diagram; b) shape of voltages at electrodes.

voltage of the anode supply). The plate voltage changes in steps. The plate voltage of one tube is a mirror image of that of the other. The grid voltage, however, changes according to another time curve. During change-over, the grid of the valve which has become non-conductive (and has a maximal

plate voltage) is charged to a minimum voltage (i. e. highly negative). Thus the grid and plate voltages are 180 degrees out of phase, as in any amplifier. In distinction to the plate voltage, this grid voltage does not remain constant but gradually increases (becomes less negative) as the C_{g_2} is being charged (or "discharged", if the absolute voltage value across C_{g_2} is considered). It is evident that the voltage across the grid has an exponential shape and that again both the grid voltages are approximately mirror images of each other.

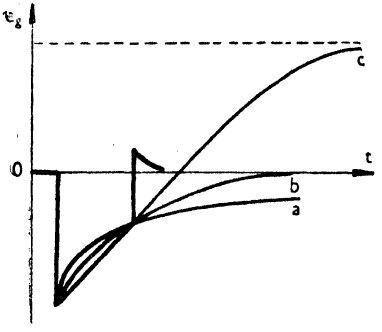

Fig. 15. Different pulse shapes at multivibrator grid. a) with negative grid bias; b) with zero bias; c) with positive bias.

The width of the positive pulse at plate A_1 thus depends upon the time constant, given by the product R_{g_1}. C_{g_1} and on the "cut-off" voltage of valve E_1, i. e. on the position of the cut-off point on the tube characteristic curve Thus the kind of the valve, the plate voltage and also the voltage at the lower end of R_{g_1} is important (Fig. 15,a). If R_{g_1} is connected directly to the cathode, the plate current will begin to flow when C_{g_1} is already considerably discharged and when consequently the discharge rate is already slow. If R_{g_1} is connected to a slightly negative bias voltage, the voltage across E_{g_2} attains the "cut-off" grid voltage even later and more slowly. This results in a decreased repetition frequency, an increased pulse width and also in a deterioration of the stability of the oscillator. The curve of the voltage drop across C_{g_2} intersects the cut-off bias level at a very sharp angle so that the point of transection is not determined very accurately (Fig. 15,b). Such a connection is of advantage only exceptionally, e. g. if the multivibrator is to be synchronised within a relatively large range of frequencies. This is really a transition to a monostable multivibrator. If, on the contrary, R_{g_1} has a positive bias (Fig. 16a), the frequency increases several times, the pulses are shorter and the moment when reversal occurs is determined much more accurately. The frequency and pulse width are much more stable, therefore, and variations drop to only 1%. In addition, the grid voltage curve is formed by shorter exponential sections than in the above examples (Fig. 15,c). Such a multivibrator may thus be used also as a source of voltage having a sawtooth wave form, but the output voltage must then be taken from the grid and not from the anode.

Frequency and pulse width controls: If $C_{g_1} = C_{g_2}$, $R_{a_1} = R_{a_2}$ and both valves have identical properties, as far as possible, then the pulse width and the quiescent interval are equal. In that case the frequency can be changed while maintaining a stable ratio 1 : 2 of pulse width to repetition interval (so called duty cycle) by changing simultaneously and to the same extent

R_{g_1} and R_{g_2} or C_{g_1} and C_{g_2}. In a non-symmetrical circuit ($C_{g_1} \neq C_{g_2}$, $R_{g_1} \neq$ $\neq R_{g_2}$) pulses longer or shorter than the quiescent interval may be obtained, depending on the valve from which the output voltage is taken. It is, however, impossible to obtain a pulse width independent of repetition frequency and vice versa. (Thus the multivibrator is not very generally useful as a stimulator and can be used rather as a fundamental oscillator, the pulses of which are

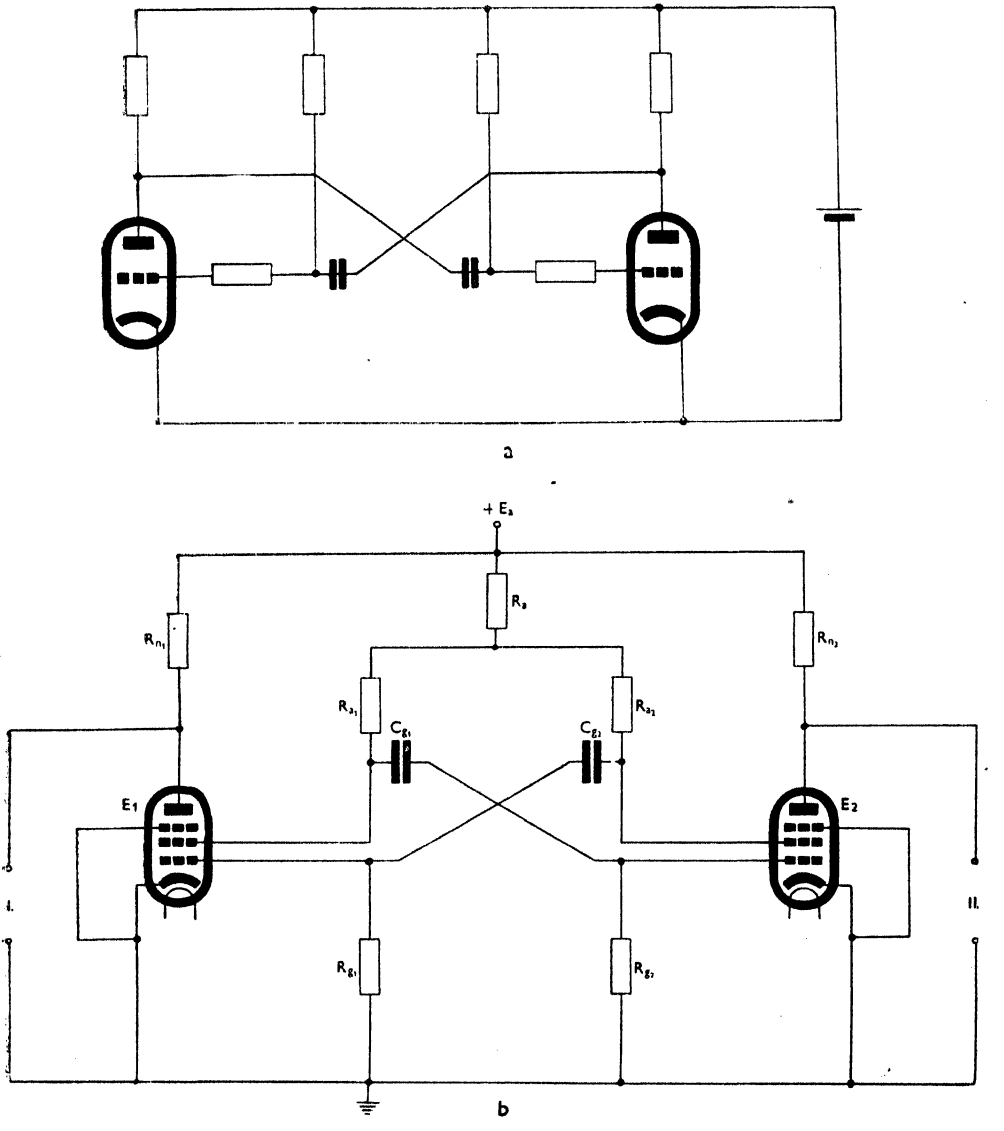

Fig. 16. a) Multivibrator with improved pulse shape and better frequency stability.
b) Electronically coupled multivibrator.

given the required width and amplitude later on.) Neither frequency nor pulse width change linearly with a change in the control elements. The multivibrator can be used for frequencies from about 0·001 c/sec. to 100 kc/sec. and more, and for duty cycles ranging from 1 : 100 to 99 : 100. If we want to attain very steep front and trailing edges, valves with a large transconductance must be used, having a high plate and screen dissipation, small internal capacitance and small anode load. Thus pulse widths of several tenths of a μsec. may be obtained.

Special types of multivibrators

The following special types of multivibrators may be mentioned:

Electronically coupled multivibrator: The multivibrator is connected to triode parts of two pentodes (the screen grids are used as anodes). The anode circuits contain loads from which the output is taken. In such a circuit the frequency of the multivibrator depends only very little on the load (Fig. 16b).

In a multivibrator giving an improved shape of oscillations the coupling condensers C_{g_1} and C_{g_2} are connected to the grids not directly but across a series resistor of 100kΩ or more, which may be blocked by a condenser with several tens pF. In such a way the grid circuit also acts as a grid limiter. The pulses are more rectangular, but the edges are not so steep as in the usual circuit (Fig. 16a).

A multivibrator with a positive bias: The common joint of the grid resistors is blocked with a large condenser (0·1 μF) to the frame and connected to the tap of a potentiometer which is situated between the poles of the D. C. source. By changing the bias voltage the frequency is regulated within a very wide range (up to 1 : 40).

There are many more modifications of multivibrators. The multivibrator with a cathode coupling, the multivibrator producing trains of pulses and Antipov's series multivibrator are worth mentioning. They are desribed in the literature (Petrovich and Kozyrev 1954, Antipov 1951, Meyerovich and Zelichenko 1953).

The multivibrator can easily be converted into a shaping circuit which either prolongs a short pulse to a constant width and definite shape (rectangular) or changes it into a positive or a negative voltage step (either positive or negative depending on the preceding state).

Monostable (one-shot, single-kick) multivibrator

The monostable multivibrator is an asymmetrical multivibrator in which one valve is constantly non-conducting because of a large negative grid bias

while the other one is constantly conducting (Fig. 17). The arrival of the trigger pulse (positive at grid E_1 or anode E_2, negative at grid E_2 or anode E_1) results in change-over of the multivibrator which remains in this reversed state (or produces a pulse) so long as the grid condenser is not discharged through the

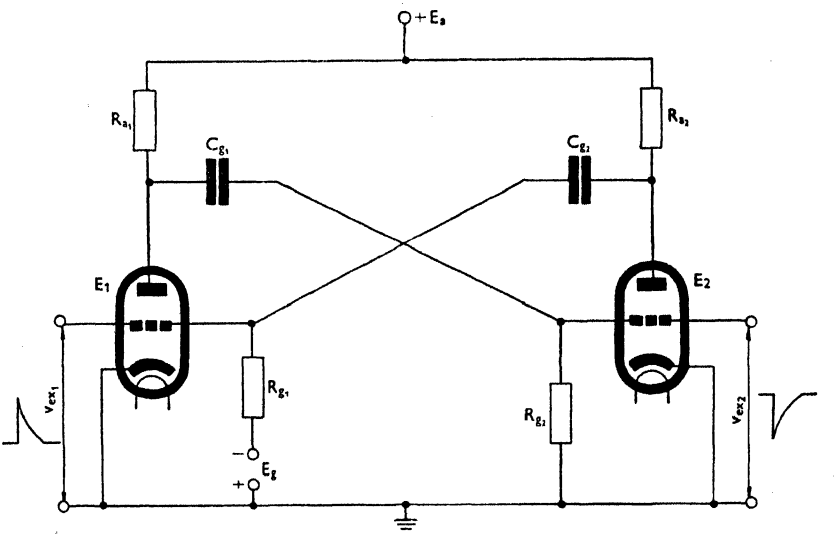

Fig. 17. Monostable multivibrator.

grid resistance. It then reverts to the original state, which lasts until the next triggering pulse arrives. Thus the monostable multivibrator has one stable and one unstable equilibrium position.

Bistable multivibrator

The bistable multivibrator (Eccles-Jordan circuit, resistance coupled trigger circuit, binary reductor) is a multivibrator with two stable positions. The circuit (Fig. 18a) is completely symmetrical. Both grids receive D. C. voltage from a voltage divider connected between the large negative bias and the anode of the other valve. The anode section of the divider is by-passed by a small condenser (10 — 100 pF). Let it be assumed that the valve E_1 carries anode current. Then its anode voltage is very low and the potential on the grid E_2 is below the cut-off value, so that E_2 is nonconducting. The voltage at its anode is very high (since no anode current is flowing). This high voltage is communicated to the grid E_1 and a maximal anode current is therefore flowing through E_1. The grid voltage is near zero, however, because of the voltage drop caused by the grid current on the series grid resistance. This state is evidently stable. If a negative pulse is applied to the grid E_1

(or to anode E_2 from where it is transmitted to grid E_1 through the condenser), then E_1 is blocked, the anode current ceases to flow and the anode voltage rises up to the values of the plate supply.

This positive pulse is transferred to grid E_2 through the condenser C_1 and resistor R_{g_1}, resulting in the appearance of an anode current at E_2. The

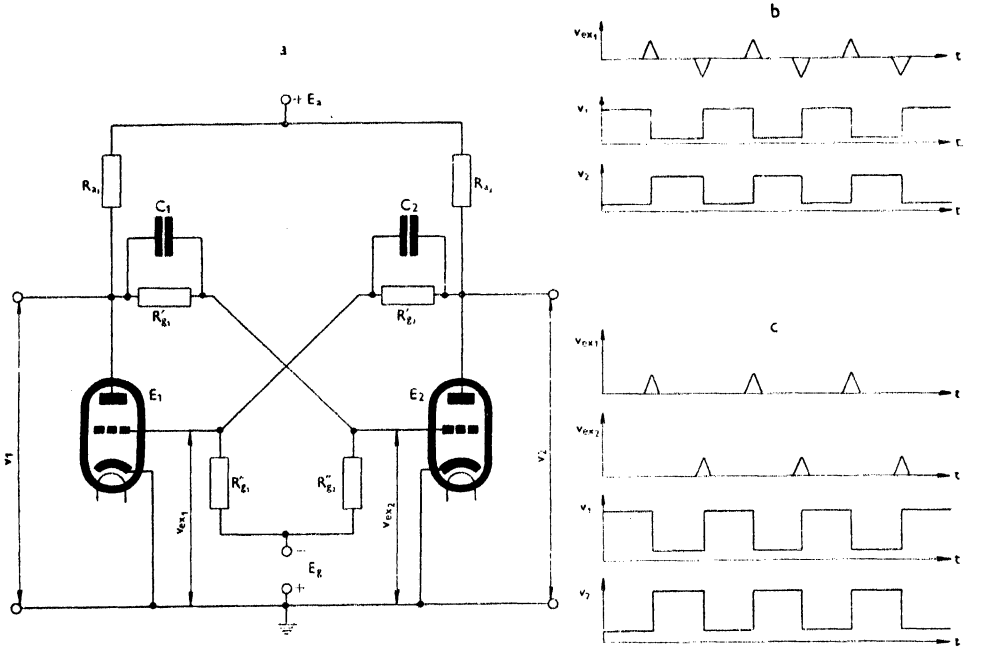

Fig. 18. Bistable multivibrator. a) circuit diagram; b) supplied with pulses of alternating polarity on one grid; c) supplied with two trains of positive pulses.

voltage at the anode of this tube decreases and this decrease is transferred through the condenser C_2 and the resistor R_{g_2} to grid E_1. Thus this new state is fixed even when the triggering pulse has passed. This second equilibrium position is also stable. Change-over of the circuit to the original state occurs only when a further triggering pulse of suitable polarity reaches an appropriate point of the circuit. In order to produce one rectangular pulse two triggering pulses are necessary. Thus a bistable multivibrator can be used for frequency division (to half the frequency of the signal).

Synchronisation of multivibrators

The synchronisation of a multivibrator, triggering of a monostable multivibrator and change-over of a bistable multivibrator must be considered.

As has already been mentioned, a multivibrator can be made to lock in with a frequency close to its own frequency (somewhat higher so that the change-over due to the injected voltage occurs sooner than automatic change-over would occur) or close to its higher harmonic or even submultiple (subharmonic) frequency. In the latter case the pulses of the multivibrator are of unequal width, since those triggered by the submultiple frequency are somewhat shorter than the others. A synchronised multivibrator is most frequently used for frequency division, i. e. it is synchronised by a frequency several times higher than its own. The higher the synchronising voltage the greater the acceleration of the multivibrator's oscillations, i. e. the further it deviates from its own frequency, until for a sufficiently large synchronising voltage, it is driven so hard that it is locked in with the injected signal. Thus it is possible, for instance, to synchronise a multivibrator using a voltage of about 1 V to a frequency 12 times lower than the synchronising frequency. For a higher synchronising voltage, the frequency of the multivibrator increases so that it is 11, then 10, 9, 8 etc. times lower than the synchronising frequency until for a synchronising voltage of about 40 V the frequency of the multivibrator reaches the synchronising frequency.

Since it is possible to inject the synchronising voltage into both valves of the multivibrator in the same or opposite phase, the multivibrator can be synchronised either with even or odd submultiples of the synchronising frequency. If the signal voltage is directed to both grids or both anodes in the same phase, synchronisation for even submultiples is accentuated. If the potentials applied to both grids or anodes are reversed in phase, synchronisation for odd submultiples is easier. If neither odd nor even submultiples are preferred, the synchronising voltage is applied only to one grid or anode. The above applies to the symmetrical multivibrator. In an asymmetrical multivibrator, it is even possible to make it several times more sensitive for a certain submultiple than for all others.

Trigger wave form

Fundamentally it is possible to synchronise multivibrators using any periodic voltage, i. e. also harmonic (sine wave) voltages. Synchronising voltage peaks with a steep wave front and an exponential trailing edge, such as are obtained e. g. by differentiation of a rectangular voltage, are most effective and also determine the moment of reversal most accurately. Pulses of the same shape (derivative spikes) are also most suitable for triggering monostable and especially for reversing bistable multivibrators. For this purpose, however, they must have a sufficiently large amplitude, exceeding the negative grid voltage (usually 10—100 V), and a sufficiently long duration (usually

0·1—10 μsec), so that after their passage the decrease of bias voltage will not again cut off the valve which has just started conducting. An exponential trailing edge is desirable, since the exciting voltage gradually falls while the internal feed-back reversing voltage gradually rises. After the first voltage step produced by the triggering pulse front, no further abrupt potential changes occur which might either interrupt the process of change-over or might produce, sit venia verbo, an extrasystolic change-over after the first one has terminated.

Triggering of monostable and bistable multivibrators

Triggering of a monostable multivibrator is simple, since the triggering pulse is applied to a single point of the circuit. It is more complex in a bistable multivibrator and consequently will be discussed in more detail.

a) Bistable multivibrator may be reversed in a similar way to monostable multivibrators: Pulses of both polarities are directed to one point (the grid or the anode — Fig. 18b). Then a negative pulse applied to the grid of the first valve, which is conducting, blocks it, while a positive pulse arriving under the same conditions has no effect. If, on the other hand, the valve is not conducting, only a positive pulse can swing its grid above the cut-off point while a negative one has no effect.

b) Two trains of impulses of the same polarity are applied, one to the grid of the first valve and the second to the grid of the second valve (Fig. 18c). After the bistable multivibrator has been reversed by a certain impulse of one train, further impulses of the same train applied to the same point remain without effect so long as the next impulse of the second train does not revert the circuit back again. The same would hold good for impulses directed to the anode.

c) The third method of reversal is fundamentally different. It is characterised by complete symmetry and formal uncertainty as to which valve will be reversed by any impulse. This means that every impulse reverses the circuit (in other words all impulses are effective) and that the direction of change-over is independent of the circuit and only depends upon the actual state of each valve (if it does or does not conduct). The triggering impulses are applied simultaneously in both valves (either to both grids or to both anodes, or — in a pentode circuit — to both suppressor grids). It is evident that the impulse at one valve tends to reverse the circuit, while the same impulse applied simultaneously to the second valve counteracts the change-over and makes it more difficult. Yet it is possible to find a certain size and width of the pulse which will regularly produce the change-over, since the conducting and non-conducting valves differ in their sensitivity to the pulse. Driving would be much more reliable, however, and the size and duration of the triggering pulses

much less critical, if they could be directed only into that valve in which they can produce a change-over and if they could be prevented from reaching the valve in which they will oppose the turning over. This is best attained by a circuit shown in Fig. 19. The negative triggering pulse is directed to the anodes of both valves through germanium rectifiers (or vacuum diodes). Impulses are applied simultaneously to the cathodes of both rectifiers having the D. C.

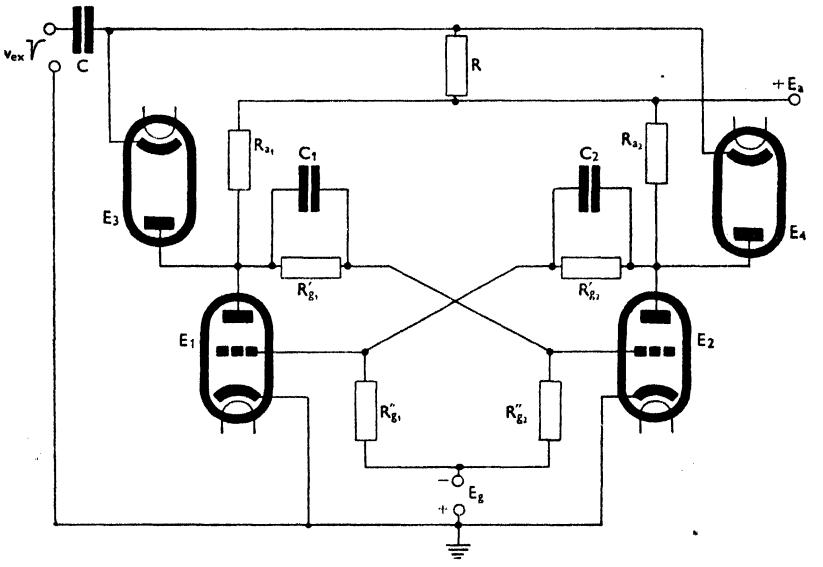

Fig. 19. Bistable multivibrator with electronic switching of pulses.

voltage of the anode supply (through R), i. e. the plate voltage of a nonconducting valve. The anodes of both rectifiers, however, have different voltages, since they are connected to the anodes of the valves, one of which is conducting, and thus its anode voltage is very low (10—50 V), the other is cut off and has the full plate voltage equal to anode supply. In other words: the rectifier connected to the blocked valve has a relative anode voltage (i. e. a potential difference between its cathode and anode) of about 0 V, while in the rectifier connected to the conducting valve the anode is about 100 to 300 V more negative than the cathode (according to the circuit and the anode suply voltage). Negative pulses having an amplitude of 50—100 V arriving at the cathode of both rectifiers can then only pass through the rectifier having zero relative anode voltage and cannot overcome the negative anode to cathode voltage of the other rectifier. Thus negative trigger impulses only reach the anode of the nonconducting valve, are transmitted through the coupling unit to the grid of the conducting valve which is thus cut off. This electronic switching of trigger impulses makes change-over considerably more accurate and less

sensitive to correct adjusting of the width, shape and amplitude of the triggering impulse. If the circuit is to be reversed by positive impulses, it is usually sufficient to change the polarity of both properly biassed rectifiers.

In addition to these completely symmetrical bistable multivibrators there are also slightly asymmetrical circuits, e. g. with a cathode coupling. They do not require a source of high negative grid-bias, but calculations and adjustment are more difficult.

Since all calculations are only approximate, it is best to test them by building a "bread-board" model, in which special care is taken to achieve symmetry. We do not check the exact absolute values of every component, but the symmetry of both R_a, R_g etc., and particularly the symmetry of valves. We choose valves that have the same characteristics — chiefly the cut-off grid voltage and the same D. C. resistance for zero grid bias (i. e. for the grid connected to the cathode).

Blocking oscillator (blocking generator, generator of impulses with transformer coupling)

Fig. 20 shows a blocking oscillator consisting of a single triode and a transformer coupled feedback circuit. The blocking oscillator somewhat

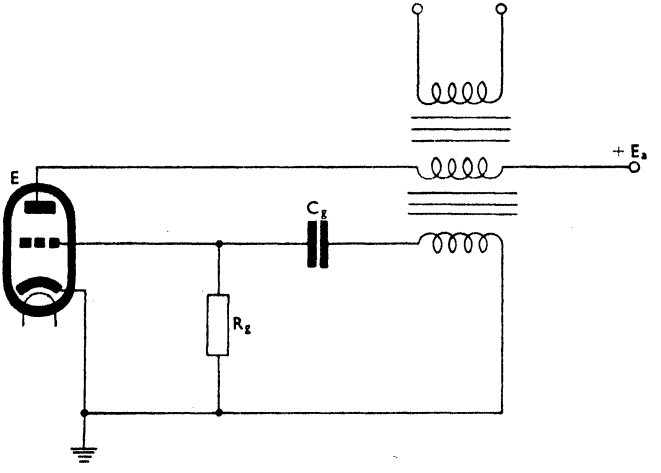

Fig. 20. Blocking oscillator.

resembles a harmonic oscillator with an inductive feedback. It differs from the latter in the fact that neither the anode nor the grid windings form a part of the resonant circuit and the transformer has a minimum stray magnetic field. Let it be assumed that condenser C_g is charged to a high negative voltage.

The valve is thus cut off and the condenser is slowly discharged through R_g. As soon as discharge has proceeded sufficiently so that the plate current starts in the valve, this small initial current causes a further increase of the grid voltage communicated to the grid by the transformer feedback. Since this feedback is very tight, the grid voltage, and thus the anode current, increases rapidly up to limiting values. This state lasts for a short time. Since the grid voltage has attained positive values, a grid current flows and the cathode current is thus divided into two parts — the anode current flowing through the transformer and producing the feedback — and the grid current charging grid condenser C_g. As voltage across C_g increases, the grid current decreases, but the anode current continues to flow, since the grid voltage has not yet dropped below zero. As soon as the grid condenser C_g is charged to such an extent that the anode current slightly decreases, this decrease in the primary (anode) coil of the transformer produces a voltage drop in the secondary (grid) coil and this is transferred to the grid through the grid condenser C_g (which a moment sooner had a high positive voltage at the condenser plate nearer to the transformer and a small positive voltage decreased by the grid current at the plate nearer to the grid). In this way the grid voltage is further reduced. Consequently the grid and anode currents continue to fall rapidly; this drop is again fed back through the transformer to the grid and so on. The anode current continues to fall very rapidly. After this current and the voltage across the secondary winding of the transformer have disappeared, a large negative charge remains on the grid condenser. This maintains the whole circuit at rest (in the blocked state) so long as the grid condenser is not discharged through the grid leak. Thus C_g discharges through R_g with a relatively long time constant C_gR_g and it is charged through the grid resistance of the valve, which is several orders of magnitude smaller. Charging corresponds to the width, discharging to the quiescent interval. The transformer increases the steepness of the rise and fall of the anode current, i. e. the slope of the leading and trailing edges of the pulse. Exact calculations of the blocking oscillator are very difficult and time consuming. Meyerovich and Zelitchenko were the first to describe this accurately (1953).

Changing of an individual element usually affects all the characteristics of the oscillator simulataneously (as in the multivibrator), but some more than others. Thus the frequency is mainly influenced by R_g, but also by C_g. C_g acts mainly on the pulse width, but also on the repetition frequency. With increasing R_g (and also C_g) the frequency falls. The pulse width increases with increasing C_g. The pulse width of a conventional blocking generator may be decreased down to 0·1 μsec., but its upper limit is also relatively low. If the pulse width is prolonged above several tens of μsec., the shape of the pulse is maintained with great difficulty. The frequency of a blocking oscillator, on the other hand, can be controlled within a wide range from several c/sec. to

tens and sometimes also hundreds of kc/sec. In every case, however, the quiescent interval is at least several tens (up to several ten thousands) times longer than the pulse width. This permits the accumulation of energy (which will be used up in the production of the pulse) in the condenser C_e (Fig. 21) separated from the anode supply and thus from the other parts of the circuit by the charging resitance R_e. Thus even for a large pulse energy a relatively small anode supply can be used (it is calculated not for maximum but for average power which is $\frac{T}{\tau}$ times smaller). The effect of the blocking oscillator on the other parts of the apparatus is also reduced. C_e is calculated so that the voltage across it does not fall during the pulse passage by more than 10—30%. R_e is chosen in such a way that C_e can be charged to nearly the full anode supply voltage during the quiescent interval. At the same time R_e must be large enough to avoid more than a minimal drop in supply voltage during the pulse passage. Let us now consider the most important part of the blocking oscillator, the pulse transformer. Very exacting demands are made on it. Its own resonance frequency must be as high as possible, i. e. the winding capacitance, leakage inductance and core losses must be small as possible. Hence it is usually constructed as follows:

a) The core material has the highest possible permeability and the least possible losses. Usually a very thin band of permalloy or other alloy is rolled into a small torroidal core. For instance, a band 0·1 × 5 mm, internal core diameter 15 mm, a total of 30 turns of the band (insulated with varnish).

b) Primary, secondary (and if necessary also tertiary) coils of approximately the same size are wound with a thin and a thickly insulated wire (for decreasing the capacitances). For a core described above, each coil has 85 turns of copper wire, ∅ 0·15 mm, EDS (enamel, double silk).

c) The whole transformer is covered with a good insulator having small dielectric losses and a small dielectric constant (e. g. ozokerite) or with bee's wax or paraffin.

Even the best transformer produces a small backswing at the end of the pulse. A bad transformer may give very large and long lasting damped oscillations. These can be considerably reduced in a good transformer by shunting one winding of the transformer with a resistance of about 5 kΩ—10 kΩ. This, however, is of no avail in a bad transformer. A still smaller resistance would help, but this would considerably distort the shape of the pulse and decrease its amplitude. Even in such bad instances shunting of the anode winding with a germanium rectifier which has its anode connected to the anode of the triode and its cathode to the opposite end of the primary winding usually improves things (Fig. 21). Its function is the following: it has no effect during the main pulse itself since no current can flow through it. As soon as the main

pulse has passed, however, and the backswing of opposite polarity is due to appear, i. e. when the triode plate becomes more positive (at the expense of energy accumulated in the transformer) than the anode supply voltage, the stored energy is destroyed through the rectifier. Thus no further oscillations occur. This arrangement sometimes makes it possible to use a conventional low frequency transformer if demands on the steepness of the front and trailing edges of the pulse are small.

It must be stressed that a blocking oscillator can oscillate only if the transformer windings have the correct polarity. If it does not oscillate, the error is usually here and it is sufficient to exchange both ends of one winding (either the anode or the grid winding).

The stability of the pulse width and repetition frequency is rather poor (5—10%) for the conventional blocking oscillator circuit. It is most easily increased, as in the multivibrator, by connecting the grid leak to a positive potential. For this purpose the potential of the anode supply is best. The repetition frequency, of course, increases approximately 10 times. The frequency can be stabilised further by synchronising it with a frequency about 15% higher. The synchronising sine wave or peak voltage can be directed to any more or less arbitrary point (Fig. 22).

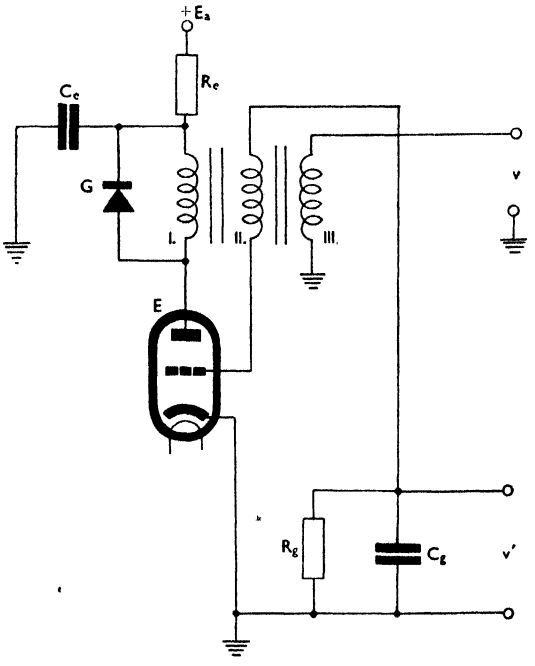

Fig. 21. Blocking oscillator with separation filter (C_eR_e), damping diode (G) and output winding on transformer (III).

The basic circuit of a blocking oscillator can be changed considerably. For instance, C_g and R_g can either be connected directly to the grid (Fig. 20) or to the other end of the secondary winding (Fig. 21). The anode can be connected either in series with the primary winding or in parallel.

The output voltage may be taken from the blocking ocillator either from the anode, from the tertiary winding of the transformer (U in Fig. 21) (more or less rectangular pulses), from the grid or from the other end of the grid winding (if it is not earthed) (U' in Fig. 21). The shape of this voltage approximates a sawtooth wave.

As in the monostable multivibrator, blocking oscillators may be blocked by a negative grid bias and triggered off by external triggering pulses. Since this is used only rarely in electrophysiology, it will not be discussed in detail (cf. Petrovich and Kozyrev 1954).

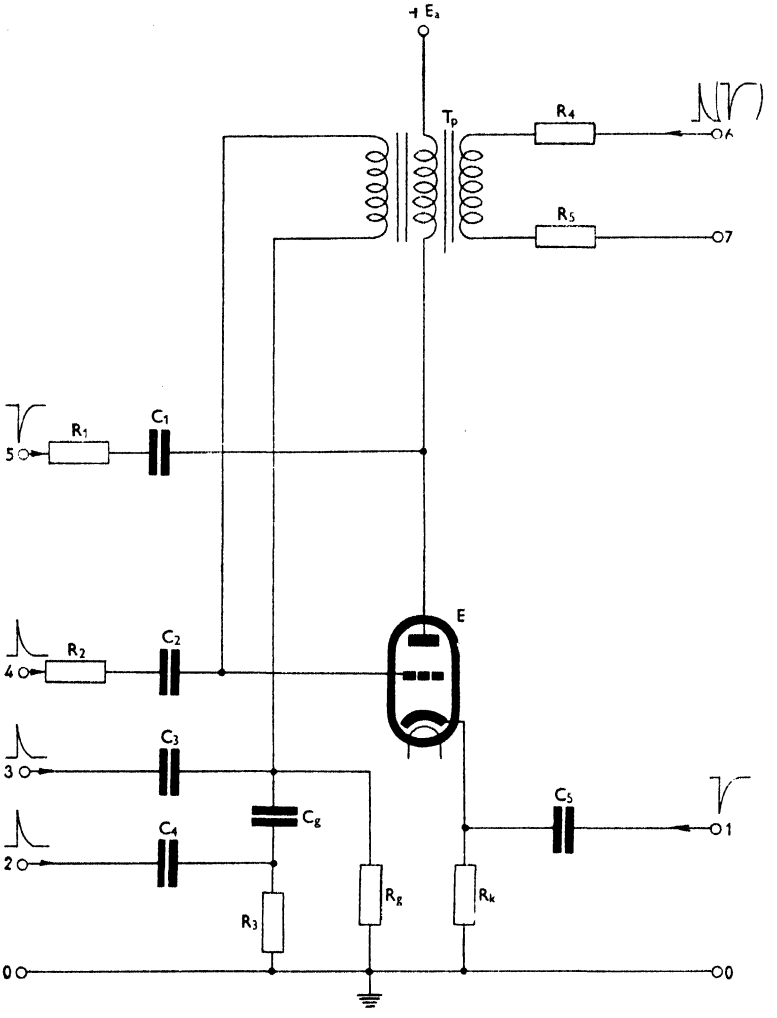

Fig. 22. Different methods of synchronising a blocking oscillator.

Glow-discharge and Thyratron oscillators

The basic component is a two to four electrode valve filled with gas at low pressure (H_2, He, Ar, Ne, Hg, Hg + Ar) with a cold or hot cathode, a so-called gas tube. If a voltage is placed across its main electrodes (cathode and anode), a current will pass through it so that at first the current is small

and rises linearly with the voltage. At a certain voltage, termed breakdown voltage, the current rises suddenly to such a high value that, if the valve were not protected by a series resistance, it would be destroyed by heat dissipation. With the series resistance the anode-to-cathode voltage falls during conduction, independently of the supply voltage, to a certain constant value. If now the source voltage is increased, the voltage across the valve remains constant and only the current increases (and also of course, the voltage drop across the series resistance). The circuit behaves as if the valve had a zero resistance. If, on the contrary, the anode supply voltage is decreased, the current also decreases, but the voltage across the valve again remains constant. Only after the supply voltage falls to that of the valve is the current flow interrupted and the valve switched off. It is evident that this extinction voltage is only slightly lower than the anode-to-cathode voltage during conduction and is considerably lower than the ignition voltage for which this large current begins to flow. This part of the ionic discharge in a gas is termed *glow discharge*. Cold cathode valves in which it is utilised for electronic or lighting purposes are called glow discharge tubes. The extinction voltage is usually between 70 and 200 V, according to the type, and the ignition voltage is by several tens of V higher. This difference between the extinction and ignition voltages can be reduced to several volts or less (e. g. in voltage regulators). Accurate measurement of the tube resistance during the glow discharge shows that it is not zero but 100 — 10 000 Ω.

The ignition voltage is not stable but depends considerably upon the number of free electrons in the gas. This means that it will decrease with external temperature, with ionising radiation etc.

The glow discharge is only maintained if the current is not too large. If, by increasing the supply voltage the current is raised considerably, the voltage across the valve begins to rise again until it reaches the second critical value (analogous to the ignition voltage attained earlier). This occurs when the temperature of the cathode attains such a value that considerable thermionic emission sets in, usually with a current of several amperes. Then the character of the discharge changes, its resistance falls to 1—100 Ω and the voltage across the electrodes (if there is a limiting series resistance) drops to about 10 V. Evidently analogous phenomena as in the glow discharge occur here. This part of the discharge is termed the *arc*. (Another kind of discharge — the spark is of similar character.) Thus a hot cathode (or at least a hot point on the cathode) is an important condition for the arc to occur. This heating is attained either by Joule heat at the electrode contact (the carbon lighting arc), by bombarding a metallic surface (usually mercury) with positive ions (ignitrons etc.) or finally by heating the cathode with an auxiliary current (thyratrons). Only thyratrons will be considered here. Further details concerning discharges in gases can be found in Kaptsov (1950, 1956).

In the oscillators considered in this paragraph the fact is utilised that the ignition voltages of the glow and arc discharge are higher than the respective extinction voltages. In glow discharge tubes this difference is about 20% of the ignition voltage, in thyratrons up to about 99%. Thyratrons, in addition to the anode and directly or indirectly heated cathode, contain a third electrode, the grid, which does not, however, control the anode current, but only the ignition voltage. The grid voltage is usually negative with respect to the cathode by tens to hundreds of volts and since the grid completely surrounds the cathode, it prevents the emitted electrons from leaving. The arc is started as follows: a) either the anode voltage rises sufficiently so that enough electrons leave the cathode through the opening in the grid. If they are accelerated enough as to evoke mass ionisation in the space between the grid and the anode, the discharge is initiated. The grid loses all influence on the discharge since it is surrounded by positive ions and thus its field is screened. The discharge is interrupted only when the anode-to-cathode voltage falls to the value of the extinction voltage and is maintained at this low value for some time (10—200 μsec.) necessary for clearing the anode-cathode space of the positive ions, which are neutralised by free electrons (deionisation time). b) The grid voltage rises (i. e. the grid bias decreases) to such an extent that with the same anode voltage, electrons begin to force their way to the anode. The further course is the same as in (a). It can be seen that for a certain anode voltage there is a definite maximum grid voltage (i. e. a minimum bias) for which the arc discharge just commences. The graphic representation of this relationship is termed the grid characteristic of the thyratron (this evidently is in no way similar to the grid characteristic of a vacuum triode, see page 95). The following characteristics must be maintained if the thyratron is to be operated properly: a) The heater voltage or current. Over- or underheating considerably decreases the tube life. On underheating, the discharge current does not flow over the whole of the cathode's surface but only through one point which is then overheated and may burn, or in indirectly heated tubes it may lose emission because the superficial oxide layers have been destroyed. b) The grid characteristic. c) The grid leak. Too large a resistance decreases the grid control, a small resistance may damage the grid. d) Maximum anode voltage. If it is exceeded, the grid loses its ability to maintain the thyratron in the blocked state. e) Maximum inverse anode voltage. If it is exceeded, the glow and then also the arc discharge is started between a positive heated cathode and a negative anode. f) Maximum average anode current. This determines the maximum permissible heating of various parts of the tube; if cooling is insufficient, the anode current must be decreased below catalogue values. g) Maximum peak current. If it is exceeded, the cathode is destroyed in the same way as when underheated. In its stead the minimum anode resistance is sometimes given or the maximum capacitance that can be discharged directly without

a protective resistance. h) Maximum repetition frequency or minimum repetition interval depends on the deionisation time; if it is exceeded, the thyratron may conduct even though the grid is at negative potential, and does not regain its proper function so long as the anode circuit is not interrupted or the anode voltage supply cut off. This failure may occasionally occur even if the thyratron is operated near the maximum frequency limit.

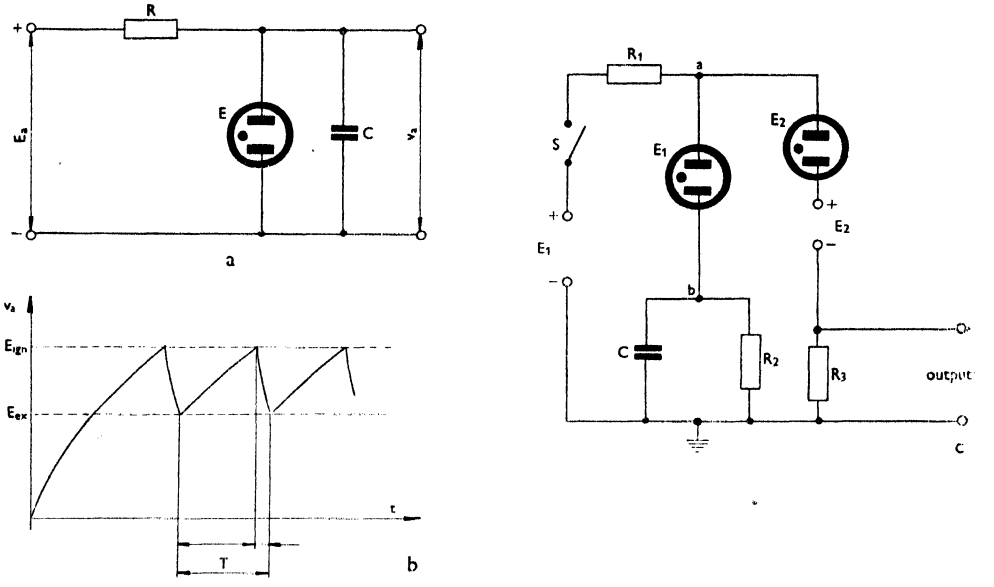

Fig. 23. a) glow tube oscillator. b) the shape of its output voltage. c) glow tube oscillator giving rectangular pulses.

Gas tube oscillators are synchronised in a similar way to the types mentioned earlier. The glow discharge tube is difficult to synchronise. This is easier for the thyratron. By changing the grid bias, any thyratron oscillator may be changed to an autooscillator or to a triggered pulse generator.

The oscillations are either sawtooth (exponential) or rectangular waves depending on the circuit. The steepness of the front (up to 10^{-7} sec.) with a large pulse amplitude (up to several hundreds or thousands V) and a small internal resistance may be of advantage. The difficulty, however, of obtaining a steep trailing edge (i. e. rectangular pulses) and controlling the pulse width, low maximum frequency (several 100 to 1000 c/sec., except for hydrogen thyratrons), poor frequency stability and limited life span are other drawbacks. Thyratrons are delicate instruments and sometimes difficult to set for an amateur. They were used often in physiology in earlier days.

The fundamental circuit of a glow discharge tube oscillator (Fig. 23a, b) needs no further comment.

The circuit of a thyratron oscillator is given in Fig. 24.

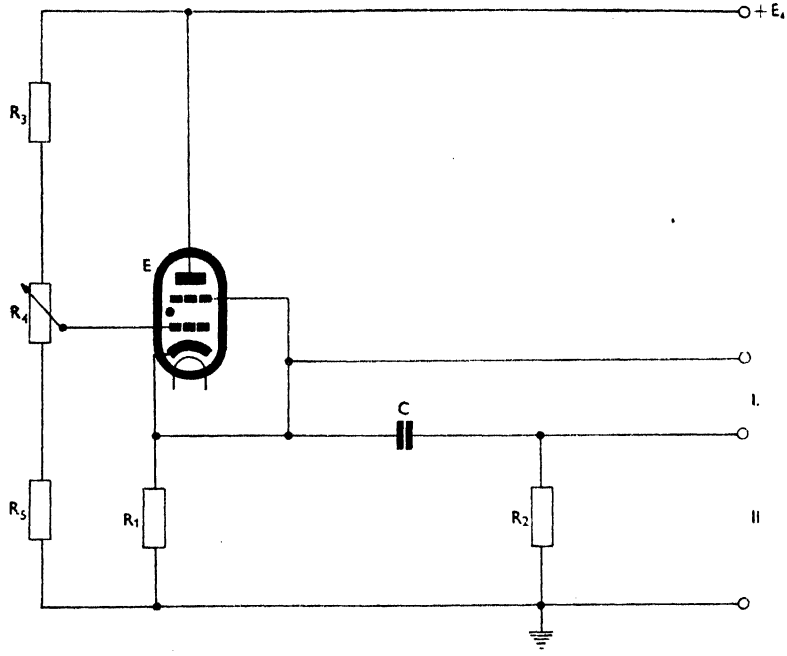

Fig. 24. Thyratron oscillator with high frequency stability. Sawtooth pulses at output I, rectangular pulse at output II. $R_1 \gg R_2$.

Transitron generators and some related oscillators

These are valve relaxation oscillators with a negative resistance.*) The transitron is the most important and works as follows (Fig. 25a): Let us start from the moment the valve is blocked. The anode voltage is the same as the supply voltage and no anode current flows because of the negative voltage of g_3. All the cathode current thus flows through the screen grid g_2, the voltage of which is very low. The negative charge from C_2 (and thus g_3) leaks through R_4. As soon as the voltage at g_3 begins to come close to zero, an anode current begins to flow, i. e. part of the cathode current ceases to flow to g_2 and begins to flow to the anode. Consequently the g_2 voltage rises somewhat, this rise is transferred to g_3 by condenser C_2. This results in a further increase in the

*) In a negative resistance, an increase in current results in a decrease in the voltage drop across it, while in a positive resistance, the voltage rises with the current.

potential of g_3, and the anode current is further increased until a strong anode current flows with a positive voltage at g_2 and g_3. Since the anode current discharges C_1, the anode voltage and then also the anode current decrease. In addition the positive charge from g_3 disappears both through the grid leak R_4 and, chiefly, at the expense of the grid current to g_3. As the anode current decreases, the grid current g_2 begins to flow. Its voltage falls and this drop is transferred through C_2 to g_3. This accelerates the rapid fall of anode

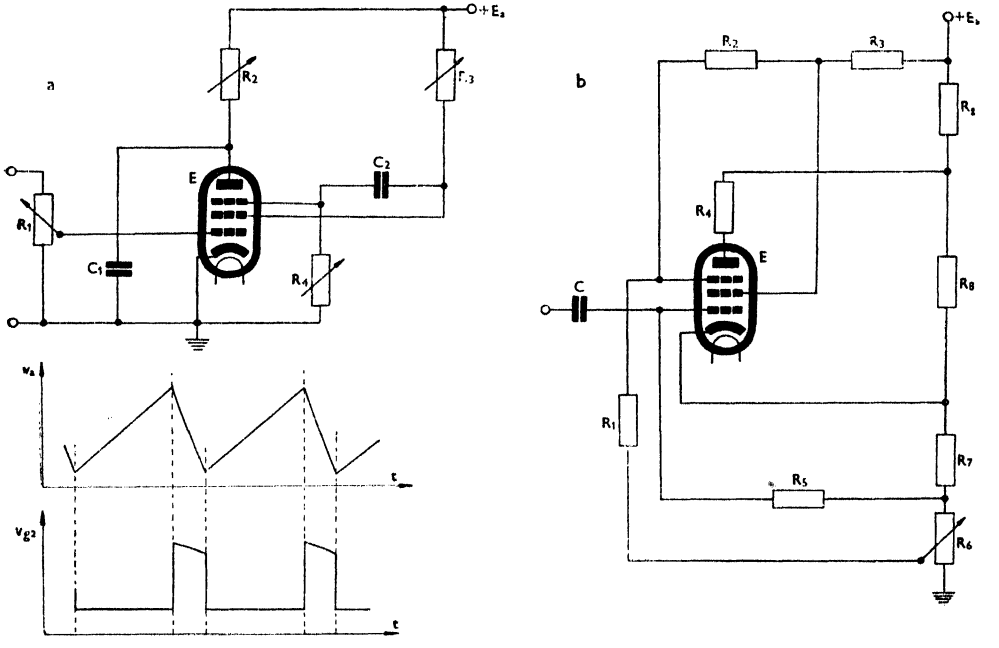

Fig. 25. Transitron. a) astable transitron and the voltages at its electrodes. b) bistable transitron.

current. The grid current g_2 is further increased and the grid voltage g_2 decreased. The tube is blocked, the anode current falls to zero, the anode voltage begins to rise as C_1 is being charged, the g_2 voltage falls considerably and the g_3 voltage reaches even large negative values. The negative charge now leaves C_2 only through R_4, i. e. at a much slower rate than the earlier positive charge. As soon as the condenser C_2 is discharged (and C_1 charged), the g_3 potential rises to zero, an anode current appears, the screen grid current falls etc.

It is evident that during the longer part of the repetition interval, C_1 is charged through R_2 and C_2 discharged through R_4. These elements thus mainly affect the frequency. The shorter part of the repetition interval is determined by discharging of C_1 and by the conductance of the tube, i. e. its characteristic, the voltages at all three grids and the grid leaks, especially

R_3, and also by the cathode resistance, if it is used in the circuit. Transitrons are often used to obtain sawtooth voltages from the anode for the time base of the cathode ray oscillograph, and then we try to obtain very rapid discharge. They may also be used for producing rectangular voltage (from g_2), and in this case, a duty cycle of $\frac{1}{2}$ or less may be obtained by a suitable choice of resistances. The synchronising voltage is usually applied at g_1.

The transitron may be blocked and changed to a triggered transitron with one or two stable positions by a suitable choice of g_1 and g_3 bias (Fig. 25b). It then fully corresponds functionally to a mono- or bistable multivibrator. In comparison to multivibrators it is simpler and can be triggered off by both positive and negative pulses. Its disadvantages are asymmetry, less sensitivity (larger and longer triggering pulses are necessary), a smaller amplitude of the output voltage (50 V or less), more difficult calculation and setting. Particularly for pulse width near the quiescent interval a large dependence of the actual pulse width not only on the preceding interval but also on the width of the preceding pulse may be observed. This may even result in even pulses being about twice as long as odd pulses, or vice versa. Triggering pulses are usually injected into the control grid circuit, which has the highest sensitivity and is not used in the feedback circuit itself. It is, however, possible to direct them to any other electrode.

In monostable transitrons the coupling between g_2 and g_3 is achieved with a condenser in the same way as in transitron auto-oscillators. In bistable transitrons a resistance coupling must be used (as in bistable multivibrators — Fig 25b).

Dynatrons are somewhat similar to transitrons. A negative resistance is formed in them as a result of secondary electron emission from the anode (see page 98). Since the dynatron effect depends to a large extent on the voltage, it is unstable and, in modern valves, is purposely avoided. Dynatrons are rarely used today. A negative resistance and thus generation of rectangular or sine wave oscillations may also be obtained with a crystal of pyrite or galenite, with a germanium diode or with a transistor. These have recently been used largely for calculating machines and similar apparatus. At present they are used more and more frequently in electrophysiological work, although they cannot replace valves in all cases (e. g. input valves). For a discussion of their use see Whitfield (1959).

Pulse shaping circuits

The pulses described above can rarely be used directly. Usually it is necessary to shape them, i. e. to adjust their shape in such a way that it approximates the ideal course with the accuracy chosen. Shaping involves three

problems: a) determination of the shape of the curve (harmonic, damped oscillations, rectangular, sawtooth, exponential etc.), b) adjustment of the time scale (width of the rectangular or sawtooth pulses, time constant of the exponential voltage drop, frequency or duration of the harmonic and damped oscillations), c) determination of the amplitude.

a) Determination of the shape

1) Harmonic oscillations may be produced α) Directly in harmonic oscillators (see p. 51). They are then further amplified only by linear amplifiers or tuned amplifiers which do not change their shape. β) Nonharmonic oscillations are changed to harmonic ones by passing them through tuned filters (either through resonant circuits, especially for higher frequencies, or through more complex *RC* filters containing often also amplifying valves, see e. g. p. 54). γ) Damped oscillations are damped so little (or damping is artificially reduced) that for certain purposes they are equivalent to undamped oscillations. Cf. the next paragraph and also p. 133.

2) Damped oscillations are nearly exclusively produced by injecting either a potential step or a short pulse into the parallel resonant *LRC* circuit. Damping is controlled by a resistance connected either in parallel to the whole *LC* circuit (e. g. a valve, germanium rectifier) or in series with *L* (resistance of the coil windings and losses in the iron core). The first stage of the appparatus in Fig. 51 shows the circuit most frequently used. The resonant circuit in the cathode of a triode is highly damped by the grid current, which is always flowing for a positive grid bias. The negative voltage step applied to the grid through C_1 blocks the cathode current. Disappearance of the cathode current (and thus of magnetic induction) in coil *L* results in oscillations in the circuit *LC*, which is only slightly damped since it is isolated by the blocked valve. When the negativity of the grid ends, the positive grid bias produces a grid and anode current which very rapidly damps the circuit. The damping of the circuit at a time when it should operate can further be decreased using a positive feedback (see p. 133). Sometimes, on the other hand, damping ought to be increased.

2a) By using a parallel resistance or, still better, a parallel diode (see p. 68) an aperiodic course is achieved, which may also be obtained by critical damping for

$$R = \frac{1}{2}\sqrt{\frac{L}{C}}$$

3) For producing square waves many different connections may be used. These were already described or will be discussed later (p. 81).

4) The same applies to sawtooth waveforms.

5) An exponential curve is most frequently obtained by charging or discharging a condenser across a resistor. Such a network is called an *RC* differentiating circuit. Quantitatively it is characterised by its time constant

$$\tau = RC$$

which determines the shape of the voltage rise or decay at the condenser

$$v_1 = E_0\left(1 - e^{-\frac{t}{\tau}}\right) \quad \text{(on charging)}$$

$$v_2 = E_0 e^{-\frac{t}{\tau}} \quad \text{(on discharging)}$$

Evidently for $t = \tau$ the voltage drops to $\frac{E}{e}$, i. e. to about $0{\cdot}37E_0$ (when discharging) or rises to $E_0\left(1 - \frac{1}{e}\right)$, i. e. to $0{\cdot}63E_0$ (when charging). See also p. 40.

Such derivative peaks are very often used for triggering monostable and bistable multivibrators or as standard pulses, as in counters, for synchronisation, or also directly as stimuli (in the older chronaximeters).

b) Time relations

The second parameter of shaping — time — is either included implicitly in the first, and controlled together with it (the frequency of harmonic and damped oscillations, time constant of exponential pulses) or controlled separately, as in the case of rectangular waveforms if the frequency, pulse width and amplitude are controlled independently. The width of a rectangular pulse may be controlled by the following means:

1) Using an astable multivibrator. Here the pulse width is not fully independent of the frequency, however. See p. 56.

2) Using a monostable multivibrator which is driven by short pulses of the chosen frequency. The leading and trailing edges are steep, but the pulse width is slightly frequency dependent and cannot be controlled accurately (variations of several %). It is difficult to control the pulse width within a wider range.

3) Using bistable multivibrators, as in Fig. 18. A sinusoidal voltage is shifted in phase by a *RC* member and both the original and the phase shifted voltage are given a symmetrically rectangular shape by limiters (see p. 81). This voltage is differentiated and the bistable multivibrator is reversed by the derivative peaks. Since the phase can be controlled very exactly (to a fraction of $1^0/_{00}$), this method of controlling the pulse length is very exact, but rather complicated. This, however, is the only method by which the pulse

length can be controlled relatively, i. e. the frequency can be changed as desired without changing the duty cycle, i. e., the constant ratio of pulse width to the repetition interval is preserved. The method may also be modified in such a way that both rectangular voltages, shifted in time, are directed into a suitable selector circuit in which they are summed.

An analogous transitron connection may be used instead of the bistable, monostable or astable multivibrator. Its advantages and disadvantages are given on p. 76.

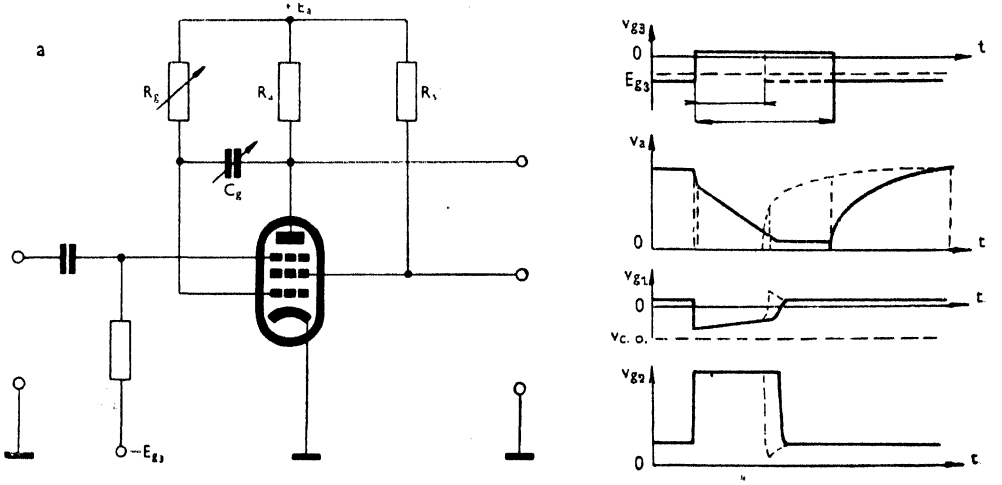

Fig. 26. Phantastron. a) circuit diagram. b) input voltage at the suppressor grid, voltages at the anode, at the control and screen grids. $V_{c.o.}$ — cut-off voltage.

4) For our purposes it is usually advantageous to use more complicated intergrating circuits known as phantastrons. The basic circuit (always termed Miller's transitron) is shown in Fig. 26.

In the blocked state the cathode current divides into that of the control grid (which is connected to the positive supply pole through the leak R_g) and that of the screen grid, which thus has a very low voltage. The anode current does not flow, since the suppressor grid has a large negative bias. Thus the anode has the full supply voltage. If a positive voltage (a long pulse) is applied to the suppressor grid, the anode current suddenly begins to flow. This results in a fall in anode voltage. This fall is transferred to the control grid through a negative feedback condenser C_g. In this way the rate of the cathode current increase is slowed. (Since the anode current begins to flow, the screen grid current decreases and its voltage rises.) The negative charge transmitted to the control grid from the anode leaks through resistance R_g, however, so that the cathode and anode current continue to rise. This rise is, of course, very slow, since the fall in anode voltage due to anode current

increase is transferred through C_g to the control grid and suppresses the anode current, which is affected more by the grid voltage than by the anode voltage (this ratio is determined by the amplification factor). This gradual and linear rise in anode current or fall in anode voltage may terminate in two ways: a) the voltage step at the suppressor grid is terminated — the valve is again blocked; b) the anode current cannot rise and the voltage cannot continue to fall, since the curved part of the characteristic is reached. Here the condenser C_g ceases to have an effect, the grid voltage again rises to positive values and thus the cathode current continues to rise rapidly. This, of course, cannot further increase the anode current, as this is saturated, but causes a rise in the control grid current and thus a rapid fall in its voltage. The anode voltage remains at the low value until the suppressor grid is again blocked.

In practice either the linearly falling part of the anode voltage (a very good time base for the oscilloscope) or the rectangular pulse at the screen grid is used. Its width depends upon the electrode voltages and on the values of R_a, R_g and C_g. Calculations are complicated and unnecessary since pulse width depends only on R_g and C_g very closely according to the equation:

$$\tau = kR_gC_g$$

where the constant k includes the effect of different parameters (the other resistances, the valve, the electrode voltages). This means, for practical purposes, that it is sufficient to determine the pulse width τ_0 in one special case and then calculate either C_g or R_g necessary for a certain different pulse width τ or determine the pulse width for any R_g and C_g. Then

$$\tau = \tau_0 \frac{C_gR_g}{C_{g_0}R_{g_0}}; \quad C_g = \frac{\tau}{\tau_0} \cdot \frac{C_{g_0}R_{g_0}}{R_g}; \quad R_g = \frac{\tau}{\tau_0} \cdot \frac{C_{g_0}R_{g_0}}{C_g}.$$

The pulse width can, of course, also be controlled otherwise, by a voltage applied to the anode through a so-called fixing diode. When the anode current rises, the anode voltage cannot fall below the potential of the cathode of the fixing diode. The end of the pulse is thus formed at this "fixed" voltage.

A further advantage of the phantastron circuit is the possibility of obtaining any desired scale (as follows from the above equations) using variable resistors or condensers, and numerically the scales for multiple ranges agree and a unipolar switch is sufficient for the range selector. What is most important, the circuit is very accurate. Variations in the anode voltage and the heating current supplies by $\pm$ 10% result in variations of pulse width by about only $\pm$ 1‰. The pulse width depends on the amplification factor of the valve, and this is probably the only drawback. This must, therefore, be checked from time to time, and calibration must be performed only after longer use (50 to 100 hrs.). If an accurate and equal scale is to be preserved even as the valve

ages or after exchanging it, a variable resistor or condenser (trimmer) must be used for adjusting the scale to the dial. If a variable resistor serves as the main tuning element, a trimmer is used for scale adjusting while a variable resistor is used if the tuning element is a condenser.

In addition to this simplest, most reliable and accurate circuit of the phantastron, more complex circuits are used frequently. These are especially suitable if the phantastron is used for shifting short pulses in time, i. e. for producing delayed pulses. This might be obtained as follows: The input pulses trigger a monostable multivibrator or transitron and this starts the phantastron. This may be achieved in a more simple way with a single valve phantastron circuit using a pentode or pentagrid. This operates simultaneously as a triggered mono- or bistable transitron and phantastron. Such connections are simpler as far as the number of valves and components are concerned, but need more careful adjustment and are usually less accurate. It is better not to use them even though more valves are then required. Reliable circuits may be found in Petrovich and Kozyrev (1954), Meyerovich and Zelichenko (1953), Markus and Zelluff (1948).

So-called transmission lines are used especially for shorter pulses of about 1 μsec. both for pulse shaping and delaying. They have the common property that pulses pass through them or are reflected by them, the pulse propagation rate in them being relatively low (i. e. much less than the speed of light in a vacuum). Their main advantage lies in their accuracy and complete independence of the input voltage etc. For our purposes they are unsuitable, since because of long pulses and delays, their dimensions are tremendous, especially if steep front and trailing edges are to be preserved. (See Meyerovich and Zelichenko, 1954, for details.) Here it need only be said that usually they consist of a number of coils and condensers (T or π networks) with more complicated coupling between the individual elements.

Limiters

Pulse amplitudes are usually determined in voltage limiters. These are nonlinear elements distorting sinusoidal or other input voltage in such a way that the output voltage has an approximately rectangular course. In other words, a limiter is an amplifier, the amplification of which changes in relation to the input voltage in such a way that the output voltage attains only two values, one of which is usually zero. The nonlinear element of the limiter is usually a vacuum valve (diode, triode, pentode), a germanium or selenium rectifier, more rarely a diode with a pure tungsten filament working in the region of saturated emission current, an iron cored coil, a thyratron, a gas discharge tube etc.

increase is transferred through C_g to the control grid and suppresses the anode current, which is affected more by the grid voltage than by the anode voltage (this ratio is determined by the amplification factor). This gradual and linear rise in anode current or fall in anode voltage may terminate in two ways: a) the voltage step at the suppressor grid is terminated — the valve is again blocked; b) the anode current cannot rise and the voltage cannot continue to fall, since the curved part of the characteristic is reached. Here the condenser C_g ceases to have an effect, the grid voltage again rises to positive values and thus the cathode current continues to rise rapidly. This, of course, cannot further increase the anode current, as this is saturated, but causes a rise in the control grid current and thus a rapid fall in its voltage. The anode voltage remains at the low value until the suppressor grid is again blocked.

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1) The series diode limiter

The diode permits current from the source to pass in one direction only, so that a pulsating voltage is obtained across resistance R as shown in Fig. 27a. If a positive or negative bias is given to the diode, the zero line is shifted above

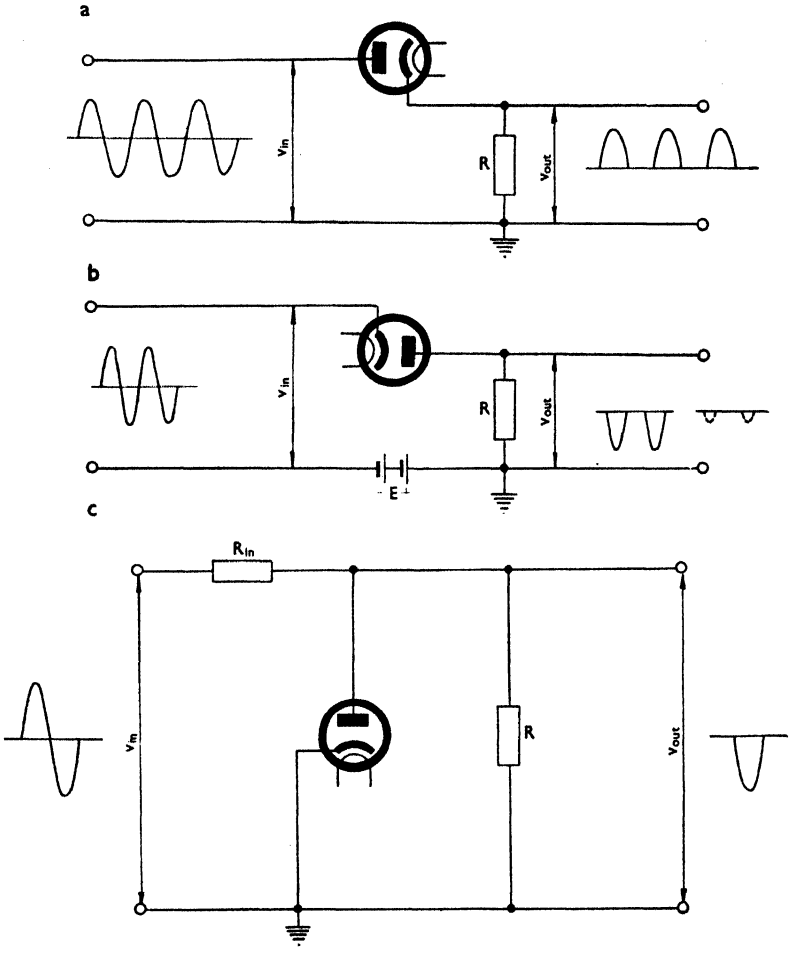


Fig. 27. Diode limiter. a) series negative limiter. b) series positive limiter with bias. c) parallel positive limiter.

or below the symmetry axis of the input sine wave voltage (Fig. 27b). If the diode is reversed, a pulsating voltage of opposite polarity is obtained (Fig. 27b). A germanium or selenium rectifier may be used instead of the diode.

By using two biased limiters the sine wave voltage may be limited from both sides, so that a voltage having a trapezoidal shape is obtained. The voltage approaches a rectangular shape the more closely, the larger the limiting, and

thus the smaller the output voltage. This is a disadvantage of all limiters working without amplification.

2) The parallel diode limiter (Fig. 27c)

The diode connected in parallel to the load R is a resistance which attains very large (infinite) values for voltage of one polarity, and very small ones

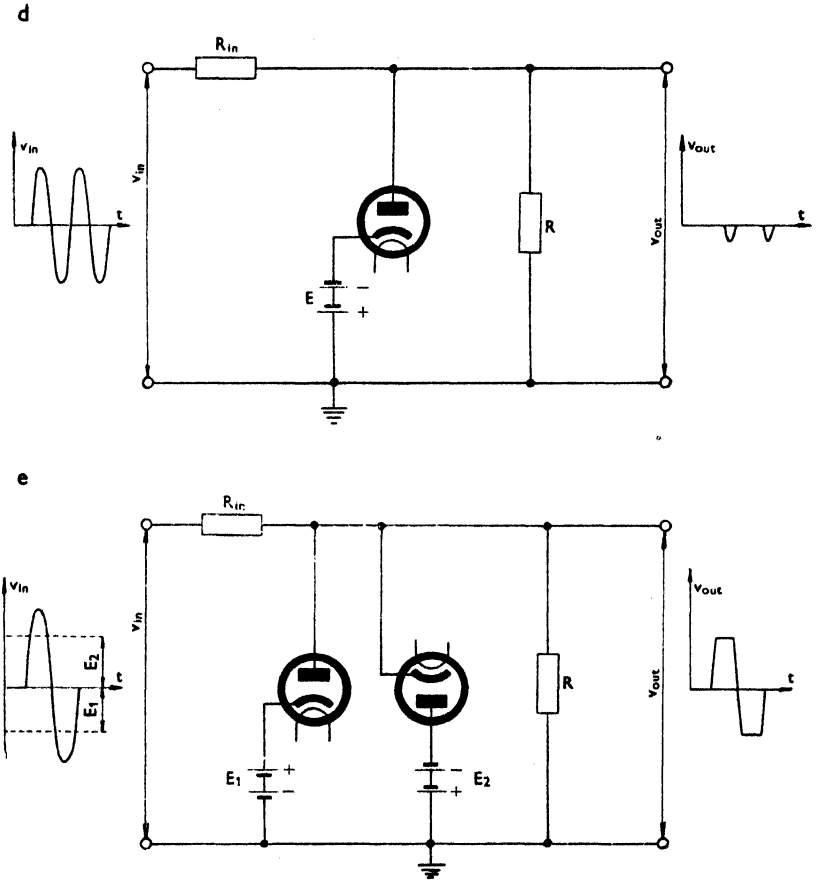


Fig. 27. d) parallel positive limiter with bias. e) parallel symmetrical limiter with bias.

(R_s — the so-called D. C. resistance of the diode, smallest in germanium rectifiers) for voltage of the opposite polarity. Thus during one half cycle the input is shunted. Good suppression is obtained for $R_s \ll R$ and $R_{in} \gg R$ (R_{in} is the internal resistance of the input signal source). The polarity in this limiter

may be changed by reversing the diode and the level of suppression may be regulated by the D. C. bias (Fig. 27d). With a parallel diode limiter symmetrical limiting may be also obtained (Fig. 27e).

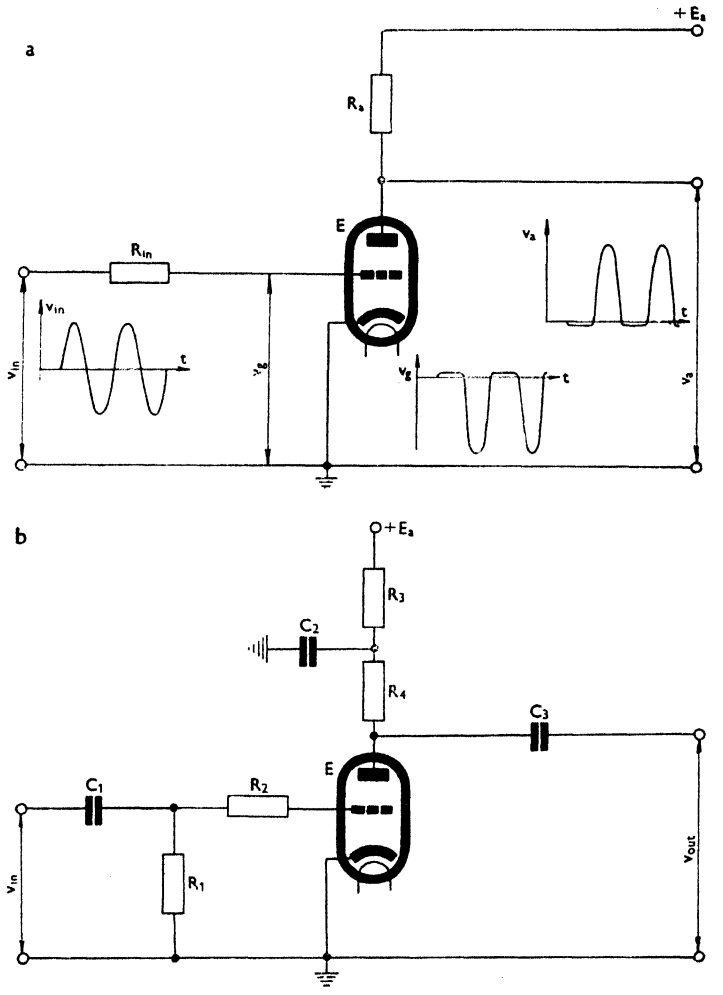


Fig. 28. Triode limiter. a) grid current negative limiter. b) grid current limiter with A. C. input and output.

Triode and pentode limiters

3) The triode limiter using grid current (Fig. 28a)

The circuit cathode — grid is really a parallel diode limiter. Due to the grid current and the drop across resistance R_g, the grid voltage and thus the anode

current are constant during the positive halfwave. The anode voltage fall. to minimum values. The negative half wave is amplified. R_g is 10^4 to 10^6 Ω.

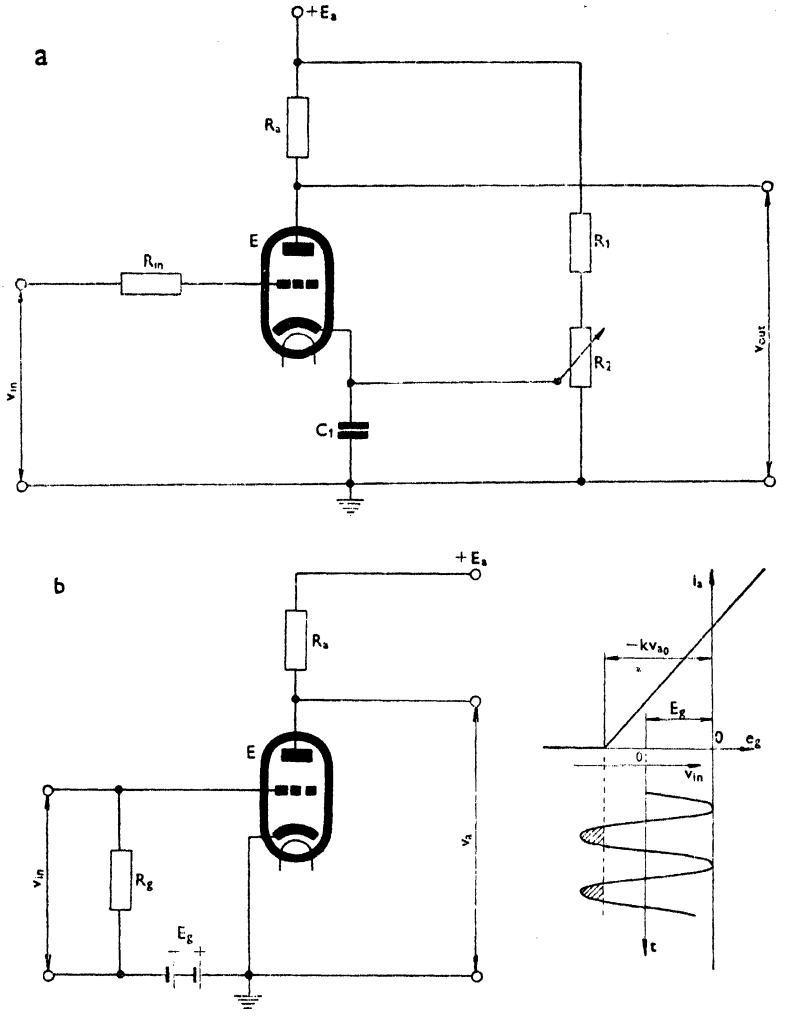


Fig. 29. Triode limiters. a) grid current negative limiter and anode current cut-off limiter with controlled level of limiting. b) anode current cutoff limiter.

4) The triode limiter using the anode current cut-off

This limits the input voltage from the opposite side from the limiter described above. The negative grid bias is chosen in such a way that the anode current disappears during the negative half waves of the input voltage and the positive half waves are amplified (Fig. 29b).

5) A limiter using shift of cathode current

In the region of zero and positive grid voltages, the valve (triode and pentode) having a large anode resistance ceases to amplify even if the source of input voltage has an internal resistance sufficiently small to compensate the grid current leakage.

6) The symmetrical triode limiter using grid current and the cut-off of the anode current (Fig. 29a)

This combination of types 3 and 4 is used more often. In a similar way any two opposite types of limiters, e. g. 4 and 5, may be combined into a symmetrical limiter. By controlling the bias the proportion of pulse width to the quiescent interval is also regulated. All types of limiters may also be designed with a pentode. The pentode limiters are mostly more efficient. Two stages of such a symmetrical pentode limiter normally are sufficient for producing a very rectangular voltage from a sine wave voltage. For limiting a voltage with a steeper course, e. g. for topping a multivibrator (or a transitron pulse etc.) one stage is sufficient.

Limiters, especially non-symmetrical limiters, are often used, after differentiating the rectangular pulses, for removing peaks of one or the other polarity. Often limiters are also used where they are not absolutely necessary, i. e. as isolating amplifiers, phase inverters etc. This lessens the danger of interference, accidental feedbacks etc.

Assembling a stimulator

We must now consider the main principles for synthesis of such elementary circuits into an electronic stimulator. First, these circuits are connected in series according to our general requirements, i. e. a so-called block diagram is made (Fig. 30). According to the stimulus parameters required and to the accuracy with which they are to be regulated, the individual circuits are now designed in more detail. This is best illustrated by an example.

A stimulator giving paired rectangular pulses is required. The absolute width of each pulse is to be regulated from 10^{-5} to 10^{-2} sec., their delay from 10^{-4} to 10^{-1} sec., their amplitude from 10^{-2} to 10^{2} V. Both pulses must be preceded, with a controllable delay of 10^{-4} to 10^{-1} sec., by the impulse for synchronising the time base of the cathode-ray oscilloscope, which has a duration of about 10^{-3} to 10^{-4} sec., and an amplitude of about 20 V or more. The accuracy of all time controls is about $1^{0}/_{00}$, that of the amplitude control

2%. The time constants of the front and trailing edges of both stimulating pulses is 2μsec for the shortest pulses and somewhat longer for the other pulses. The output pulses may be applied to the same or to different electrode pairs. This system of pulses may be repeated with a frequency than can be controlled from 10^{-1} to 10^{3} c/sec (accuracy $1^{0}/_{00}$) or is produced only once (by pressing a key). To satisfy these requirements, the following principles are to be observed.

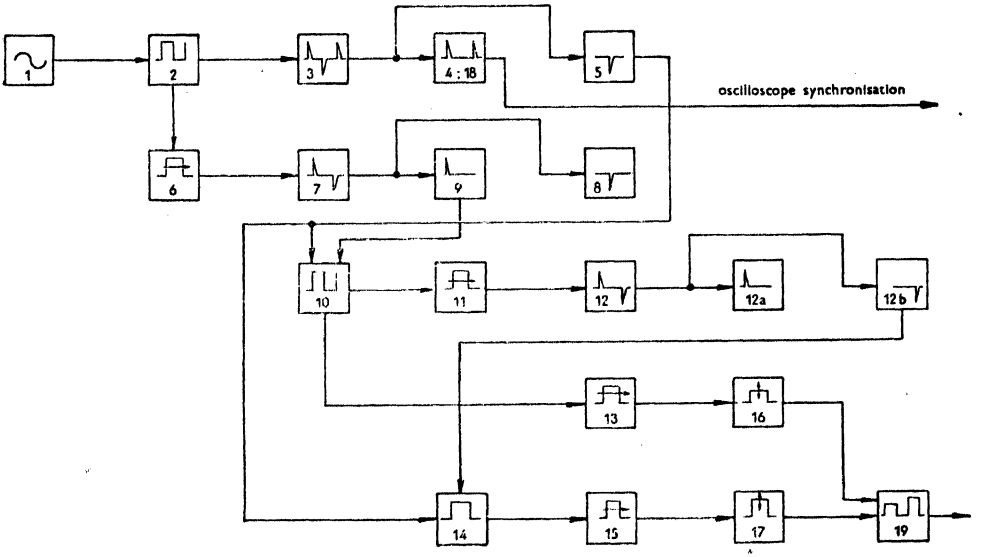


Fig. 30. Block diagram of complex stimulator.

A) In view of the required accuracy, the generator of the fundamental frequency must be harmonic. In view of the large frequency range and the very low lower limit, a symmetrical *RC* oscillator will be best (see p. 53).

B) In view of the requirement of triggering the stimulus with a key, it is necessary that the whole cycle occur, after triggering with one potential step. The return to the original state can be attained either by a step of opposite polarity or automatically.

C) In view of the required accuracy of time relations, all these relations must be controlled by phantastrons.

D) In order to attain simple graduation of dials (readings without calculations, graphs etc.), the corresponding times must be directly determined by phantastrons. Now the diagrams of the time relations for a frequent stimulus are drawn (Fig. 31).

The sine wave voltage (1) of the oscillator is changed to a rectangular one (2). Using differentiating circuit (3) and limiters, peaks for synchronisation of the cathode ray oscilloscope (4) and bistable multivibrators (5) are obtained.

A variable delay of the first pulse, i. e. the time by which the synchronising pulse precedes the first stimulating pulse, is produced by the phantastron (6). The width of the first stimulating pulse must be controlled independently of this delay. Consequently we use derivative peaks of the trailing edge of the

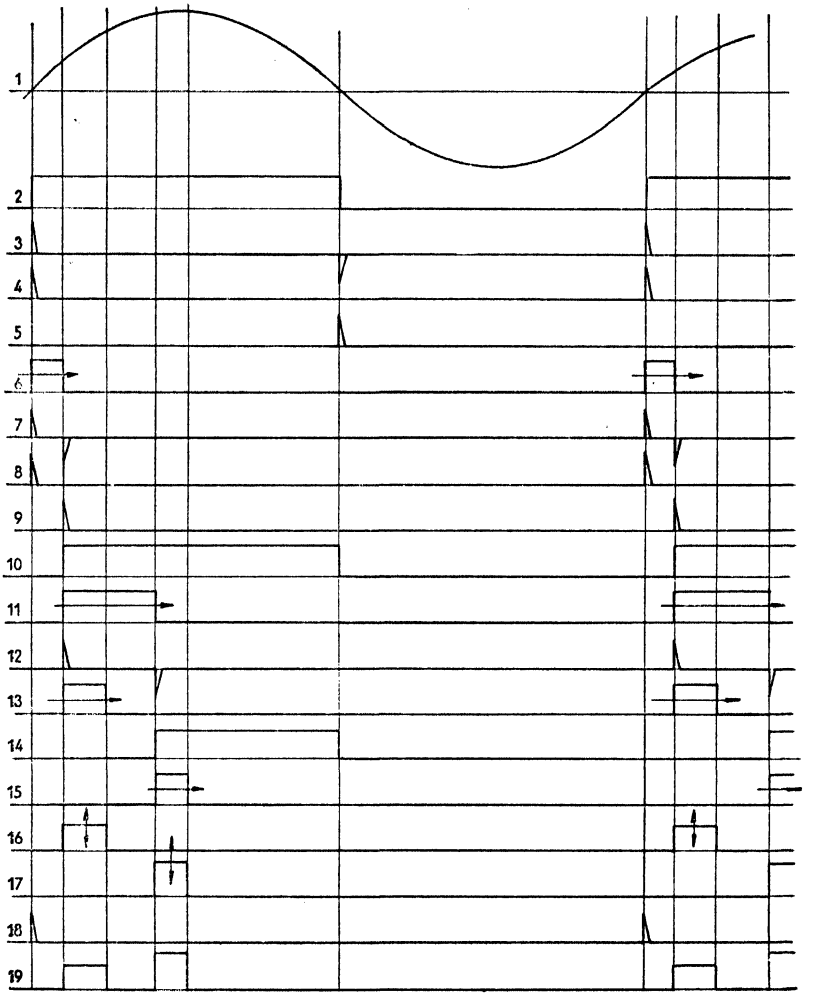


Fig. 31. Time diagram of oscillator shown in Fig. 30. The row numbers indicate the block diagram stages (for details see text).

phantastron pulse (9) and the derivative peaks (5) of the basic rectangular waves (2) for the formation (in the bistable multivibrator) of the first main pulse (10). This is shortened by the phantastron to the required width of the first stimulating pulse (13). The same main pulse (10) is used to drive a further phantastron, the pulse of which (11) determines the delay of the

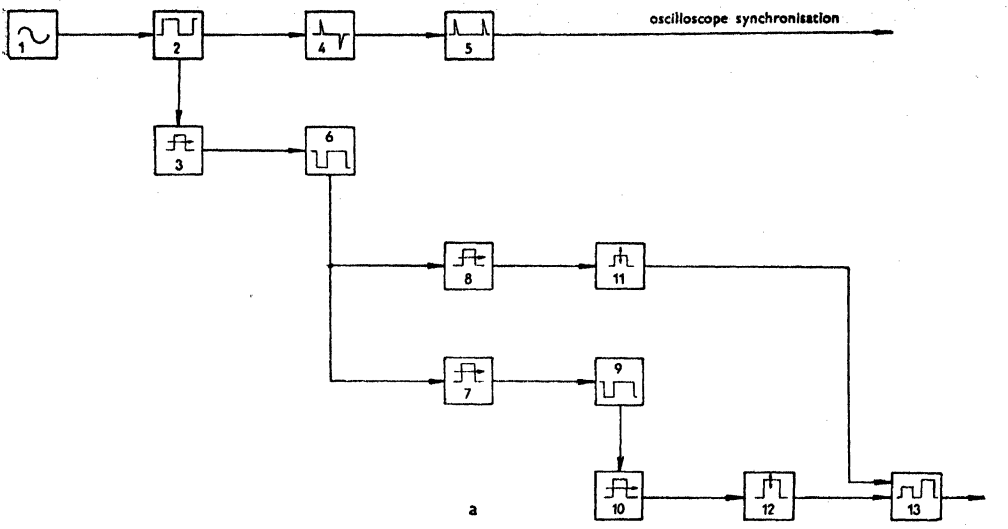
front edge of the second main pulse (14) from that of the first one (10). By differentiating the phantastron pulses, peaks (12) are produced, the second of which is used for triggering a further bistable multivibrator (14). The end of the pulse of this is determined either by the peak terminating the first half cycle of the basic frequency (5) (if the whole second pulse lies in the first half cycle), or by the peak terminating the second half-cycle (4) (for long or very much delayed second pulses). Thus the second main pulse (14) is obtained, from which the second stimulating pulse width (15) is formed by the phantastron.

This ends the first part of the rough design, the part determining the time relations. Now both pulses are formed and their amplitude, polarity and mutual relationship determined. Each pulse is directed separately into a separating limiter and then into an amplifier, where their size and polarity (16, 17) are determined. If both pulses are to be supplied to the same electrodes, they must be lead into a linear mixer (19). Now the number of the oscillator ranges is determined. Because of the high accuracy required, an oscillator with several, not-too-wide ranges must be used, e. g. 0·1 c/sec. to 0·3 c/sec., 0·3 c/sec. to 1 c/sec., 1 c/sec. to 3 c/sec., 3 c/sec. to 10 c/sec., 10—30 c/sec., 30 to 100 c/sec., 100—300 c/sec., 300—1000 c/sec., i. e. altogether 8 ranges. Phantastrons must be made in a similar way. They must have about 6 ranges (for an accuracy of 1% 2—3 ranges would suffice). Time constants of the circuits are then chosen according to the required slope of the front and trailing edges of the pulses, etc.

The requirements of this example can of course also be met in many different ways. For less strict demands another example is given in Fig. 32.

Several simple commercial stimulators are constructed in such a way that they can be connected in series so that one stimulator triggers the next and thus the stimulating apparatus may be adapted to even the most complicated requirements, e. g. trains of paired pulses etc. If requirements are not so strict it is, of course, possible to use each of these units as a separate apparatus.

Another very important auxiliary arrangement must be described when dealing with stimulators: radiofrequency stimulator, radiofrequency transformer or radiofrequency output unit (Schmidt and Dubert 1949) (Fig. 33). This is an arrangement permitting complete resistive and capacitative isolation of the stimulating electrodes from earth (cf. p. 149). It is based on the fact that the stimulus is transmitted to the electrodes without wires (as in broadcasting). The transmitter is an anode modulated Hartley oscillator (cf. p. 53). Its anode receives positive impulses from any stimulator. If such an impulse reaches it, the oscillator begins to oscillate for a time corresponding to the pulse duration with a frequency of about 10 Mc/sec. The receiver consists of a coil (at a distance of 1—2 cm from the oscillator coil) and a condenser. It is tuned by moving the powdered iron core or with a trimmer. A germanium diode forms the detector. After filtration to remove the remaining high fre-



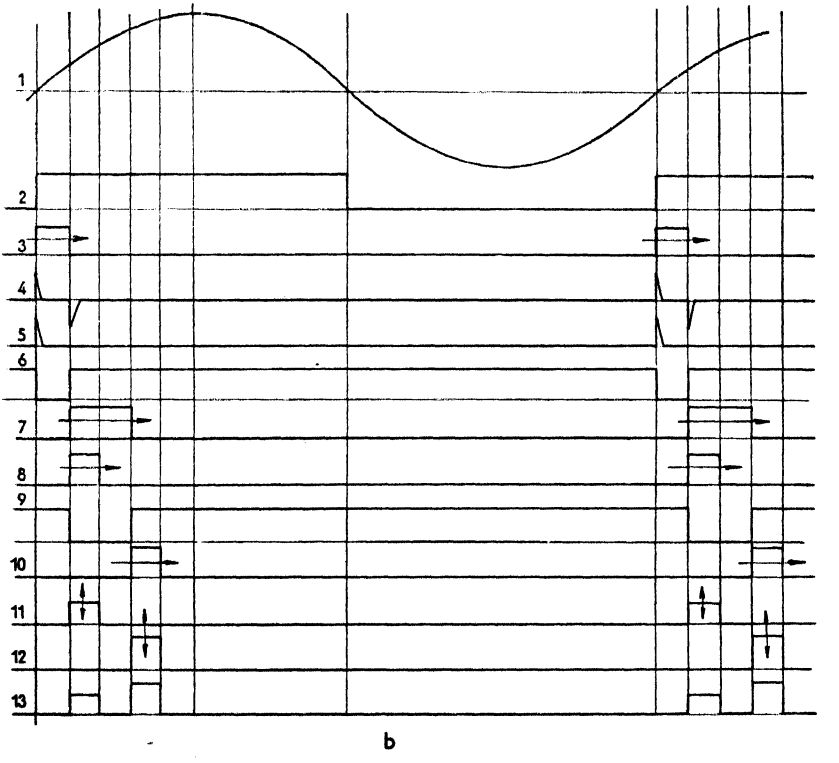


Fig. 32. Block diagram of simple stimulator (a) and its time diagram (b).

quency voltage, an impulse of the same duration as that modulating the oscillator, is applied at the stimulating electrodes. Its amplitude, however, is only a fraction of that of the modulating pulse, but is proportional to the latter (from a certain minimum value). The minimum amplitude of the modulating pulse necessary for oscillating the oscillator is 1—10 V, depending upon the type of valve and the design of the apparatus. It may happen if modulating voltage is reduced by radiofrequency transmission to one third, that

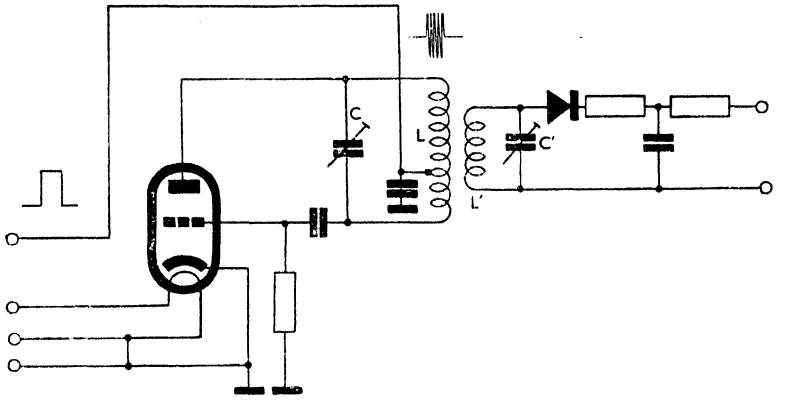


Fig. 33. A radiofrequency-coupled output stage of a stimulator.

the amplitude of the output pulse is still too high. It is then necessary to connect a voltage divider to the output. The heating voltage for the valve is either taken from the modulating stimulator or from a battery.

Recording technique

The second cardinal task of electrophysiology is to study electrical signs of vital processes either in cells or their parts or in tissues, organs or whole organisms. This is done by using recording apparatus. Before describing the individual typical components of such apparatus demands made upon them in general, and how far these can be realised will be discussed.

The fundamental characteristics of signals and their sources

Usually a change in the potential difference between two parts of a living object is considered as an electrical expression of vital functions. It must, of course, be remembered that a change in potential difference is always accompanied by a number of changes in other electrical properties (resistance, capac-

itance etc.) and often also of non-electrical characteristics (optical, mechanical etc.) that have not yet been studied sufficiently. Such a change is called an electrical impulse in the theory of communication (which forms the basis of applied electrophysiology). A single electrical impulse is characterised by its shape, amplitude and further, by characteristics of its source, particularly its internal resistance. The amplitude of impulses recorded in electrophysiology lies between 0 (practically, several μV) and 0·1 V for nerve and sensory cells, nerve and muscle fibres (excepting the electric organs of some fish).

The time course of the amplitude changes can be developed into the so-called frequency spectrum according to the principles of harmonic analysis, which are described in most radiotechnical and physical text books and the practical procedure is given in textbooks of higher mathematics. Here it is sufficient to state the qualitative rule that each electric impulse is formed also by oscillations of a much higher frequency than is the repetition frequency and the inverse value of the pulse width. The participation of these "higher harmonics" is the larger the more square or pointed the impulse shape. How far the whole of this "frequency spectrum" is to be respected is often an important question in electrophysiology. A further important characteristic of impulses is the internal resistance of their source, i. e. the internal resistance of the tissue. Its significance can be illustrated by an example: If the size of an impulse recorded from the whole nerve is measured with an apparatus (e. g. cathode ray oscilloscope) having an internal resistance of 100 000 Ω the result will be roughly the same as with an apparatus having a resistance of 1 000 000 Ω. It follows that the internal resistance of the impulse source (of the nerve) is considerably smaller than 100 000 Ω. If, however, microelectrodes are used to record impulses from a single fibre, the first apparatus will show nothing and the second only very low values. An apparatus having an internal resistance of 10 MΩ or more will show considerably larger amplitude. Thus the internal resistance of a single fibre is about 10 MΩ or more. It is evident that the internal resistance of the impulse source does not only depend upon its nature, but also considerably upon the external medium in which the source is situated (conductive, nonconductive), on the size and shape of the electrodes, etc. It even seems that the tissues may have a different internal resistance for different frequency components of the impulse. It follows that it is sometimes necessary to increase the internal resistance of the input circuit of the recording apparatus to extremely high values.

Recording apparatus

The recording apparatus consists of several components with different functions. Impulses from the tissue are lead into the input circuit, which must not distort them. This is followed by one or more (or no) middle stages which

change the impulse (usually only its amplitude) in such a way that it can be transferred to the output stage connected with the actual recording or measuring instrument. Demands on the individual components differ according to these different functions.

Input circuits

The following are the most important requirements:

1) The input circuit must not affect the studied substrate. It is especially important that it must not take up too much energy nor supply energy to the substrate.
2) The input circuit must pass on impulses without distortion, i. e. the output amplitude must be exactly proportional to the input amplitude. This means that no frequency component must be suppressed or accentuated and no voltage not corresponding to the input voltage must be transmitted. For details see below.
3) The input circuit must amplify the energy of the input signal. In some cases this is achieved by the output impulse having a considerably larger amplitude than the input impulse. At other times the amplitude remains practically unchanged or even decreases, but the output resistance is much smaller than the input resistance. Since power is $\frac{e^2}{R}$, power is also amplified in this case. The first two requirements can be fulfilled only in part and the corresponding compromise must be considered for each case separately.

Middle stages

Requirements are somewhat less strict:

1) undistorted transmission (see above),
2) the greatest possible amplification (usually voltage amplification).

The output (power) stage

1) must have the necessary amplification (of voltage or power),
2) must compensate for the distortion occurring in the recording system.

The recording or measuring system

1) must permit registration or measurement within the whole required frequency and amplitude range,
2) must either work without distortion, or its distortion must be compensated for by the preceding amplifier.

The following are the most commonly used instruments: The rotating coil instrument (Deprez-d'Arsonval), mechanical recording system with a moving coil, magnet or iron, a cathode-ray tube. For details see p. 123.

Biological amplifiers

All stages except the last one are usually constructed as valve amplifiers. They will now be discussed and the types which can be used for the different stages will be described.

Valves

The basic component is a valve — a pentode or triode. Their functions will be reviewed first, especially those important for our purposes.

The diode. The perfectly exhausted glass vessel contains a heated cathode and a cold anode (plate). Electrons leaving the cathode either remain concentrated around the cathode in the empty valve space (space charge) or reach the anode and can be lead off from it. The number of electrons reaching the anode during unit time (anode current) depends on the composition and temperature of the cathode and on the potential difference between the cathode and the anode. If the anode is negative to the cathode, no anode current flows. If the anode voltage is raised, a very small current begins to flow already with a small negative voltage and becomes definite with zero voltage. As the anode voltage continues to rise the anode current rapidly increases until all electrons released from the heated cathode reach the anode — the region of saturated current is attained. The current cannot rise any further (there being no further electrons available). The graphic representation of this relationship is termed the anode characteristic. The saturated current can only be increased by increasing the temperature of the cathode. The cathode is heated electrically. The cathode is either formed by the heated filament itself (directly heated cathode) or the cathode, in the form of a cylinder, is heated by an insulated spiral inside it (indirectly heated cathode). In electrophysiological instruments indirectly heated valves are used nearly exclusively. The saturated current

depends: 1) On the material of the cathode surface. Consequently the majority of indirectly heated cathodes are covered by a layer of the oxides of Ca, Sr or Ba. These emit electrons at relatively low temperatures, several hundreds degrees Centigrade. They cannot, however, be loaded with a saturated current, which destroys them within a fraction of a second. 2) On the temperature. (Thermionic emission rises rapidly with increasing cathode temperature).

Diodes are used to rectify A. C. currents. Today selenium, germanium or silicon rectifiers often replace them (unless a practically infinite resistance for the inverse voltage or a very high inverse voltage value is required).

Triode. This has a third electrode — the control grid — between the cathode and anode. The anode current does not only depend on the temperature of the cathode and the anode voltage, but also on the grid voltage, i. e. on the voltage difference between the grid and cathode. This is shown in Fig. 34b, d. The most important relationship is that between the anode current and the grid voltage, the so-called grid-anode characteristic (Fig. 34b). It can be seen that a change of the grid voltage has a much greater effect upon the anode current than the same change of the anode voltage. This is the key to understanding the function of a triode as an amplifier: a small change in grid voltage causes a large change in anode current. In series with the anode is an anode load across which the anode current evokes a voltage drop having a D. C., and an A. C. component. The A. C. component corresponds to the A. C. component of the grid voltage, but is larger and of the opposite direction (the phase of the anode voltage is shifted 180 degrees from that of the grid voltage). The suitable size of the anode load must be chosen according to the tube properties. For that purpose some of the triode parameters must be defined: Penetration coefficient

$$D = \frac{\Delta V_g}{\Delta V_a} \quad (I_a = \text{const.})$$

states by how large a factor the anode current is affected more by the anode voltage than by the grid voltage. $1/D = \mu$ is called the amplification factor and gives the theoretically maximum possible amplification.

The mutual conductance or transconductance

$$g_m = \frac{\Delta I_a}{\Delta V_g} \quad (V_a = \text{const.})$$

indicates the extent to which changes in grid voltage evoke changes in anode current. The anode resistance

$$R_i = \frac{\Delta V_a}{\Delta I_a} \quad (V_g = \text{const.})$$

shows how a change in anode voltage influences the anode current.

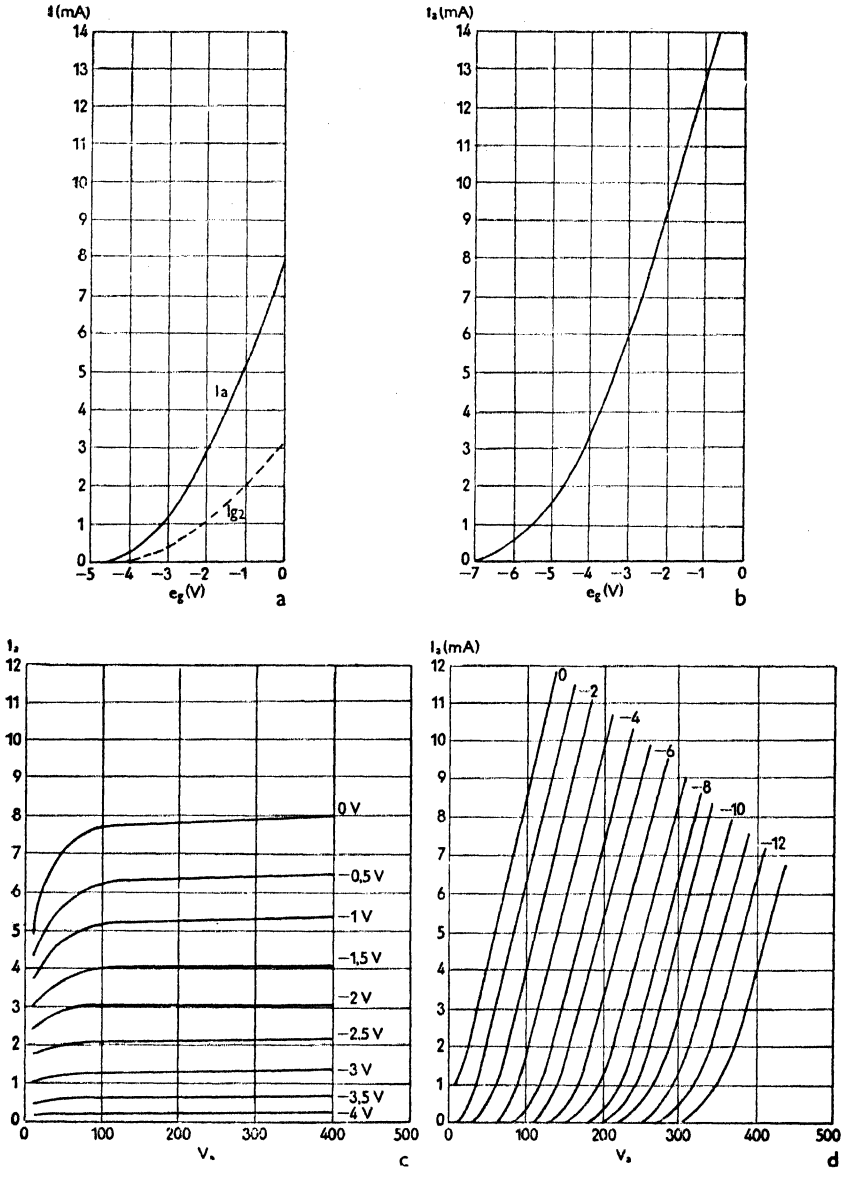


Fig. 34. Characteristics of pentode EF6. a, c — connected as pentode. b, d — connected as triode (screen and suppressor grids connected to anode). a, b — grid characteristics. Anode voltage 150 V, screen grid voltage 100 V. c, d — anode characteristics (control grid voltage is a parameter and is given for each curve). The difference between a triode and pentode is best seen when comparing c and d.

It follows from these definitions that

$$D \cdot g_m \cdot R_i = \frac{\Delta V_g}{\Delta V_a} \cdot \frac{\Delta I_a}{\Delta V_g} \cdot \frac{\Delta V_a}{\Delta I_a} = 1 \quad \text{(Barkhausen's equation)}$$

It must be underlined that, except for the penetration coefficient (D), these parameters are not constant, but depend on the conditions under which the valve is working (Fig. 35).

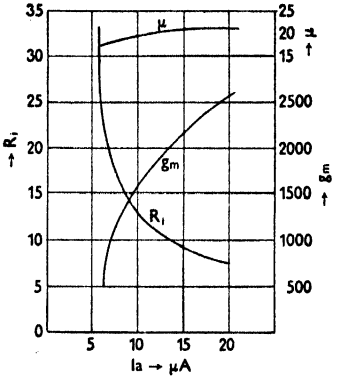


Fig. 35. Dependence of triode coefficients on anode current. Abscissa: anode current. Ordinate: scale for anode resistance in kΩ (left), transconductance in μA/V or microohm (right), amplification factor (top right), Curve R_i — anode resistance; g_m — transconductance; μ — amplification factor.

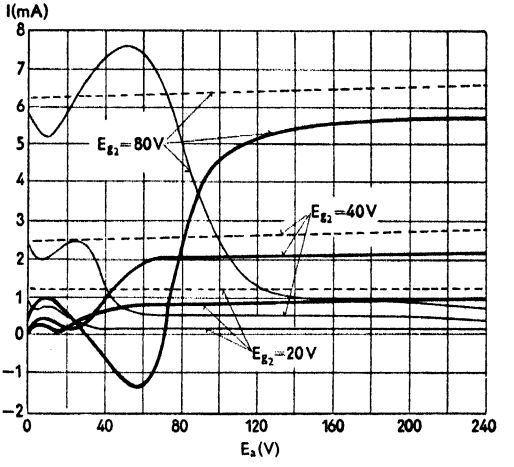


Fig. 36. Tetrode characteristics. Abcissa: anode voltage. Ordinate: current. Full heavy line — anode current curve (I_a), full thin line — screen grid current curve (I_{g_2}), interrupted line — cathode current curve (I_k). All curves for 3 different screen grid voltage values (20, 40, 80 V). The initial waves (left) in curves I_a and I_{g_2} are due to secondary emission. A negative anode current with a minimum for E_a 50 V is seen on the curve for E_{g_2} 80 V.

If a small anode load R_a is chosen, even a large change in anode current causes only a small change in voltage drop across it

$$\Delta V_a \approx R_a \ \Delta I_a \text{ , when } \Delta I_a = g_m \ \Delta V_g \text{ and thus } \Delta V_a \approx R_a g_m \ \Delta V_g$$

In this case the amplification will be small. If, on the other hand, a very large R_a is chosen, the anode voltage will approach zero and the amplification will again be small, since for a small anode voltage the anode current and the transconductance, which is its function, fall considerably. It can be demonstrated that maximum voltage amplification is obtained for $R_a = R_i$, which for the majority of triodes is equal to several tens of kΩ.

Pentode. It is evident from the above that the amplification could be increased considerably by increasing R_i, i. e. by decreasing the effect of changes in anode voltage on changes in anode current. This is achieved by introducing a further grid, a so-called screen grid, between the control gird and the anode. The screen grid has a voltage near to that of the anode and acts, therefore, as a kind of first anode. Since it has a constant potential, the electron current between the control and screen grid is not affected by changes in the anode voltage (Fig. 34c). (This is the case for triodes, where the drop across the anode load R_a causes variations of anode voltage and decreases the effect of the control grid on the anode current.) The screen grid accelerates electrons, but intercepts only about 10% to 20% of them. The remainder pass through its openings and thus reach the anode nearly independently of the anode voltage, i. e. also independently of the voltage drop across R_a. The latter can thus be chosen to have very high values, up to several MΩ. In other words, the anode resistance is high. Such a valve is termed *tetrode* or screen-grid valve. In addition, however, to the above advantages, tetrodes have a considerable drawback due to secondary emission of electrons from the anode (Fig. 36). The electrons falling onto the anode release so-called secondary electrons because of their high kinetic energy. There may even be several secondary electrons to a single primary one. These secondary electrons enter the space between the screen grid and the anode and if the former has a larger voltage than the latter, they may return to the screen grid. Thus a negative anode current is produced: more (secondary) electrons leave the anode than are trapped to it. This is called the dynatron effect and is usually very unwelcome. It can be eliminated either by a special arrangement and shape of the electrodes in so-called beam tetrodes, or by introducing a further, so-called suppressor or antidynatron grid. This has a negative voltage with respect to the anode (it is usually connected with the cathode). Rapid primary electrons pass through it without difficulty while slow secondary ones are returned to the anode by negative voltage of the suppressor grid. They do not release further "tertiary" electrons at the anode on their return, since their kinetic energy is small. Such valves are termed *pentodes*.

Some interference phenomena in amplifiers

When designing amplifiers the following phenomena have to be considered:

Noise

A voltage may be found at the output of every valve which does not correspond to any voltage applied to the input. This is called noise voltage. It has several causes and differs according to them.

Thermal noise. Every conductor contains free electrons which have thermal movement. At a certain moment more electrons may move in one than in another direction. Thus a voltage due to an instantaneous excess of electrons arises at one end of the conductor as compared to the other end. This noise voltage continually varies and changes its polarity. Theoretically it contains voltages of all frequencies from 0 to ∞. Hence the noise voltage is usually determined only for a certain frequency bandwidth. If the resistance of the conductor does not depend on the frequency, then the r. m. s. thermal noise voltage (in volts) is

$$V = 2\sqrt{kTR\,\Delta f}\,,$$

where k is Boltzmann's constant ($k = 1{\cdot}38 \cdot 10^{-23}$ Joule . degree^{-1}), T is the absolute temperature of the conductor (in degrees Kelvin), R is the resistance of the conductor in ohms, Δf the bandwidth of the measured frequency in c/sec.*) Thermal noise is found not only in all valves, but also in all circuits. Noise can never be reduced below values calculated from the above equation. This equation mainly serves to give a rough indication of demands on the recording apparatus and to evaluate the final product.

The equation shows that the only practical possibility of reducing thermal noise is to decrease load resistance and to narrow the passband of the amplifier. In addition to thermal noise, there are other noise forms in resistances, caused by changes in the quality of the resistor. This resistor component is the largest in graphite and carbon resistors, the smallest in wire-wound ones.

Valve noise. The current in a valve is produced by electron emission from the cathode. The number of emitted electrons is determined statistically. It varies and thus causes fluctuation in mean anode current, which produces so-called shot noise voltage across the anode load. The spectral density of the shot noise is similar to that of thermal noise. The noise current is (in amperes)

$$i = 5{\cdot}64 \cdot 10^{-9}\sqrt{I_{a_0}\Delta f} \quad (A;\ A;\ \text{c/sec.})$$

(where I_{a_0} is the mean anode current). This holds only if all electrons are immediately taken over by further (positive) electrodes. Usually, i. e. with a negative grid voltage, electrons accumulate at the cathode and thus form a so-called space charge. This evidently considerably decreases the shot noise. The equivalent noise resistance of the valve equals an equivalent grid leak, which would produce at the output of an ideal valve a noise equal to the noise of the valve at room temperature (300 degrees Kelvin) and for the same band width Δf. For a diode with a saturated current

*) After substitution (for 20°C)

$V = 0{\cdot}13\sqrt{R\ \Delta f}$ (μV_{eff}; kΩ; kc/sec.)

$$R_{eq.} = \frac{1}{20\, I_{a_0}}$$; for a diode with a space charge

$$R_{eq.} = \frac{1}{30\, I_{a_0}}$$; for a triode with a space charge

$$R_{eq.} = \frac{2{\cdot}5}{g_m}$$, where g_m is mutual conductance in amperes per volt.

The noise of pentodes is considerably higher than that of triodes because of the variation in the distribution of the cathode current between the anode and the screen grid. The equivalent noise resistance is equal to:

$$R_{eq.} = \frac{I_{a_0}}{I_{a_0} + I_{g_0}} \left(\frac{2{\cdot}5}{g_m} + \frac{20 I_{g_0}}{g_m^2} \right)$$

where I_{g_0} refers to the mean screen grid current.

Other less important sources of noise are irregularities in the oxide layers of the cathode and their changes (flicker noise), ionic cathode and grid currents (for imperfect vacua), thermal and secondary electron emission of "cold" electrodes, etc.

Valve hum is due to imperfect filtration of anode voltage on the one hand and the magnetic field of the heating filament on the other. In order to suppress it completely in the first stages of the most sensitive amplifiers, valves must be heated by D. C. current from a battery or a regulated rectifier.

Microphony. Microphony is due to mechanical vibrations of the valve. The vibration of the electrodes (especially of the grid) considerably changes the characteristics, which depend upon the electrode distances. In some of the newest valves microphony has been nearly completely eliminated. Others must be mounted resiliently.

Other interferences. In addition to the above mentioned, most obvious disturbing phenomena, attention must also be paid to other frequent sources of interference, which are most undesirable in electrometric amplifiers. In most cases they are due to the following: Secondary electron emission from the anode and the screen grid (see above). Thermal emission from the control grid especially in valves having a high transconductance, where the grid is very close to the heated cathode.

Leaks between the electrodes inside the glass envelope (these are often due to getter — a layer of reactive metals of groups I and II which ar eintroduced into the envelope to remove the last traces of gases) and also outside (dirt, moisture) or even in the glass (the leak is the larger, for the same glass properties, the larger the metal-to-glass seals).

An imperfect vacuum results in frequent collisions of the electrons coming from the cathode and going to the positive electrodes with gas mole-

cules. In this way other electrons and especially positive ions flowing to the negative electrodes (the cathode and the grid) are formed. The ion current flows in a direction opposite to that of the electrons. Ions cause most disturbances at the grid. They produce a negative grid current. With a high grid leak resistance, the grid is charged positively. This results in an increase in anode current and may also cause an electron (positive) grid current and secondary emission. Consequently the grid resistance for the usual circuits and valves cannot be increased above 2 MΩ (0·5 MΩ for the power valves). The grid currents (positive and negative, i. e. electron and ionic) may considerably affect the tissue between the electrodes and hence must be reduced to a minimum, particularly when working with single fibres or single cells. This will be discussed later. A strong ionic current also rapidly destroys oxide cathodes.

Elements of A. C. voltage amplifiers

Coupling of the amplifier stages. Frequency characteristic.

The majority of amplifiers are composed of several stages, the coupling of which must be discussed.

The input voltage is applied to the grid, which requires a constant D. C. bias for correct function. These two voltages, the signal and the bias, must be separated so as not to affect each other, particularly in order to avoid the bias producing a current flowing through the measured object. This may be achieved in two ways:

Fixed bias

A constant bias from a stable voltage source, e. g. a dry cell. This is directed through a large resistance (grid leak) to the grid. The other pole is connected to the cathode. The measured A. C. voltage is applied, through a condenser, also between the grid and the cathode. For the D. C. bias voltage the condenser represents an infinitely large resistance and it does not, therefore, influence the object. The measured voltage, however, is connected to a load (the grid condenser in series with the grid leak), which takes up energy from the object. This may be excessive, especially if the internal resistance of the object is too large, as in the case of single fibres, or when recording with microelectrodes. (In such cases some electrometric device must be used.) This energy is dissipated in the grid leak and in the valve (when the grid current is flowing). In addition, phase and frequency distortion occurs. This may be explained as follows: The condenser and the grid leak form a voltage divider (Fig. 37).

The first (upper) part of the divider — the condenser — produces a voltage drop depending on the frequency. The resistance R_g does not depend upon the frequency. Thus the amplitude and phase of grid voltage depend not only on the input voltage, but also on the latter's frequency.

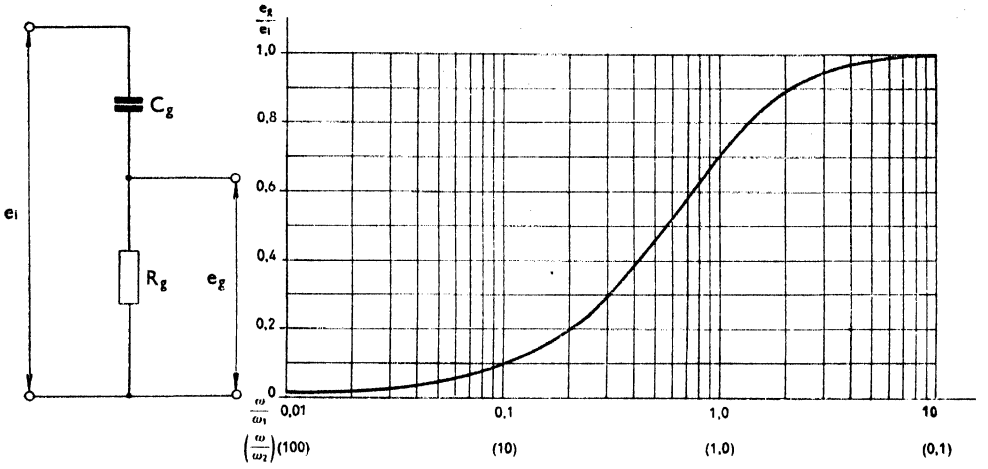


Fig. 37. Decrease in the amplitude of a low frequency signal caused by a grid condenser. Abscissa: relative frequency $f_r = \dfrac{\omega}{\omega_1} = \omega RC$. Ordinate: ratio of grid to input voltage $y = \dfrac{e_g}{e_i}$ (Meaning of symbols explained in text. The same curve is valid also for the upper limiting frequency ω_2 when using the lower abscissa with the scale $\dfrac{\omega}{\omega_2}$).

Lower limiting frequency

If the harmonic input voltage is e_i, the grid voltage e_g, its frequency f, the angular frequency $\omega = 2\pi f$, the phase shift of e_g to e_i is φ, and the grid leak R_g, then

$$e_g = e_i \frac{R_g}{Z} = e_i \frac{R_g}{\sqrt{R_g^2 + \dfrac{1}{\omega^2 C_g^2}}}\,; \quad \tan\varphi = -\frac{1}{C_g R_g}$$

and since the power $P = \dfrac{e^2}{R}$, then (after squaring and dividing by R)

$$P_g = \frac{e_g^2}{R_g} = \frac{e_i^2 R_g}{R_g^2 + \dfrac{1}{\omega^2 C_g^2}} \qquad (1)$$

Let us now find the frequency ω_1 for which the power at the grid falls to half its original value (calculated for direct coupling without the grid condenser).

Without the condenser

$$P_0 = \frac{e_i^2}{R_g}$$

With the condenser

$$P_g = \frac{e_g^2}{R_g} \quad (*)$$

$$P_g = \frac{1}{2} P_0, \quad P_0 = 2P_g, \; 2P_g = \frac{e_i^2}{R_g} \quad (**)$$

by division of (*) and (**) we obtain

$$e_i^2 = 2e_g^2$$

and by substituting in (1)

$$\frac{e_g^2}{R_g} = \frac{2e_g^2 R_g}{R_g^2 + \frac{1}{\omega_1^2 C_g^2}}$$

$$1 = \frac{2R_g^2}{R_g^2 + \frac{1}{\omega^2 C_g^2}} \qquad R_g^2 + \frac{1}{\omega_1^2 C_g^2} - 2R_g^2 = 0 ; \quad R_g^2 = \frac{1}{\omega_1^2 C_g^2}$$

$$\frac{1}{\omega_1} = R_g C_g ; \; \omega_1 = \frac{1}{R_g C_g} ; \quad T_1 = \frac{1}{f_1} = \frac{2\pi}{\omega_1} = 2\pi R_g C_g$$

This frequency, for which the power falls to $\frac{1}{2}$ and the voltage to $\sqrt{\frac{1}{2}}$ (i. e. both by 3 dB) is termed the lower limiting frequency or the lower half-power frequency f_1 and is defined by the product RC, the time constant (see p. 40)

$$\tau_1 = R_g C_g, \quad \text{i. e.} \quad \tau_1 = \frac{1}{\omega_1} = \frac{T_1}{2\pi}$$

Let us now consider the phase shift (see formula p. 37) for the limiting frequency

$$\tan \varphi_1 = -\frac{1}{\omega_1 C_g R_g} = -\frac{1}{\frac{1}{\tau_1}\tau_1} = -1 ; \quad \varphi_1 = -\frac{\pi}{4} = -45 \text{ degrees}$$

The distortion for the limiting frequency follows from the above equations: The amplitude decreases by 3 dB and the phase is shifted by one eighth of the period. Both values change much more for lower frequencies. When amplifying harmonic voltages only the amplitude distortion is of importance. Usually 3 dB is considered as a permissible limit. When amplifying periodic non-harmonic voltages which, according to Fourier, may be considered as composed of many harmonic components with definite amplitude and phase relations, the phase shift for the limiting frequency may cause a considerable distortion of the wave form, if this frequency is one of its important components. In this case, the demands on eliminating phase shift are much greater than in the

former case. This conclusion holds generally, i. e. also for the upper limiting frequency (see p. 105).

Let us repeat: The size of the time constant in the grid circuit is chosen according to the lowest frequency transmitted in such a way that:

$$T_1 = \frac{1}{f_1} = \frac{2\pi}{\omega_1} < \tau_1 = R_g C_g$$

R_g is chosen as large as the valve permits, especially its vacuum, thermal grid emission, leaks between the electrodes and the condenser leak (see p. 101 and p. 105).

Self bias (cathode bias)

The measured voltage is again applied to the grid through the condenser, not against the cathode but against the frame (earth). The grid is connected through the grid resistor R_g with the frame and not with the bias source. Bias is obtained by a voltage drop across the cathode resistor R_c, caused by cathode current. This positive cathode bias is, of course, equivalent to the negative grid bias (Fig. 38). This method has several advantages and disadvantages: Advantages: No special highly stable bias source is necessary. Considerably larger grid leaks may be used. Disadvantages: Amplification is decreased by the negative feedback: For a more positive grid voltage a larger cathode current is flowing and a larger voltage drop is formed across the cathode resistance. The cathode is thus more positive to the frame and grid, i. e. the grid more negative to the cathode, as compared to the grid-cathode potential without the signal. Thus the positive change in grid voltage is decreased by the corresponding change of voltage drop across the cathode resistance, which is proportional to the signal. This negative feedback decreasing the sensitivity can, however, be suppressed by preventing the cathode voltage from varying and maintaining a constant average cathode voltage. This is achieved by connecting a condenser C_c in parallel to the cathode resistor. The condenser is alternately charged and discharged and thus maintains a more or less constant cathode voltage (analogously to a filter condenser in an anode supply rectifier). A detailed analysis shows that, as for the grid condenser, the size of the cathode condenser is defined by the time constant $\tau_1 = R_c C_c$ (the cathode resistor R_c and condenser C_c). The time constant determines the limiting frequency for which the decrease in amplitude by 3 dB and the phase shift by $\frac{T_1}{8}$ (i. e. 45 degrees) occurs. Since, however, R_c is small (hundreds of Ω), the necessary capacitances are usually large and often even tremendous for frequencies used in physiology: tens to thousands of μF, which is difficult to obtain.

Interstage coupling

The coupling of the anode circuit to the following stage is again achieved using a condenser which is connected between the anode of the first and the grid of the second valve (Fig. 38). Mathematical considerations are somewhat more complicated, but since the anode load (R_a) is usually much smaller than the grid leak (R_g) it is possible, as far as transmission of the lower end of the

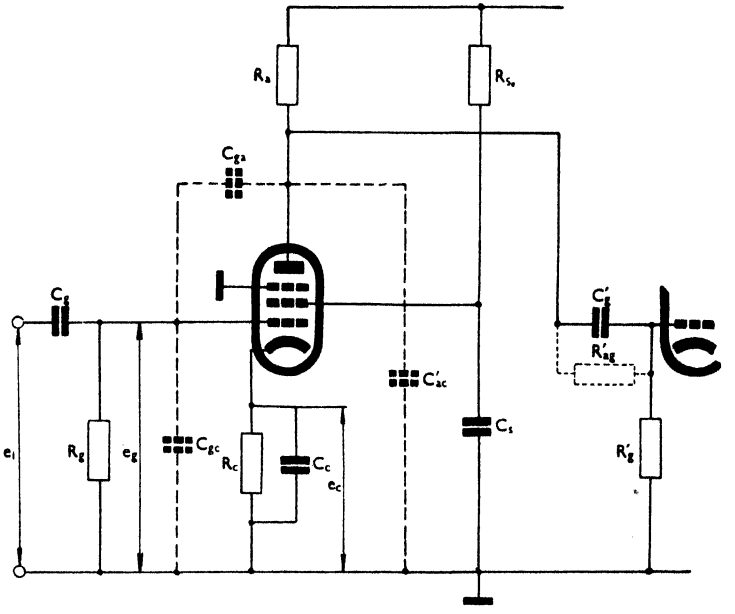


Fig. 38. Coupling between amplifier stages. For desription see text.

frequency range is concerned, to consider the time constant of the grid circuit of the following stage as essential. It is important, however, that the condenser C'_g be connected between the high positive potential of the anode and the negative grid. Since every condenser also has a leakage resistance R'_{ag}, it may happen that the grid obtains an evident positive potential which considerably worsens its function, particularly when using a fixed grid bias. This usually occurs when using very large condensers of inferior quality and large grid leaks.

Upper limiting frequency

The upper limit of the transmitted width is determined by a decrease in the amplification due to stray capacitances of the valve electrodes and the conductors carrying current to them. These are a) the grid-cathode (and grid-

frame) capacitances, b) the anode-cathode (also anode-screen grid and anode-frame) capacitances. Both are usually connected in such a way that the grid-cathode capacitance is actually connected in parallel to the anode-cathode capacitance of the preceding stage and they thus are added. The resulting capacitance C'_{ac} is then connected in parallel to the valve. It is charged or discharged with every change in anode current. Changes in anode voltage are thus delayed with respect to the grid voltage by this capacitance (resulting in a phase shift). At the same time amplitude distortion occurs. Again the time constant τ_2 determines these effects:

$$\tau_2 = R_{eq}C'_{ac} = \frac{R_i R_a R'_g}{R_i R_a + R_i R'_g + R_i R'_g} (C_a + C'_{gc})$$

where τ_2 is the time constant, R_{eq} the equivalent resistance for the anode resistance (R_i), anode load (R_a) of the first valve and for the grid leak resistance of the following valve (R'_g) connected in parallel. C'_{ae} is the equivalent capacity for the parallel connection of the anode-earth capacitance of the first (C_a) and the grid-earth capacitance of the subsequent valve (C'_{gc}), i. e. the leak (parasitic) capacitance (C'_{ac}) of the anode circuit. The decrease by 3 dB occurs at the upper limiting frequency or upper half power frequency

$$\omega_2 = \frac{1}{\tau_2}$$

The voltage falls to 70·7% or the power to 50% with respect to the medium frequencies (for which neither this decrease due to leak capacitance nor the decrease due to the grid condenser described above play a part). The phase shift of the output voltage from the input voltage is $+\frac{T}{8}$ or + 45 degrees, i. e. numerically the same as the distortion due to the input condenser C_g for the lower limiting frequency, but of opposite sign.

The above relations are important for designing the amplifier. Consider, for instance, that $C'_{ae} = C_a + C'_{gc}$ is at least 100 pF (often much more) and that it cannot be decreased to any considerable extent. If the anode resistance of the pentode is completely utilised, this being several MΩ, and if $R_a = R_i = R'_g$ then for

$$R_a = 3\ \text{MΩ},\quad R_{eq} = 1\ \text{MΩ}\quad \text{and}\quad \tau_2 = 10^{-10} \cdot 10^6 = 10^{-4}\ \text{sec.}$$

and thus $\omega_2 = 10^4$ and $f_2 = \frac{\omega_2}{2\pi} = 1600$ c/sec. which is often a value that is too small. Consequently (and also in order to reduce the anode supply voltage) a considerably smaller anode load is chosen — about 0·1 MΩ. Then $R_a \ll R_i$ and $R_a \ll R_g$ and thus $\tau_2 \approx R_a C'_{ac}$. The capacitances C_g and C'_{ae} can often be changed by connecting several suitable condensors with a selector switch. Thus the lower (by high pass filters) and upper (by low pass filters) limiting

frequencies can be controlled in order to reduce noise and interference by reducing the bandwidth of the amplifier. More selective filtering can be attained with *LC* filters (Terman 1943, Smirenin 1950).

Screen grid circuit

For pentode amplifiers a further factor affecting the frequency characteristic must be considered. This is the screen grid circuit. The screen grid has a lower potential than the anode. The potential difference is usually obtained by a drop across screen grid load due to the screen grid current. The screen grid current, however, varies with the control grid voltage, as does the cathode current considered previously. Thus negative feedback arises again, as at the cathode resistor. It can be simply suppressed by blocking the screen grid. Again $\tau_{s_1} = R_s C_s$ where $R_s = \frac{R_{si} R_{se}}{R_{si} + R_{se}}$ is the equivalent resistance for the parallel connection of the screen grid resistance (R_{si}) and of the external screen grid load (R_{se}); C_s is the blocking condenser. It is necessary that

$$\tau_{s_1} \gg \frac{1}{\omega_1}$$

(ω_1 is the lower limiting frequency), otherwise phase shift and amplitude distortion occur at low frequencies.

Various types of A. C. voltage amplifiers

1) We have now described the most important amplifier circuit, the single-ended amplifier with resistance-capacitance coupling ("the *RC* amplifier"), which is very often used in electrophysiology. Triodes are usually used for the first stage of the most sensitive amplifiers. Pentodes are used for the further stages.
2) In the *LC*-and *RLC*-coupled amplifiers, an inductor is used instead of the anode resistor. It is very rarely used in electrophysiology.
3) Transformer coupled amplifiers use the primary winding of a transformer as the anode load. The grid of the following stage is connected to the secondary winding.
4) Tuned amplifiers use resonant circuits, tuned transformers, band-pass filters etc., instead of anode load (or grid leak). They transmit only a very narrow band width.

The amplifiers described above are single ended. They amplify a voltage applied between the grid and the cathode.

5) Balanced, symmetrical or push-pull amplifiers. Both valves of any stage of a push-pull amplifier are connected in exactly the same way. The amplification is only double that obtainable with one valve, but the stability of the amplifier is considerably increased. This circuit is used in nearly all EEG amplifiers and most D. C. amplifiers. For more detailed description of its operation see p. 116.

Power amplifiers and impedance transformers

The voltage amplifiers described above also amplify power, which is not consumed, however, since the input impedance of the following stage is always considerably larger than the output impedance of the preceding one. For some purposes it is necessary to transform an electric signal into the mechanical movement of an ink writer. It is then necessary to amplify the power and not the voltage. The output impedance of the amplifier must be matched to the input impedance of the ink writer (see p. 135). In this case the power amplification is maximum and may attain about 25% for triodes and about 50% of the anode dissipation for pentodes. The anode dissipation is the heat produced by electrons hitting the anode. Since the work performed by one electron is equal to its kinetic energy (proportional to the accelerating anode voltage V_a) and the number of electrons is determined by the anode current I_a, anode dissipation is equal to $I_a \cdot V_a$. Calculating and designing a power amplifier is comparatively difficult. Many insufficiently defined or variable factors must be considered and an approximate solution must be found fulfilling many conditions, some with more and some with less accuracy. Up to recent times nearly all power-amplifiers were inductance-coupled (by transformers and chokes) if the load impedance, i. e. impedance of the recording system, was low. For purely resistive anode load, capacitance coupling was used. Both approaches were difficult and awkward, especially for wide-band power amplifiers with a very low lower limiting frequency. The necessary inductances and capacitances were large and it was usually necessary to wind and test several large and expensive transformers. Only recently have direct-coupled power amplifiers been constructed, using anode loads of medium size (10^2 to 10^3 Ω). These undoubtedly will be used frequently in electrophysiological work.

Electrometer amplifiers

Power amplifiers may be regarded as impedance transformers since their input impedance is much larger than their output impedance. Hence they can also be used for matching impedances, if no amplification is required.

Often, however, demands for this are high: to attain input impedances of $10^8 - 10^{10}\,\Omega$ and more. In other words: the input signal of 0·1 V (the maximum usual in electrophysiology) may cause a maximum grid current of $10^{-9} - 10^{-13}$ A. This is usually solved by using special electrometer circuits with electrometer valves. If demands are not extreme, however, satisfactory results may be obtained with conventional valves and, on the contrary, even with special valves success is not guaranteed if care is not taken to avoid many pitfalls. Consequently, only the most usual circuits will be described and more attention will be paid to how to avoid failure.

Cathode follower

The most usual electrometer circuit is a cathode follower (Fig. 39). The input voltage is applied to the triode grid, which is connected to the negative pole of the anode voltage supply through a very large resistance R_g. The anode is connected to the positive supply pole directly without any intervening impedance. The load resistance R_c is connected in the cathode and is much larger than the usual cathode resistance used for obtaining grid bias (see p. 104), usually 10^4 to $10^5\,\Omega$. With a zero grid voltage a cathode current flows. This produces a drop across the cathode resistor so that the cathode has a positive potential. The grid is thus considerably negative to the cathode, since the cathode resistance is very large. The cathode current is thus very small and the cathode voltage follows the changes of grid voltage. It is in phase with the latter and has nearly the same amplitude — hence the term cathode follower. The cathode follower does not amplify the input voltage, but decreases it. Its output impedance, however, is very small. If an external load is connected in parallel to the cathode resistor, the cathode voltage decreases and the grid becomes less negative to the cathode. This increases the cathode current and consequently also the voltage drop across the cathode resistor. In

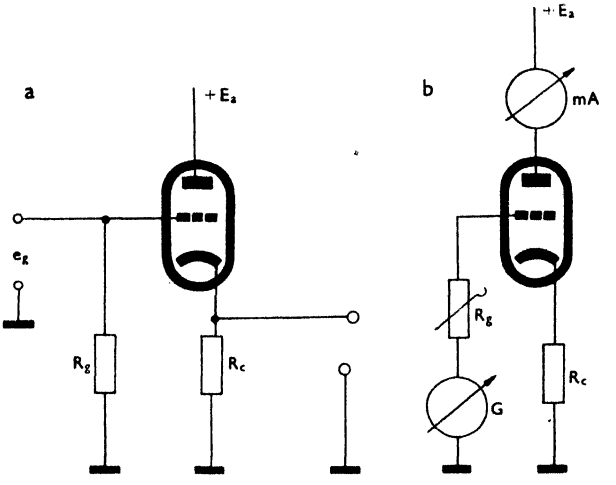


Fig. 39. Cathode follower. a) basic circuit. b) measuring grid current.

other words: the effect of connecting a load in parallel to the cathode resistor is compensated for to a considerably extent by a negative feedback, so that the effective internal resistance of the cathode follower is considerably smaller than its cathode resistor. As already stated, the input voltage is always larger than the output one. For the amplification to be large (in reality for the decrease to be small) valves having large transconductance must be used.

Cathode follower input

Conditions at the amplifier input are very important. The input resistance must be as large as possible. The grid is connected not only to the measured voltage, but also to all other electrodes of the valve through the resistance of the glass envelope and by the tube-element capacitances. In addition, different e. m. f. are connected to it (e. g. thermal emission of electrons). Let us now only consider those currents which may influence the grid, when the grid voltage $e_g = 0$ and the ways in which each of them, and thus also their sum, may be reduced to less than 10^{-12} A. The following currents are mainly concerned: the insulation leakage current between the electrodes, electron grid current, ionic grid current, thermionic grid emission (see Fig. 40).

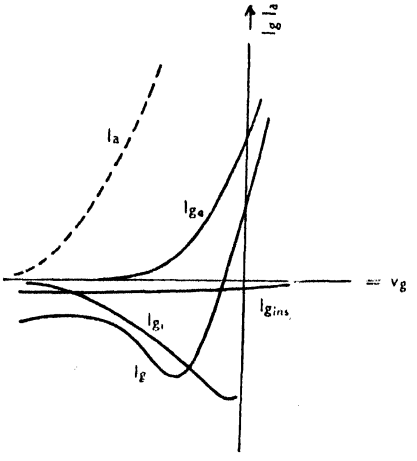


Fig. 40. Grid currents of triode (Tsarev 1953). Abscissa: grid voltage. Ordinate: current. I_a — anode current (interrupted line), I_g — total grid current, I_{g_e} — electron grid current, $I_{g_{ins}}$ — leakage grid current, I_{g_i} — positive ion grid current.

1) Currents due to interelectrode leakages. a) Anode-to-grid leakage R_{ag}. For $E_a = 100$ V, R_{ag} must be more than $10^{14}\ \Omega$, for $E_a = 10$ V, $R_{ag} > 10^{13}\ \Omega$. The current I_{ag} flows from the anode to the grid. This current direction (to the grid) will be termed negative. b) Cathod e-to-grid leakage — R_{cg}. The current direction is the same, its size depends on the potential difference between the grid and the cathode. This may vary but is always smaller than E_a.

2) Electron grid current. It flows from the grid to the cathode and is thus positive. It increases exponentially with increasing grid voltage $I_{ge} = kI_k^{\alpha e_g}$ (the proportionality constant k depends on the distance of the grid from the cathode, on the grid structure, α depends mainly on the temperature of the cathode). If this current is to be maintained at the lowest possible value then a) the valve must not have an extremely

high transconductance, b) R_c must be as high as possible, c) heating voltage must be decreased.

3) Ionic grid current. This flows to the grid and is thus negative. Its absolute size depends mainly on the gas pressure p and the anode current. $I_{gi} = c \cdot p \cdot I_a$ (c mainly depends on the kind of gas). It is important that this current occurs only at a certain V_a, when the kinetic energy of the electrons suffices to ionise the gas molecules.

4) Thermionic grid emission again produces a negative grid current and depends on the material of the grid and its surface impurities, on the cathode-to-grid distance and on the temperature of the latter. It is very effectively suppressed by underheating the valve.

Valve selection

An analysis of all kinds of grid currents gives the following results:

1) The valve must have

a) the best possible insulation of the grid and of other electrodes,

b) the best possible vacuum,

c) electrodes not soiled with getter or cathode paste and if possible, specially prepared (gilded grid, ground cathode, etc.),

d) a grid not too close to the cathode.

In addition to special electrometric valves, older types with fine electrode leads inside the envelope and the external grid terminal on the top of the valve come up to these requirements. The valve, and especially the base, must, however, be thoroughly cleaned and all screening varnish, dirt (soot) and ions (from the soldering paste) must be removed carefully.

Individual pieces must be chosen with care, since differences in the above parameters may be very great.

2) It is advantageous to underheat the valve slightly.

3) The circuit must be arranged in such a way that the grid currents of different directions, all decreased to a minimum, also cancel each other as far as possible. Different currents are different functions of the main variables (e_g, I_c) and parameters (E_a, R_c, p, T, and perhaps the potentials of other electrodes). Consequently, compensation cannot be obtained for the whole range of operation. It is necessary to maintain all individual grid currents at very low absolute values and to compensate for $e_g = 0$, when the same absolute error due to grid current results in the largest relative error in e_g.

It is best to proceed as follows: From a larger supply of valves, with the bakelite bases, top caps and screening varnishes removed, all those having a large anode-to-grid leak are excluded. The leak is measured in the cold valve with a voltage of 500 V and a sensitive galvanometer. All connections and the galvanometer must be carefully insulated, otherwise the surface resistance

TABLE 2

A valve NF2 in a cathode follower with cathode load 775 kΩ

E_a(V)	I_a(μA)			I_g(10^{-9} A)		Heating voltage
	Rg = ∞	Rg=50 MΩ	Rg = 164 Ω	Rg=50 MΩ	Rg=164 Ω	
0	—	—	—	15	—	
0	—	—	—	14	—	
3	0	0·8	7·5	+13	—	
4·5	0	1·6	8·3	+11	+129	
6	0	3·2	9·2	+10	+ 60	
10·5	0·2	8·8	11·5	+ 4	+ 7	9 V
19·5	3·0	16·8	16·8	+ 0	+ 0	
30	cca55	22	22	— 0	— 0	
60	400	35	35	— 0	— 0	
80	660	47	47	— 0	— 0	
120	—	68	68	— 0	— 0	
10·5	0·1	6·1	8·3	+ 2	+ 5	
19·5	2·5	12·2	12·2	0	+ 0	6 V
30	25	16·1	16·1	— 0	— 0	
10·5	1·6	10·4	15·0	+ 4	+ 14	
19·6	17·6	20·4	20·4	+ 0	+ 0	12 V
30	100	26·4	26·4	— 0	— 0	
10·5	0·9	12·0	18·0	+ 8	+ 21	
19·5	13	22·8	22·8	+ 0	+ 0	15 V

of the working table may be measured instead of the valve leak. This possibility must always be checked. The significance of removing the base and the screening varnish can be seen from the following measurement: A valve NF2 had an anode-grid leak $1{\cdot}5 \cdot 10^{10}$ Ω, after removing the base and washing off the screening varnish with acetone $5 \cdot 10^{11}$ Ω. After thorough drying in an oven at 60°C, the leak resistance rose to $5 \cdot 10^{12}$ Ω. Thus the resistance increased 300 fold altogether.

The vacuum is checked in the remaining valves. This is done by measuring the difference ΔI_a for a high anode voltage (at least 200 V) by which the anode current I_a increases when a large resistance 10—100 MΩ or more is inserted into the grid lead connected directly to a fixed bias of about—2 V. The positive ions are attracted by the negative grid and on inserting a resistance they accumulate at the grid, charge it positively and thus increase the anode current. If the characteristics of the valves are reasonably equal, then the value $\frac{\Delta I_a}{I_a} = c \cdot p$ is a measure of the gas pressure in the valve as the reader can easily demonstrate himself (see p. 111). It is suitable to measure with the maximal catalogue values of I_a, which must be the same for all valves. Usually all valves with a measurable positive $\Delta I_a/I_a$ (due to the ionic current)

as well as those with a negative $\Delta I_a/I_a$ (caused by excessive electron current) should be discarded. In pentodes it is often better to measure ionic current in the suppressor grid with a sufficiently large negative voltage at it.

The selected valves are connected in the cathode follower one after the other, and the total grid current is measured for different anode voltages with a very sensitive galvanometer (Fig. 39b). At the same time the anode current is also measured with a less sensitive instrument. The heating voltage is usually chosen as half to two thirds of the catalogue value. The measurements are arranged into a table for each valve as shown in Tab. 2. Valve NF2 connected as a triode in the cathode follower with R_c 75 kΩ. For circuit diagram see Fig. 39b, heating voltage 9 V.

Let us analyse the table in detail. With zero anode voltage, the initial "diode" current of the valve is measured. At a low anode voltage, only a positive electron grid current and a thermionic emission (negative) current are flowing through the valve. The ionic current is not flowing, since the kinetic energy of the electrons is not sufficient to ionise the gas. The positive grid current greatly predominates. Hence for $R_g = \infty$ (i. e. the grid disconnected) or even $R_g = 50\,\mathrm{M\Omega}$, the grid voltage falls considerably (grid is charged with electrons) and the anode current decreases. From 4·5 V, however, the grid current decreases and so does its effect on the anode current, because the electron grid current falls to some extent with rising anode voltage, as electrons more attracted by the anode miss the grid more easily and also because an ionic current begins to flow. Between 20 and 30 V the positive and negative grid currents are in approximate equilibrium and therefore the total grid current is zero. With higher E_a the ionic current continues to increase. This is seen indirectly from the large increase of I_a for $R_g = \infty$. E_a is chosen in such a way that $I_g = 0$ and I_a is constant for any R_g or only falls slightly for $R_g \to \infty$. Valves for which this E_a is too low are discarded. The rest are grouped according to the V_a. For a balanced amplifier (see p. 115) valves with I_a values closest to each other are selected in pairs from the above groups.

Cathode follower construction

First all components must be perfectly insulated. The leakage of the condensers (if used) must be very small, the valve envelope clean. If requirements are very strict, a drying substance (P_2O_5, silicagel) is placed into the apparatus box.

The input and output of the follower may be connected as an A. C. amplifier with capacitance coupling or a D. C. amplifier with direct coupling. In the A. C. circuit the grid leak resistor is usually also used to prevent charging of the grid with the grid current; it is not connected, however, to the frame, but to the tape of the cathode resistor. Its equivalent value then exceeds

several times its nominal value and must be larger than the maximum source resistance R_i, i. e. 10^8—$10^{11}\,\Omega$. In D. C. amplifiers the grid resistor is usually omitted.

Frequency response of the cathode follower

The upper limiting frequency and losses due to parasite capacitances, especially the grid-to-anode, grid-to-cathode and grid-to-frame capacitances, must now be considered. They can be wholly substituted by a single grid-to-frame capacitance C which is connected as a load to the measured source. Its apparent resistance Z connected in series to the internal resistance of the source R_i represents (as mentioned on p. 105) a branch of a frequency dependent divider. The voltage across Z falls with the frequency and for

$$\omega_2 = \frac{1}{R_i C} = \frac{1}{\tau_2}$$

decreases to 0·707 of the source voltage. It is difficult to decrease C below 10 pF. If R_i is again $10^8\,\Omega$, the upper limiting frequency

$$f_2 = \frac{1}{2\pi\, 10^8 .\, 10^{-11}} = 160 \text{ c/sec.}$$

It can be seen that the upper limiting frequency is relatively low*). The input resistance of the cathode follower is then parallel to this capacitance and because of its high value it does not affect the limiting frequency.

Nevertheless an attempt is often made to obtain even higher input resistances for followers. This is so because the loading of an electrophysiological source due to the grid current of the measuring instrument is qualitatively quite distinct from loading the source with the input capacitance. A grid current, especially in a direct coupled instrument, evokes polarisation of structures, i. e. changes their physiological state, while the loading with the input capacitance usually causes distortion of the shape of the measured potentials, suppression of the higher frequency components and changes in the phase relations between the different components. The above also indicates how to connect the cathode follower with the tissue and the next stage. The input capacitance must be decreased as much as possible. A valve is chosen with the lowest capacitance of the grid to the other electrodes, i. e. a physically small valve, and this is brought as close as possible to the measured object so that the grid lead is only a few centimeters long. The grid connection is usually not screened. If screening is necessary, then the whole cathode follower and the measured object must be screened together, or the grid lead screening is con-

*) By modification of the grid circuit, however, the input impedance (including also the input shunting capacitance) can be considerably increased. For details see e. g. Gray 1954.

nected to the cathode. Sometimes, however, the object must be screened from the heat produced by the valve. Insulating opaque screens (not metallic sheet) are used in such cases. All connections in the grid circuit are as short as possible and of thin wire. A longer screened cable is used to connect the follower to further stages of the amplifier. This cable also carries the supply voltages. Heating is usually D. C. from the accumulator, the anode voltage is also usually taken from the battery and is very low.

Balanced cathode follower

In the same way as the usual triode or pentode amplifier with an anode load, the cathode follower also has an A. C. output voltage, superimposed upon a D. C. voltage, produced by the flow of the mean cathode current through the cathode resistance. A coupling condenser may also be used to separate the D. C. voltage. It is, however, adavantageous to use a pushpull circuit, where the input voltage is applied between the grids of symmetrically connected valves and the output voltage is taken from between their cathodes (Fig. 41a). Thus the output voltage is symmetrical with respect to the centre of the cathode resistances and a coupling condenser is no longer necessary in the output. For a balanced cathode follower, the connection may also be made as follows: cathode currents may be increased while maintaining high cathode resistances and a low anode voltage by either not earthing the anode voltage supply at all ("floating anode voltage") or by earthing a certain positive potential lying somewhere between the positive and negative supply poles, instead of connecting the negative supply pole to the common joint of the grid resistors with the frame.

This gives the frame, i. e. the grids, a positive bias, which is compensated for by an increased voltage drop across the cathode resistor, i. e. by an increase in cathode current.

When building a balanced follower, identity of the valves is essential. It is necessary 1) to obtain with the same transconductance of the valves and with the same cathode resistances exactly equal cathode currents. This may be achieved by a) correct choice of valves, b) proper choice of the operating parameters (size of R_c, E_a, I_c, i. e. earthing of a certain potential, see above), c) adjustment of the valve characteristics, e. g. by using tetrodes or pentodes instead of triodes, and by changing the second grid voltage with a potentiometer in such a way that the cathode currents are exactly equalised (Fig. 41b). The same effect may be obtained by adjusting the heating of the valves. If the substrate is to be affected minimally, all that has been stated on the preceding pages concerning grid currents must be respected. If the grid currents of the two valves are equal, they cancel each other. Nevertheless it is best

to maintain each at its lowest value, avoiding procedures that might also change other valve characteristics. It is better to measure each valve separately and not only the resultant current in the input circuit. New valves must not be used. They should be aged artificially; according to requirements, valves are aged for 100—1000 hours with the catalogue current. They are then permit-

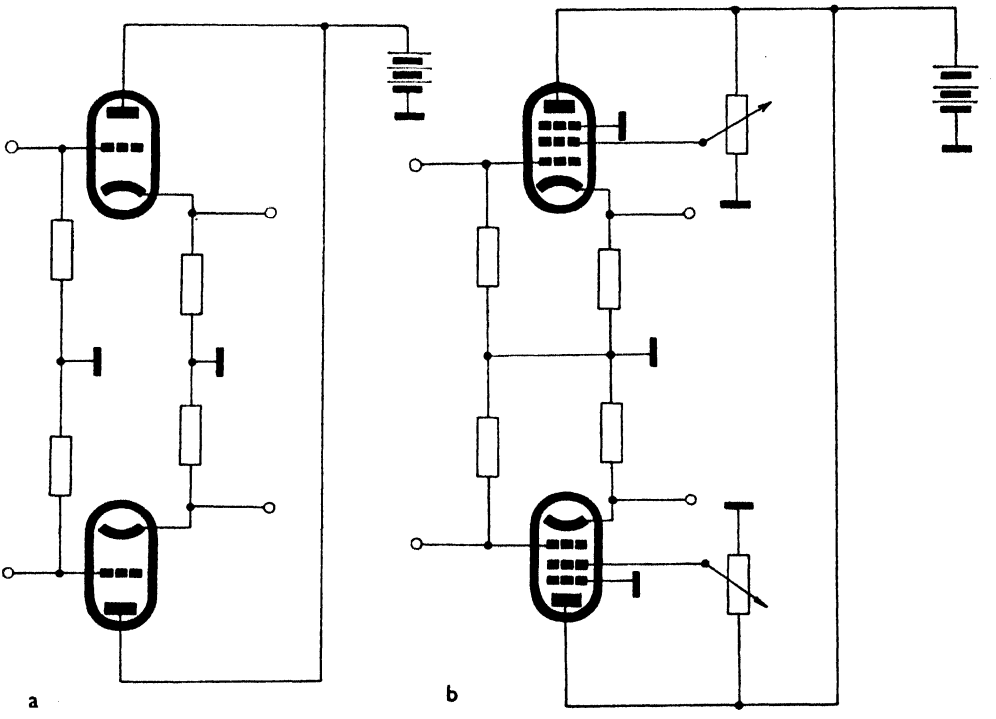


Fig. 41. Balanced cathode follower. a) basic circuit. b) equilibration of valves by changing the voltages at the screen grids.

ted to rest for several days and again used for about 50 hours with such a voltage and current that will be used later. With such precautions, valve characteristics change only little and slowly. The first aging stabilises the cathode. During rest and the second aging with a considerably smaller current, the vacuum is improved.

D. C. amplifiers

The causes limiting the transmitted bandwidth from below and the significance of the time constant have been discussed when describing A. C. amplifiers. If the amplifying stages are coupled together, amplitude and phase

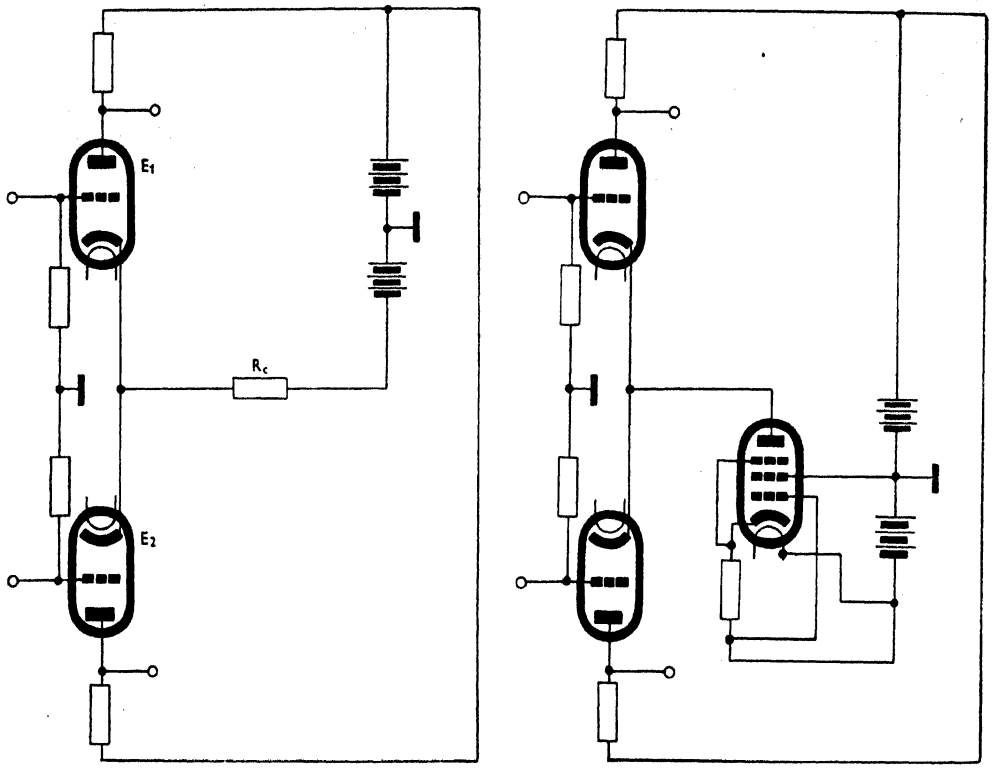


Fig. 42. D. C. amplifiers. a) increase in cathode current with a negative cathode bias. b) pentode as cathode resistance.

distortions occur at each stage (or even several times in each stage — at the control grid, cathode and screen grid). Thus the time constant τ_1 of the whole cascade amplifier is of necessity much shorter than any of the time constants τ_{1i} of the individual stages. Approximately,

$$\frac{1}{\tau_1} = \frac{1}{\tau_{11}} + \frac{1}{\tau_{12}} + \frac{1}{\tau_{13}} + \ldots + \frac{1}{\tau_{1n}}$$

It can be shown that it is best to choose $\tau_{11} = \tau_{12} = \ldots \tau_{1n}$. Then $\tau_1 = \frac{\tau_{11}}{n}$.

For obtaining the lowest limiting frequency, n must be as small as possible, i. e. the number of capacitances must be small. These have two functions: to prevent the occurrence of negative feedback (C_c, C_s) and to separate the A. C. from the D. C. component (C_g). Fig.42a shows a balanced amplifier without condensers. It is relatively easy to do without the first kind of capacitances. The negative feedback at the cathode is avoided as follows: If the grid voltage of

one valve rises, the cathode current in this valve increases and this should result in an increase in cathode voltage (i. e. grid bias drop). This is the basis of negative cathode feedback. Since the cathode resistor R_c is common for both valves, the grid voltage in the second valve falls at the same time. Thus its cathode current also decreases, and if both valves are identical, the common cathode current remains nearly constant as does the drop across the common cathode resistor. Consequently, no negative feedback occurs. The negative feedback in the screen grid circuit is eliminated in the same way, i. e. by a common resistor (Fig. 43, R_s).

Discrimination

It is evident that this circuit is much more sensitive to a difference in voltage between the grids than to a difference in voltage between the two "connected" grids and any point on the axis of symmetry of the circuit (e. g. earth). Thus if a positive voltage is applied between the connected grids and the frame, the cathode current of both valves increases, resulting in a rise of the drop across the common cathode resistor, which limits the increase in cathode current. In this case a negative feedback does occur, which is the larger the larger the common cathode resistor. This negative feedback is important not only for the common grid-to-frame voltage changes, but also for the anode voltage variations, which are even more essential.

It is therefore best to choose a high common cathode resistor and screen grid load. It is usually necessary to compensate for the increased cathode drop by using a high negative cathode voltage supply, as described for the cathode follower, and often also a high anode voltage. This prevents the cathode current from falling below an acceptable value. It can be seen that the role of the cathode resistor is to maintain a constant current. The current will be the more constant the greater the role of the constant cathode resistor in comparison to the variable valve resistances. If a common current of 1 mA, which is relatively small, must flow through the valves, an E_c of 1000 V is necessary for $R_c = 1\ \mathrm{M\Omega}$, which is excessive. Hence occasionally a current stabiliser is used (usually a pentode) instead of the high R_c. Let us recall the description of the pentode, the anode current of which does not depend on the anode voltage. In the circuit shown in Fig. 42b, a common cathode current from both amplifying valves flows through the pentode, which has a more or less constant control and screen grid voltages and thus a constant anode current independent of the anode voltage, i. e. independent of the anode resistances of the amplifying valves. For E_c equal to about 100 V, the current stabilisation by the pentode is about the same as that achieved with a 2 MΩ resistor, which for the same current of 4 mA would need an E_c of 8000 V. Although this connection has

many advantages, it is used only little, since often an R_c of $10^4 - 10^5\,\Omega$ is sufficient and also because the pentode has considerable noise, ages, complicates the power supply (heating has a high potential against the frame), etc.

Up to the present, the circuit behaviour has been described when connecting the measured voltage symmetrically between both grids. As was shown, the potential difference between the "electrical centre" of the grids and the frame is hardly important. If a certain potential difference against earth is to be measured, one grid is connected to earth, the frame is also earthed and the second grid is connected to the measured potential. The asymmetry of this connection is automatically eliminated by the negative feedback across R_c, so that the output voltage is again symmetrical. If the input signal is applied between earth and both grids connected together (i. e. in-phase in both valves), the output voltage is less by several orders of magnitude than with the same signal applied between the grids (i. e. in anti-phase in both valves). The ratio between theese two output voltages is the discrimination ratio.

Interstage coupling in D. C. amplifiers

One stage of a direct coupled amplifier has been described. The lower limiting frequency of such an amplifier is evidently 0 c/sec., and hence it is termed D. C. amplifier. All that has been said for *RC* amplifiers holds for the upper limiting frequency. If a multistage D. C. amplifier is to be constructed, the coupling between the stages remains to be designed. It was shown that a high negative cathode voltage must be supplied to the amplifier in order to compensate for the drop across R_c. The same compensation may be obtained with a positive voltage supplied to both grids. For coupling the first and second stage, such a positive voltage is at hand: the anode voltage of the first stage (Fig. 43). Thus grids of the 2nd stage are connected directly to the anodes of the first and their positive D. C. voltage is compensated for by a large R_c. The third stage may be coupled in a similar way. This is more difficult for the 4th stage, which then requires a larger R_c and higher anode supply voltage. This complicates the power supply considerably. In a four stage amplifier it often suffices, however, to arrange the first two and the second two stages as two D. C. amplifiers and to couple those with a condenser. The time constant of this single coupling can be maintained at a high value much more easily than one composed of many separate time constants, particularly since R_g is very high and hence a relatively small C_g is sufficient.

The arrangement of a multistage D. C. amplifier used for the most delicate work is thus the following:

1) A D. C. cathode follower with battery supply. This may be disconnected. Amplification 0·8.

2) A D. C., battery-supplied triode preamplifier directly coupled with the cathode follower. When working without the follower, the E_c must be increased. Amplification 50.
3) The first mains-supplied D. C. pentode stage. Amplification 200.
4) The second mains-supplied D. C. pentode stage. Amplification 200.

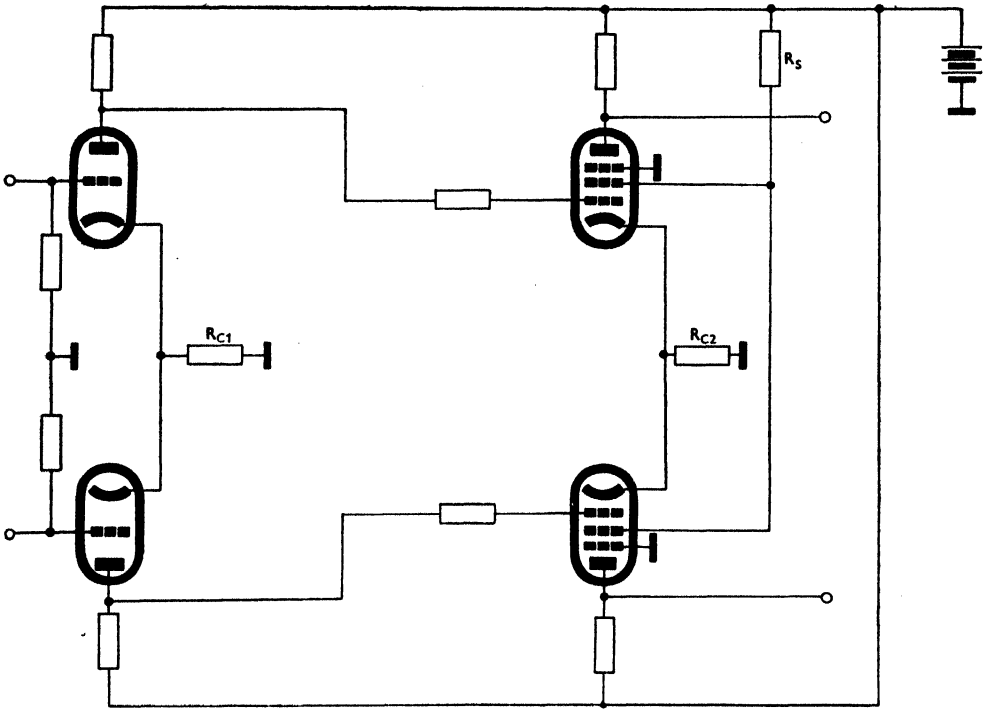


Fig. 43. Two-stage D. C. amplifier.

5) The output is connected a) to the cathode ray tube. For a total amplification of 2×10^6 and a sensitivity of the screen 1 mm/V the total maximum sensitivity is 2 mm/μV, b) or to the D. C. power amplifier operating a mechanical recording system.

Valves for the D. C. amplifier must again be carefully selected in exactly similar pairs, especially for the first stage. Valves with the lowest microphony must be taken. Some firms supply paired valves, and it is worth while paying extra for these. The stability of the zero position is the most important criterion of the quality of a D. C. amplifier. This is expressed as the ratio of the equivalent input voltage which would cause the same change in the output voltage as is produced by the amplifier instability to the time during which this shift occurs. Zero stability is measured (and amplifiers may be used) only after stab-

ilisation, which occurs only a long time after switching on (15 min. to 3 hours). The stability of zero is most affected by variations in heating voltage in the first stages. Hence the heating supply must also by carefully regulated. This is usually attained by the use of a large by-pass accumulator (which is constantly recharged by the rectified and filtered mains heating supply).

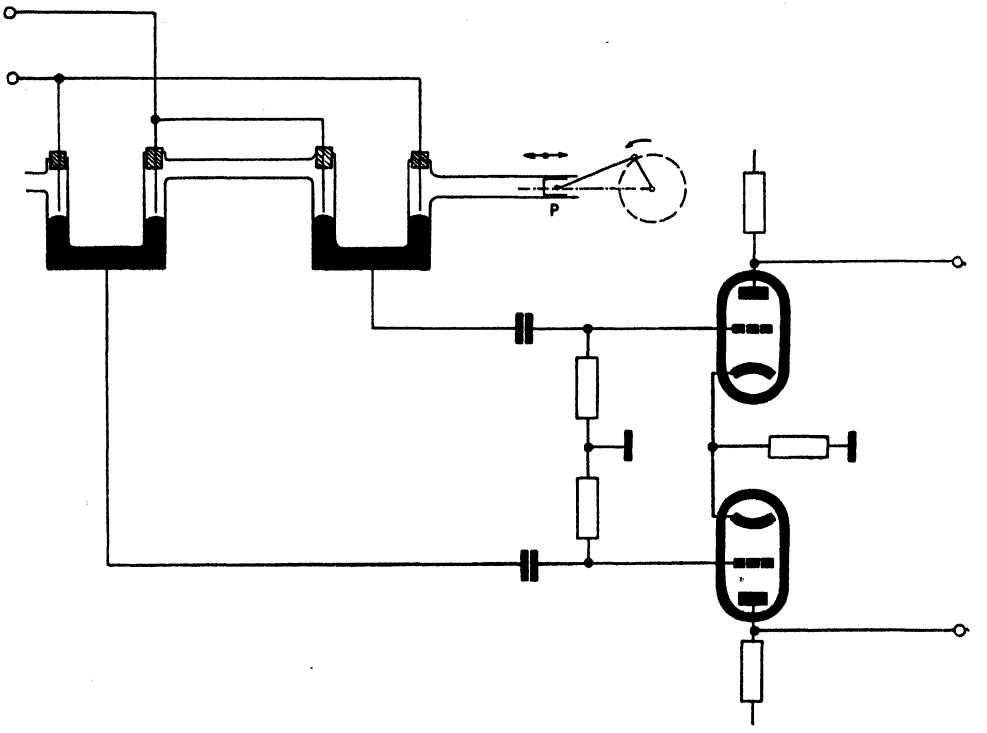


Fig. 44. Simple mercury commutator for amplifying D. C. voltage with an A. C. amplifier. *p* — a motor driven piston. By regulation of the mercury level the input in the zero position may be either short circuited or disconnected.

Amplifying D. C. voltages with A. C. amplifiers

In some cases, especially if only a relatively low upper limiting frequency of 1—100 c/sec. is sufficient, but a high zero stability and high input resistance are required, the D. C. voltage can be amplified with A. C. amplifiers after first changing it to an A. C. or intermittent D. C. voltage. This may be achieved with mechanical choppers or commutators. Fig. 44 shows a mechanical mercury commutator and its connection to the A. C. amplifier. Function is reliable, but the time constant is large and the upper limiting frequency is low, since the mercury oscillations are fairly slow. More rapid switching may be obtained with spring contacts operated by an electromotor, a compressed air motor or

an electromagnetic relay. The electromotor must be at a sufficient distance from the contacts and must be screened. It is then often possible to construct an amplifier perfectly tuned in a narrow frequency band (e. g. a synchronous detector) and thus to increase the signal-to-noise ratio. By appropriate connections of the mechanical contacts, several inputs can be connected one after

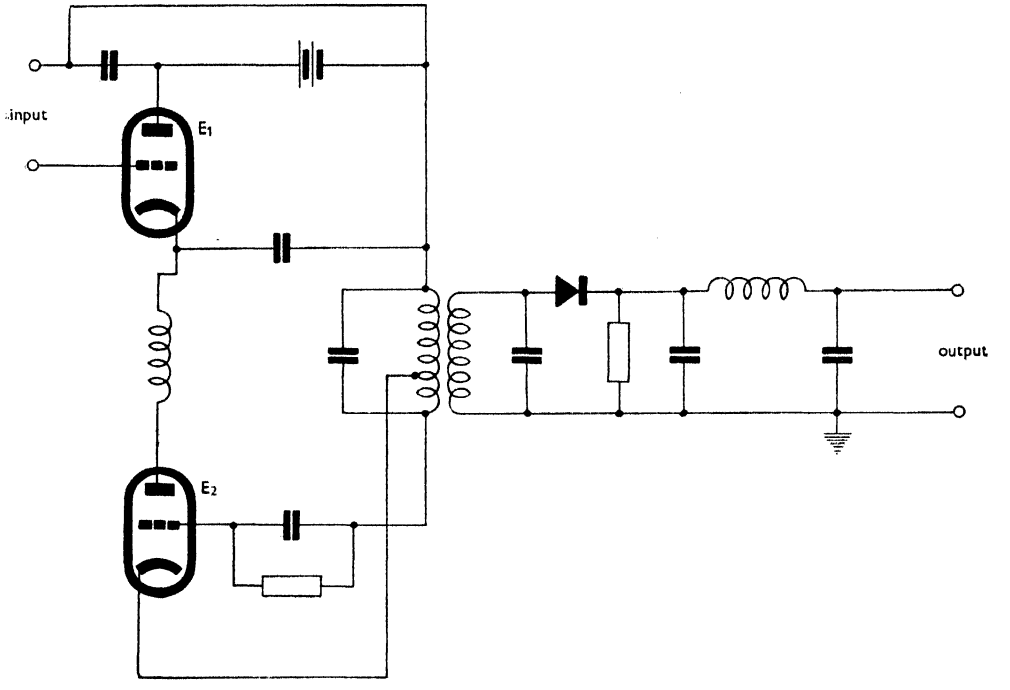


Fig. 45. Radiofrequency-coupled amplifier according to Haapanen. E_1 — modulator, E_2 oscillator. This amplifier has a coupling condenser in the input, but an D. C. amplifier can be designed on similar lines.

the other to a common amplifier input, and thus a multilead recording can be obtained with a single amplifier (Zachar 1955).

The change of D. C. voltage to A. C. modulated voltage may also be done purely electrically in a mixer. The regulated A. C. voltage is applied to one grid and the measured D. C. voltage to the other. The so-called radiofrequency amplifier is an interesting modification of this technique (Haapanen et al. 1952). It was shown in the chapter on stimulators (page 89) that complete resistive and capacitative isolation of the stimulating electrodes from earth is of advantage, and that this can be attained most easily using a radiofrequency stimulator or transformer. In some cases it may be nearly as advantageous to separate the recording electrodes from earth, although this is more

difficult. Fig. 45 shows a "radiofrequency amplifier" which makes this possible. The first valve is connected as a cathode follower acting as an impedance transformer and modulation amplifier, the cathode load of which is a Hartley H. F. oscillator. The oscillator is modulated by the follower so that the amplitude of the H. F. oscillations is proportional to the measured voltage. The heating and anode supplies are accumulators or dry cells. Neither the apparatus nor the power supply are connected to earth. An H. F. fransformer is used for coupling to the further conventional stages of the amplifier. The primary coil of the transformer is the oscillator coil and the secondary coil is connected in the demodulator circuit. A germanium rectifier usually serves as the detector. In view of the fact that up to the moment of detection the signal is continuously weakened, the adjustment is fairly delicate. For conversion of D. C. to A. C. see also p. 141. The literature see p. 30.

Recording devices

The recording system is the last stage of an electrophysiological recording apparatus. This always transforms electrical to mechanical energy. The movement is recorded in various ways. The recording system is characterised by its sensitivity, by the frequency characteristic and also by the constancy of the deflection. Sensitivity, however, may be expressed in various ways: in amperes or volts per unit length or unit angle of the deflection. It is more correct, however, to express sensitivity in watts or joules (as the product of watt sensitivity and of the period of mechanical oscillations) necessary for a certain angular deflection. When giving the sensitivity, the equation of motion of the recording system must also be considered. This accurately characterises the functional relationship between the sensitivity and acceleration (angular or linear). In general it may be stated that the higher the sensitivity the lower the acceleration, and thus the longer the oscillation period, but, of course, only in apparatus of the same class. In moving magnet apparatus the oscillation period increases with the square of the sensitivity, in moving coil apparatus with the square root of the sensitivity. Although today only a few types of recording instruments are used, some of the older ones, which have often been ignored unjustly, will also be metioned. Recording apparatus may be classified according to many criteria:

I. According to the basic function:

A. Systems sensitive to current:
1) Magnetoelectric
a) with a moving magnet
b) with a moving conductor (usually a coil)

2) Dynamoelectric
   a) dynamometers
   b) cathode-ray tubes with magnetic deflection
3) Soft iron instruments, etc. (electromagnetic)
4) Hot-wire meter, etc.

B. Systems sensitive to voltage
1) Mechanical electrometers
2) Molecular electrometers (Lipman's capillary electrometer, Kerr's cell, piezoelectric instruments)
3) Electronic electrometers (valves, electron-ray indicators, cathode ray tubes with electrostatic deflection)

II. According to the method of reading and recording:

A. Direct reading instruments
1) Pointer instruments
2) Mirror instruments
   a) with objective reading from the scale
   b) with subjective reading in a telescope
3) Microscopic instruments
   a) with objective reading (projected image)
   b) with subjective reading (visual microscope)
4) Cathode-ray tubes

B. Instruments with photographic registration — The same instruments as in A with facilities for photographic registration

C. With mechanical recording
1) Ink writing instruments
2) Printing (point after point) instruments
3) Instruments writing with a hot needle on wax paper or with a high voltage discharge on specially prepared paper, etc.

III. According to sensitivity

IV. According to the frequency range

Instruments still of use today are described below and divided according to IV and III. Some remarks concerning criterion II will be made at the end.

1) The slowest instruments:

Rotating coil instruments (galvanometers and milliamperemeters). Sensitivity up to 10^{-11} A/mm/m, oscillation time 20—0·5″, internal resistance from several Ω to several kΩ. A so called aperiodic resistance must be connected

in the external circuit for obtaining the most suitable damping. They are used to measure D. C. currents (thermocouples, photocells, etc.) and for measuring D. C. voltage (e. g. in connection with a cathode follower).

Moving magnet instruments. They are often more sensitive, but more delicate. A. V. Hill (1937—1938) used them to measure temperature changes in nerves and muscles.

Quadrant electrometer. Oscillation time is the same. Sensitivity up to 10^{-4} V/mm/m depending on the connection.

2) The medium speed (10^{0}—10^{-2} sec.) instruments:

Moving coil instruments. Rein's galvanometers (1940) with rotating coil, which in comparison to Moll's and to loop galvanometers are very sensitive.

Loop galvanometers are robust and simple and well damped mechanically. In all of them recording is photographic with the help of a mirror.

With a moving coil. Constructed in a way similar to dynamic loudspeaker. The coil is wound with thin wire and has a resistance up to several hundred or thousand Ω with a central tap. It is connected as a cathode load into balanced cathode follower power amplifiers. Recording may be mechanical with ink etc.

Moving magnet or soft iron instruments. Coil resistance about 10^{4} Ω. They are connected as anode resistances in balanced pentode power amplifiers. Mechanical recording. Both these types are mainly used in clinical electroencephalographs. They may also be matched to the output with transformers.

Capillary electrometer. The surface tension at the boundary between mercury and diluted sulphuric acid changes in dependence upon the potential difference between the two substances. Up to about 0·1 V no current flows. Recording is photographic with a projecting microscope or subjective in a measuring microscope. Voltages below 1 mV can also be measured. This was used for ECG recording before good amplifiers were introduced.

3) Very fast instruments. Oscillation time below 0·01 sec.

String galvanometer. The "coil" is reduced to one fine filament which oscillates with the current passing through it between the two poles of a strong horseshoe electromagnet. The movement is perpendicular to the lines of magnetic force. Recording is photographic with a projection microscope. Sensitivity is high. After calculating sensitivity and oscillation period, the relative sensitivity is higher than for the best moving coil galvanometers. This is due to the fact that the filament acts simultaneously as a conductor, a "former" of the coil, and the directing spring and carries no mirror. The filament is either

a thin platinum wire ($\varnothing$ 1—3 μ — Wollanston's wire), a gilt quartz fibre or an aluminium band. The last mentioned is most suitable, since it has a very good conductivity-to-mass ratio, is more flexible than a wire with a circular shape having the same conductivity and tension strength and can be damped more easily with air. It is of special advantage that the sensitivity and the oscillation time of the string galvanometer can be regulated by stretching the filament. The filament is delicate, may be easily torn and is difficult to exchange. It may be well to remind the reader that in many institutes long-discarded string galvanometers can be easily modernised by replacing the electromagnet poles by modern iron alloys, permitting even higher induction in the slit between them, and by using new quartz or aluminium fibres. Such an instrument used with one or maximally two D. C. amplifying stages makes it possible to attain the same absolute sensitivity with a better signal-to-noise ratio and a better zero constancy than the best multistage amplifiers with not very sensitive recording devices (e.g. c.r.tube), unless of course extreme demands are made with respect to the upper limiting frequency.

Piezoelectric electrometer. The amplified voltage is put across piezoelectric plates (today ceramically treated barium or alkaline-earth titanates are used because of their high mechanical strength). A mirror is connected to the plate. This enlarges the deflection optically. Recording is photographic.

4) Inertialess instruments

Only *the cathode-ray tube* operates practically without inertia (Fig. 46). This is a valve in which electrons are concentrated into a conical beam by a series of electrodes. The vertex of the cone falls on the fluorescent screen which glows at the point where the electrons hit it. The location of this point, however, varies according to the shape of the electric or magnetic field through which the electron beam passes. In cathode-ray tubes with electrostatic deflection, the path of the electrons is controlled by two pairs of plates, deflecting the beam vertically and horizontally. The amplified input voltage is applied across the vertical plates. The beam is deflected towards the positive plate proportionally to the potential difference between the deflecting plates. If the beam is permitted to move only in one direction, the movement of the spot is recorded on a film moving at a constant speed perpendicularly to the deflections of the beam. The variation in measured voltage is thus obtained on the film as a function of time. If only a relatively short interval is to be recorded, a constantly rising voltage, a so-called sawtooth wave, is put across the other pair of plates, usually deflecting the beam from left to right. Curves are thus plotted directly in Cartesian coordinates. The ordinate indicates the immediate voltage value while the abscissa shows the moment when this voltage was attained. Electrical circuits for the time-base may be very different. Several

of the circuits described in the section on stimulators are used most frequently: thyratron time-base, blocking oscillator circuit, asymmetrical multivibrator with positive grid bias, transitron or phantastron circuits. If a periodic phenomenon with a frequency F is observed, a sawtooth voltage of frequency $f = \frac{F}{n}$ is applied to the horizontal plates, where n is a positive whole number. For

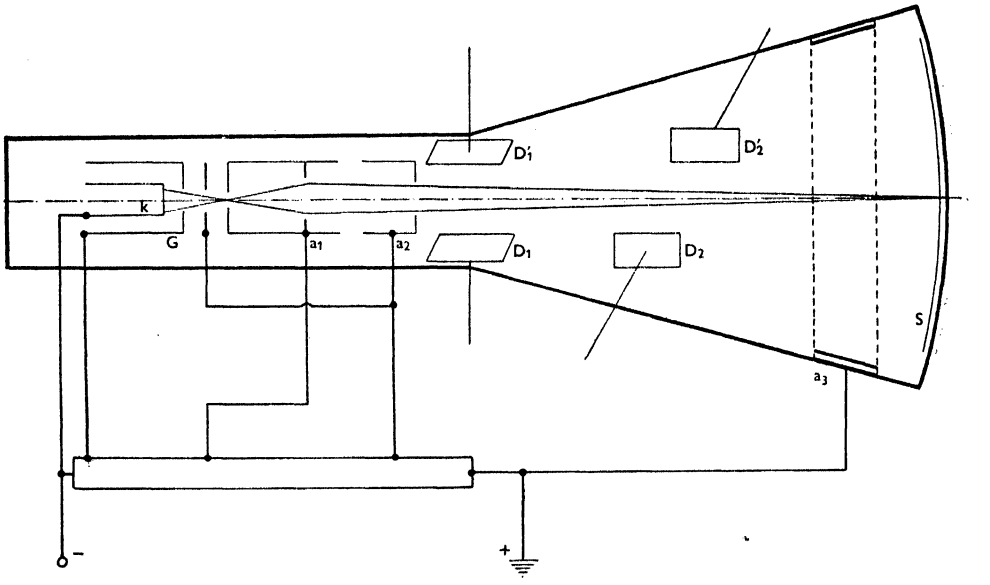


Fig. 46. Cathode-ray tube. k — cathode, g — grid, a_1 — first anode, a_2 — second anode, D_1D_2' vertical deflecting plates, D_2D_2' horizontal deflecting plates, a_3 — third anode (not essential), S — fluorescent screen.

visual recordings, a frequency larger than 20 c/sec. is best. For $f < 20$ c/sec. and for visual observation of nonrecurrent electrical phenomena, the screen should have a long persistance of the trace. This, of course, prevents a clear record on moving film or paper but does not matter when photographing single sweeps on stationary film. Often it is necessary to have both visual control and photographic documentation. In that case a moving film recording may be made by adding a "photographic" cathode-ray tube to the "visual one". The horizontal plates of the former are, of course, without voltage. Alternatively the "visual" screen with the time base may be photographed onto a film moving slowly in a vertical direction. The recording obtained is in oblique coordinates. See Petráň (1952b) for automatic transformation into rectangular coordinates.

Cathode-ray oscilloscope

The cathode-ray tube (see above) with the amplifier for the vertically deflecting plates, the sawtooth voltage generator for the horizontally deflecting plates and corresponding power supplies are connected together in the cathode-ray oscilloscope. The following are the main characteristics: Diameter of the screen, maximum sensitivity in volts (at the amplifier input) per 1 cm beam deflection, the type of amplifier (A. C. or D. C.), their input resistance and frequency characteristic, the frequency range of the sawtooth oscillations of the time base, possibilities for synchronisation, etc. In A. C. oscilloscopes, the sensitivity is often given in r. m. s. values (V_{eff}/cm), which may be misleading. A sensitivity of 1 V_{eff}/cm is approximately the same as that of a D. C. oscilloscope given as 3 V/cm (the transformation factor is $2 \cdot \sqrt{2}$).

Today D. C. oscilloscopes are preferred, since they are more universal and mainly because the central position of the spot on the screen is stabilised immediately, while in A. C. apparatus stabilisation takes some time, depending on the time constant, so that if an interference (e. g., the stimulus) enters the input of an amplifier having $\tau = 10$ sec., it is blocked and it lasts sometimes 30 seconds before the spot reappears on the screen. (For a antiblocking device see Baumann et al. 1956.)

Often two phenomena are to be recorded simulataneously with one cathode-ray oscilloscope. For such purposes a double beam cathode-ray oscilloscope is used, having either a cathode ray tube with two independent electron guns and deflecting systems, or containing a so called electronic switch connecting the vertical plates alternately to the two amplifier outputs in such a way that it first draws a short part of one, then a short part of the other curve. Each curve is thus composed of discrete points. It is also possible to connect the electronic switch as a special apparatus to a single beam oscilloscope. It then has a controllable switching frequency (e. g. from 0·1 c/sec. to 50 kc/sec.). Although the switching decreases the time resolution for higher frequencies and the curves are less exact in details, the arrangement is less expensive than the double beam apparatus, and the two curves have exactly the same time scale. In double beam cathode-ray tubes this is not the case, since both beams are independent of each other and the voltage outputs of both time-base generators may show different amplitudes and distortions. There are, of course, other ways for obtaining two records with one cathode-ray tube e. g. by splitting one electron beam into two.

Modern oscilloscopes together with their amplifiers are usually already so sensitive that for electrophysiological purposes only one or two stage battery preamplifiers (sometimes with a cathode follower input stage) are sufficient. If the oscilloscope is constructed in such a way that it can stand a higher positive voltage of both input terminals against earth, then direct coupling

between the preamplifier and the oscilloscope may be used. Gain is usually controlled only in the oscilloscope. The oscilloscope must be constructed in such a way that single sweeps can be started at suitable moments, i. e. when the triggering impulse enters the oscilloscope.

The function of the time-base and different methods of synchronisation

In electrophysiology the time-base is usually synchronised with the stimulator in such a way that the stimulator gives a short pulse which triggers the sweep generator (one sawtooth wave is produced). The stimulus is applied only after a controllable delay, so that it arrives when the time-base is already running. The time-scale is controlled in the oscilloscope with controls marked "width" and "time-base frequency" (although the frequency is determined by the stimulator).

The synchronisation switch must be switched over to position "external synchronisation".

If a periodic phenomenon is to be observed, the frequency is adjusted as described above so that $f = \frac{F}{n}$, usually $f = F$. The input voltage or another voltage coupled with it (external synchronisation) is used for synchronisation or, alternatively, the synchronising voltage is taken from the amplifier for the vertically deflecting plates (internal synchronisation). If a small part of the curve is to be analysed in more detail, the voltage of the time-base is increased with the "width" control to a multiple of the voltage needed for the full screen deflection of the beam. With the "horizontal" control, such a D.C. voltage is applied to the horizontally deflecting plates so that the chosen part of the curve appears on the screen.

Supplementary equipment

Amplitude calibration

Often it is sufficient to measure the amplification of the amplifiers or the sensitivity of the whole apparatus once and for all, and to provide its controls with a scale. In other cases, where there are more control elements, if exact documentation is required, it is better to use a calibrator. This is usually an accurate voltage divider with several steps fed from one dry cell (Fig. 47). The calibration voltage is chosen with the selector switch and by pushing the push-button, this voltage is applied to the amplifier input. The calibrator is

connected either directly to the input instead of the object or in parallel to the object (the internal impedance of the calibrator is so small that it practically short circuits the measured tissue and the recording thus contains no

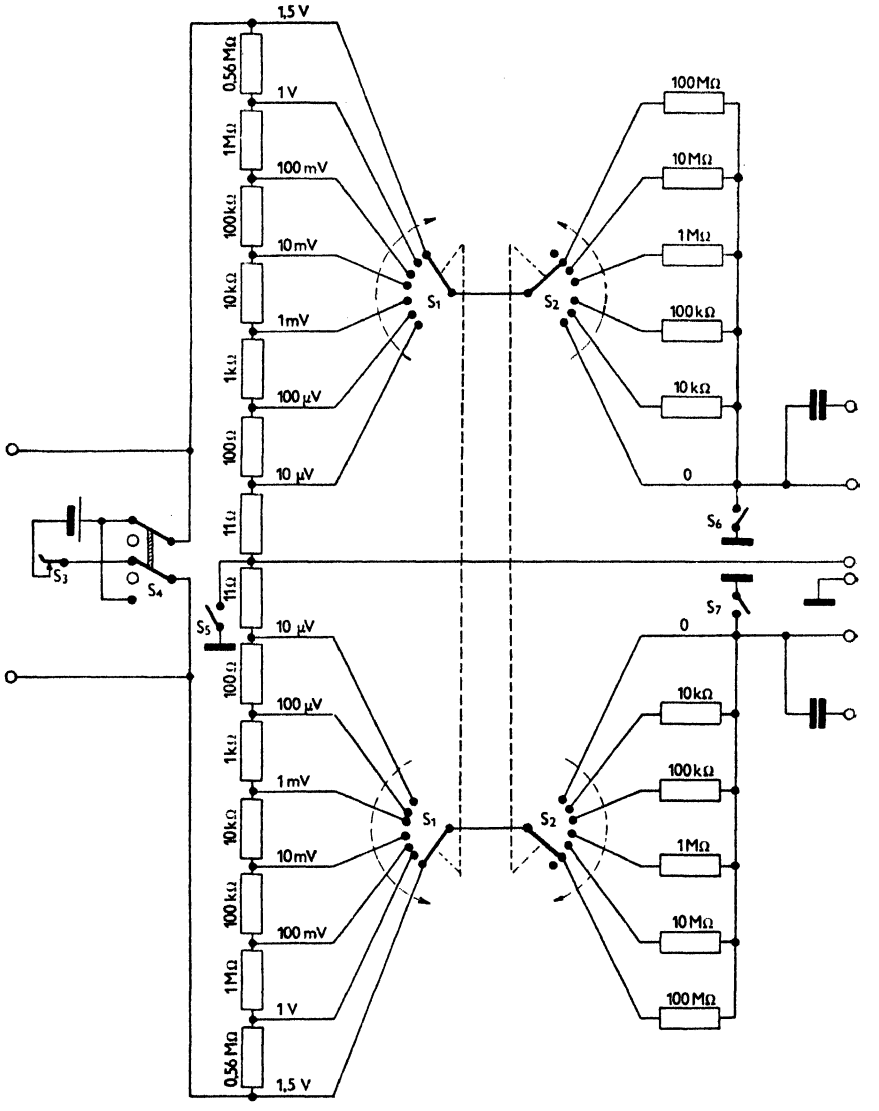


Fig. 47. Calibrator. S_1 — voltage control, S_2 — input resistance control, S_3 — key, S_4 — commutator. With the aid of plugs and switches and the commutator of input voltage, a calibrating voltage of any polarity may be directed either between both amplifier grids, between one grid and the frame, or between the connected grids and the frame, either directly or across a condenser. Thus the sensitivity, discrimination ratio, linearity, noise, etc., can be determined. As voltage source a dry cell or an external supply is used.

bioelectrical potentials but only the calibration voltage) or the calibration voltage is connected in series to the bioelectrical potential (the recording is shifted upwards or downwards). In addition the time constant can be estimated from the shape of the output.

Time mark

Time calibration is usually more important than voltage calibration and may be performed in several ways:

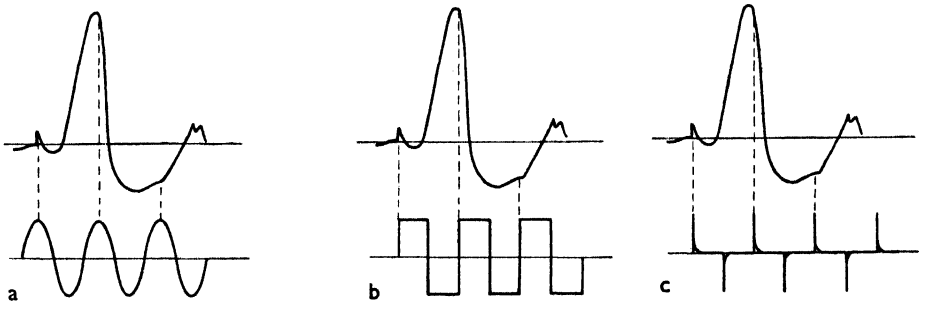


Fig. 48. Time marking in a double beam oscilloscope. Upper curve — action potential. Lower curve — time signal: a) from tone generator, b) from rectangular wave generator, c) from b) after differentiation.

1) Occasionally a periodic voltage of known frequency, e. g. 50 c/sec. from the main, 1000 c/sec. from e.g. a tone generator, is recorded instead of the measured voltage. The accuracy of time determination is small, maximally 10%.

2) On the second beam of a double beam cathode-ray oscilloscope a sine wave voltage from a tone generator is registered. Accuracy is small. A number of exact parallel lines must be drawn which mark the moments of the same phases of the second curve on the first curve (Fig. 48). Since the phase of a harmonic curve is difficult to determine accurately, interpolation must be used. It is, therefore, better not to use harmonic voltages but rectangular or short sharp pulses. These may easily be produced from the sine voltage of the tone generator even without valves (fig. 50). This increases the accuracy from 5% to 0·5% of the period of the A. C. voltage used.

3) The brightness of the beam is modulated using sine wave voltage or, better, short pulses applied to the modulation terminals of the oscilloscope. Evaluation is easier but accuracy is not higher.

4) Short sharp pulses are injected into the vertical deflecting system. They may be applied to the amplifier input (if an asymmetrical input is used) in a push-pull amplifier directed to the free grid (or the suppressor or screen

grid of the first stage) or directly to the plates. The last possibility gives the advantage of stable amplitude. The result is a tracing as shown in Fig. 49bc. Compared to (3) there is less interference with the recording (shorter pulses are sufficient), but accuracy is only slightly increased.

5) All the above methods have a common disadvantage: The initial phase of the calibrating voltage, the delay between the first pulse of the time

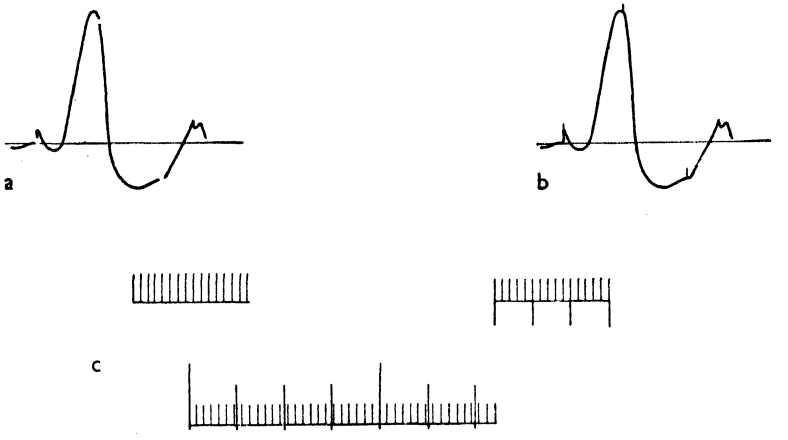


Fig. 49. Time mark in a single beam oscilloscope: a) using modulation of beam brightness, b) using mixing of signal with short sharp calibration pulses, c) different arrangement of time scale using short pulses (diagramatic). Left: distance between lines 40 μsec. Right: 40 μsec. and 200 μsec. Bottom: 40 μsec. and 250 μsec. By their interference further markings (1000 μsec.) are automatically formed.

mark and the stimulus artifact, is accidental. Consequently the number of whole periods of the calibrating frequency must be determined for each curve and an interpolated part at the beginning and the end must be added. This is more elaborate and less accurate than synchronisation of the calibrating frequency with the stimulus in such a way that the time mark is always started by the stimulus in the same phase, as far as possible, in the zero phase. Since the duration of a single recorded curve is usually considerably shorter than the interval between the sweeps, this is easily obtained. The circuit diagram is shown in Fig. 51: The stimulus excites the resonant circuit *LC*, its damped oscillations are amplified, limited to a rectangular shape, differentiated and the derivative peaks are used either to modulate the beam brightness (see 4) or are mixed with the signal (see 5). The drawback is that the damped oscillations soon fade so that only such a calibrating frequency can be used that gives 10 time marks on the recording at the most. Damping of the resonant circuit can, however, be reduced by introducing a positive feedback. Increased damping must then be introduced at the end of the sweep so that the circuit

is resting at the moment when it is to start oscillating again. The whole arrangement is shown in Fig. 52. The input exciting voltage must remain negative for the whole period during which calibrating pulses are to be formed. If there is no such voltage available (e. g. from the stimulator) then itmust be produced, e. g. by triggering a monostable multivibrator or transitron with a stimulus or synchronising pulse from the oscilloscope. The pulse must be slightly longer than the time during which the spot passes across the screen. Negative pulses are directed to the grid of a triode E_1 having a positive D. C. bias. An oscillation circuit is connected in its cathode LC. The valve is blocked by a negative impulse to the grid. The anode current disappears and this results in damped oscillations with frequency $\omega = \frac{1}{\sqrt{LC}}$ appearing in the circuit. The voltage from the cathode is directed to the grid of a further valve E_2, the cathode of which is connected to a top of the coil L of the resonant circuit. During the positive period of the current passing through the coil, a positive voltage appears at the cathode. This produces a rise in current also in the second valve. This current, however, also passes through the coil. Thus there is positive feedback, the size of which is controlled by a variable resistance R in such a way that losses in the coil are compensated. When the negative pulse perishes, a cathode current begins to flow through the first valve having a positive bias. At the moment when the cathode is negative due to oscillation of the resonant circuit, a larger current passes (the grid is more positive to the cathode) and produces a voltage drop across the coil in the opposite direction: the cathode becomes more positive. Thus this is an ordinary negative feedback which very rapidly suppresses the oscillations of the resonant circuit (within 1—3 periods). This may also be explained as follows: the first

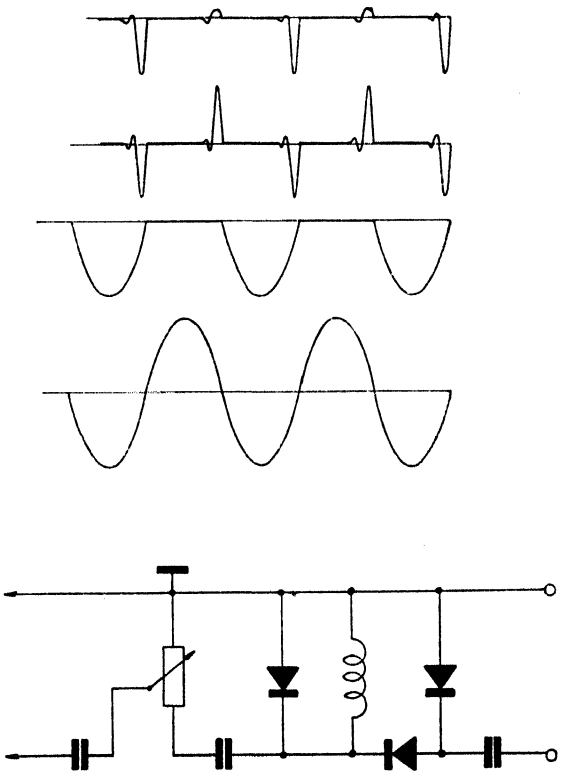


Fig. 50. Apparatus without valves for producing time calibration peaks from sine voltage.

valve connected in parallel to the resonant circuit, acts as a damping resistance consuming energy accumulated in the resonant circuit. This damping resistance is infinitely large for a negative grid voltage and very small for a positive grid voltage.

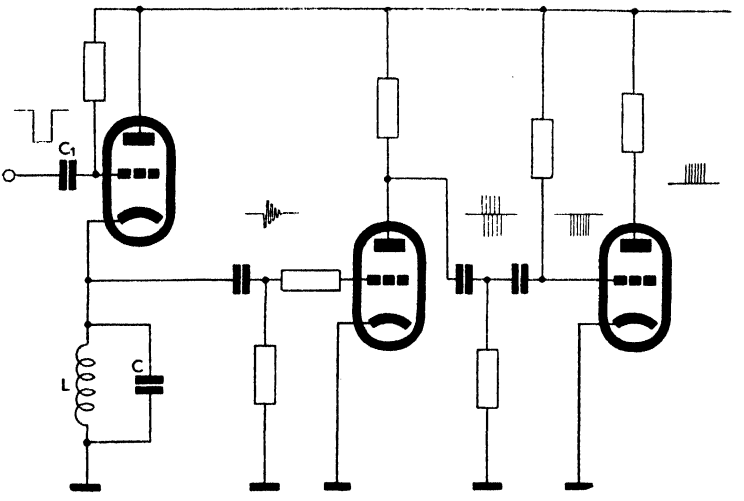


Fig. 51. Production of synchronised calibration peaks using a resonant circuit.

As compared to the usual generators, this arrangement has one further advantage: the frequency stability is considerable (up to 1 part per million) without any special precautions. If, however, similar absolute accuracy is required (e. g. for measuring the conduction velocity), the apparatus must be calibrated with a quartz crystal oscillator.

Practically, the apparatus is usually constructed with several coils and condensers, or even several first stages which can be selected according to the different speed of the time-base. A limiter D, differentiating circuit $C'L'$ and limiter-amplifier E_3 follow. The sharp peaks thus produced are amplified and applied directly to the vertical plates (see 4) or to the cathode-ray tube grid (or to the "modulation" terminal, see 3). It is also possible to have two simultaneous time marks, one more and one less dense, one on each side of the recording or a kind of time nonius making it possible to determine easily a large number of scale units (Fig. 49c) even for fine division. The accuracy of such a time marker is very high and fundamentally depends only on the sharpness of the trace. The treatment of results, particularly visual measurements, is incomparably more rapid and easier than for all other types of time markers. The greatest advantage of this arrangement is that the time mark needs no control and takes up no functional place in the experimental arrangement which might be used otherwise (e. g. one beam of a double beam oscilloscope).

On the contrary it is here possible to inject the time mark on both beams: Method 4, however, is more suitable than method 3 for a double beam oscilloscope with an electric switch. Another circuit for synchronised time marker is described by Saunders (1954).

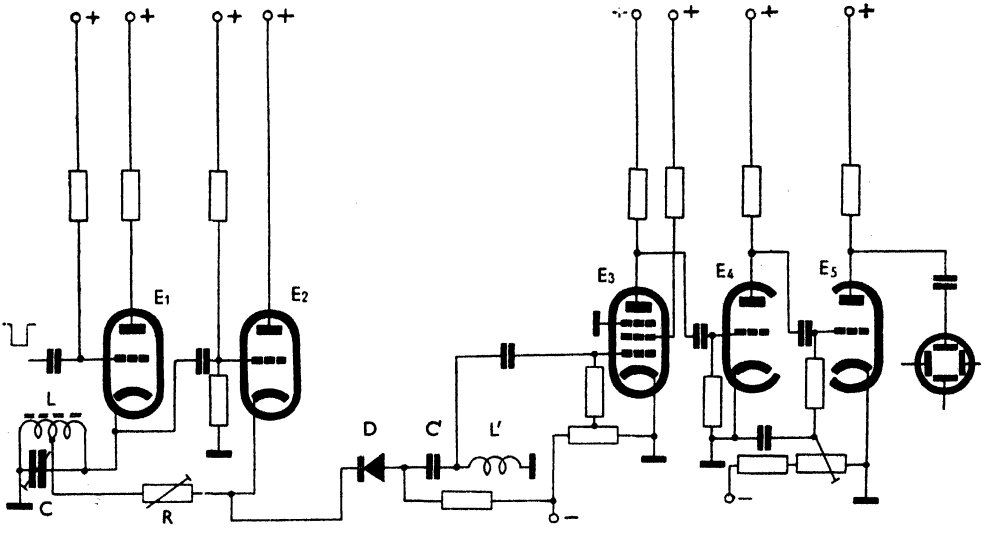


Fig. 52. Synchronised time mark with positive and negative feedback.

Properties of mechanical recorders

Attention must also be paid to some characteristics of mechanical recording instruments. The most important are sensitivity and the oscillation time. Not taking the effect of mechanical resistances and friction (e. g. of the pen and paper) into account at first, the equation of equilibrium for an instrument having a constant current sensitivity is

$$D \,.\, \alpha = kI \quad (*),$$

where D is the directive force, which is determined by the banding strength of the spiral springs (or analogous components) maintaining the moving coil in equilibrium state, α is the angular deflection. Thus $D \,.\, \alpha$ is the moment of the directive force equilibrating the moment of the second force proportional to the current I and a constant k given mainly by the shape of the magnetic field and the coil construction. The sensitivity is then $\frac{\alpha}{I} = \frac{k}{D}$, and thus inversely proportional to the directive force .

The oscillation time $T = \text{const.} \frac{J}{D}$, where D is again the direction force and J the moment of inertia, given by the mass of the movable parts and the distribution of this mass with respect to the axis of rotation. It follows that for instruments which are the same from the electrical aspect, i. e. having the same k and R_i (resistance of the coil), the sensitivity is proportional to the square of the oscillation time and inversely proportional to the moment of inertia $\frac{\alpha}{I} = c \cdot \frac{T^2}{J}$. Since, however, equal instruments have the same moment of inertia, T^2D is a constant for instruments of the same type or $T^2 . \text{const} = \frac{\alpha}{I}$.

Thus, without changing the electrical characteristics, it is possible to increase the sensitivity only by decreasing D while T increases at the same time. If the sensitivity is thus raised λ times, the oscillation time increases $\sqrt{\lambda}$ times. If, on the other hand, the original oscillation time T_0 is to be shortened without changing the electrical characteristics of the instrument, its shortening to $\frac{T_0}{\lambda}$ can only be realised by a corresponding increase of D_0 to $\lambda^2 . D_0$. This means that the sensitivity is reduced to $\frac{1}{\lambda^2} \cdot \frac{\alpha}{I}$ (where $\frac{\alpha}{I}$ is the initial sensitivity). The oscillation time can, of course, be shortened to the same extent also by decreasing J without changing D, i. e. while preserving the sensitivity. This is achieved by reducing the mass of movable parts of the instrument. It is then necessary to decrease the moment of inertia by $\frac{1}{\lambda^2}$ for the oscillation time to be reduced by $\frac{1}{\lambda}$. It is also possible to shorten T_0 to $\frac{T_0}{\lambda}$ by simultaneously decreasing J_0 and increasing D_0.

If now the moment of inertia in mechanical recording instruments is considered, especially the fact that for a mass point $J = mr^2$ (m = mass, r = radiusvector) and for a body $J = \int_0^R mr^2 \, dr$, the following possibilities are seen: m may be decreased by substituting aluminium for copper in the moving coils. The mass of the ends with the largest r, the writing pen and its lever, must be reduced. Here every milligram is important, while decreasing the mass of the axis is of no importance. If demands on shortening the oscillation period are extreme, it is often preferable to decrease the sensitivity by shortening the recording lever and thus decreasing considerably the moment of inertia. It is also possible to increase D and to raise the sensitivity by increasing the power amplifier output to the recorder.

Just as in the microscope, where high resolution is much more desirable than enlargement alone, so also in the recorder it is most important to increase

discrimination power. This is given not by the absolute amplitude of the tracing but by its fidelity, particularly for the smallest details of amplitude and time.

The first important characteristic is thus the ratio of the thickness of the trace to the amplitude of the record. A line 0·1 mm thick and a recording 1 cm wide are equivalent to a line of 0·5 mm and a 5 cm wide recording. In the first case the moment of inertia may be several times smaller for the same coil.

The second important factor is the rigidity of the lever. A lever which is too long, thin and elastic may give a different deflection for rapid and for slow movements: it may oscillate mechanically. This error must be smaller than other errors and inaccuracies.

The third important group of parameters is mechanical friction. In instruments writing on paper, friction in the bearings is usually negligible in comparison to other resistances. The most important is friction of the pen against the paper. This is $F = \beta p$ where p is the pressure of the index (pen) on the paper and β the friction coefficient, dependent upon the kind of recording (ink, wax paper, etc.) and also on the quality of the surface of the index and paper. F is then the force braking the movement of the pen. The friction braking produces a moment of force $M = rF$, where r is the length of the lever. If, for instance, when the current is increasing, the pen moves from left to right then, on first approximation, its final position α_1 will be such that

$$\alpha_1 = k\frac{I_1}{D} - \frac{rF}{D}$$

If now the current begins to fall, the deflection remains constant up to the moment when

$$\alpha_1 = k\frac{I_2}{D} + \frac{rF}{D}$$

By subtracting both equations we obtain:

$$k\frac{\Delta I}{D} = \frac{2rF}{D}$$

i. e.

$$\Delta I = \frac{2rF}{k}$$

where ΔI is the error in the current measurement. If ΔI is inserted into the upper equation (*) (page 135) the error of the deflection is obtained

$$\Delta\alpha = \frac{2rF}{D}$$

$\Delta\alpha$ is the angular error, which it is better to transform to the linear error Δy. Thus $\Delta y = r\ \Delta\alpha$ and hence

$$\Delta y = \frac{2r^2F}{D}$$

This shows how the length of the lever (r) again considerably worsens the quality of the tracing and that a thin line is preferable to a large deflection.

The problem of damping is also important. This should be such that the equilibrium position is attained as fast as possible and without overshoot (aperiodic movement). Damping differs according to construction: eddy currents, induction, shunt resistances, negative feedback of the amplifier, pneumatic or hydraulic damping. The mechanical properties and damping of the recorder are best tested with rectangular pulses.

Time mark and other markings (stimuli, injections etc.) are also performed mechanically. Either a synchronous motor moves the pen directly or the pen is fixed to the anchor of a telephone relay which receives impulses from any contact clock.

Mechanical recordings are made on constantly moving paper. The paper is either moved by an arrangement built into the apparatus (EEG, ECG etc.) or by a kymograph. The maximum utilisable speed is 50—100 mm/sec.

Photographic recording

For photographic recordings an optical kymograph is used. Sensitive paper is usually used in mirror instruments and string galvanometers and film in the cathode-ray oscilloscopes. This moves at a speed of 100—500 mm/sec. If more rapid movement is required, there are unnecessarily large losses when starting and stopping the apparatus. These should be limited in such a way that the motor, having a large fly wheel, runs at constant rate and movement of the film is started by a rapidly acting clutch. Often, however, a more simple device is better. This needs no time to get started and is always prepared for recording. A large drum with a turn of paper or film rotates rapidly continuously (1000—10 000 or more mm/sec. at the circumference) and is protected from light by a screen box etc. but for small opening through which the recording beam is to pass. This opening is closed by a photographic shutter which is set in such a way that, on opening, nearly the whole length of film is exposed. This shutter is synchronised, e. g. with the stimulator, or is controlled manually. This arrangement can easily be improvised, e. g. from a bicycle wheel.

When recording with an optic kymograph, the image of the bulb filament is projected by a strong convex lens onto the lamp slit and the image of the slit is then projected by an achromatic convex lens via the mirror of the measuring

instrument onto the kymograph slit. It is best to have the lamp as close as possible to the mirror. The sensitivity is regulated by changing the distance between the mirror of the recorder and the kymograph slit (the length of the so-called optical lever).

The time and amplitude marks are important in optic kymographs with paper recording, since the paper changes its dimensions by up to 10% during treatment and the motor is usually also not driven quite regularly. The amplitude mark is either a system of lines on the cylindrical lens in the slit of the kymograph or a system of thin wires extending into the slit. Both sharply interrupt the trace whenever the beam exactly passes the first, second and every further centimeter of the slit length. One then interpolates between these points in the recording. The time mark may be simply made by another beam and a mirror as in mechanical recordings or by illumination of the whole slit at a certain moment. This gives a system of black time coordinates across the whole width of the recording. The slit may also be covered at certain intervals. This gives white time coordinates perpendicular to the white amplitude marks. Both methods are best applied by using a wheel with cut out sectors rotated by a synchronous electromotor.

For the most accurate work, geometrical errors must be considered, arising in the recorder because of transformation of linear coil movement to rotary movement of the mirror and the tangential error inherent in every mirror registration.

The optical kymograph for recording with a cathode-ray oscilloscope has no slit, but a good photographic objective which projects the screen of the cathode-ray tube onto the plane of the film (see p. 126).

When working with optic kymographs it is sometimes not possible to place lamps, mirrors and beams of some of the recording systems into a position and direction suitable for direct registration. It is then necessary to change the direction of the beams and sometimes also to change the plane of the beam deflection. Directional changes are attained by the use of good plane mirrors having a silver- (or aluminium-) plated front surface (to prevent the formation of a double picture) or by the use of prisms (rectangular, pentagonal and others). For changing the plane of deflection without changing the direction of the beam, so-called reversing systems are used, usually Wollaston's prism. When this is rotated by an angle α about its long axis, the image of the slit rotates by 2α about the beam axis. If such a prism is, for instance, placed in front of the objective of the camera, the way of recording from the oscilloscope screen may be changed without turning the camera or oscilloscope box and only rotating the prism through 45 degrees. Either the time axis coincides with the direction of the film movement (recording without the time-base), or the time axis is oblique to the film movement (using the internal time-base). For details see page 126.

Simultaneous recording of electrical and non-electrical quantities

It is often necessary to register both action potentials and the mechanical reactions of living tissue or physiological (non-electrical) stimuli.

This is most simply done in mirror recordings, were a myograph or a similar arrangement may also be a mirror instrument and the corresponding curve is recorded with the same optic kymograph.

The transformation of non-electrical quantities into electrical ones

If mechanical recording and a cathode oscilloscope are used, it is usually necessary to transform the corresponding mechanical deflection into electrical voltage or current. This can be done in many ways (Zhdanov 1952, Klensch 1954), the simplest of which are described below.

1) *Electromagnetic transformation.* Mechanical movement is transformed into the movement of a magnet inside a coil, or of a soft iron core increasing or decreasing the magnetic flow in coils on a permanent magnet. Examples:

a) Isometrical myograph. The muscle is stretched by a thread, the end of which is fixed to the centre of a telephone membrane (earphone). On contraction, the movement of the membrane induces a voltage in the telephone coils. By amplifying this, a voltage sufficient for recording with an oscilloscope may be obtained.

b) Isotonic myograph. A weight, with a bar magnet below it, is fixed to the muscle. The magnet enters a flat coil. Movement induces a voltage in the coil. In both cases the voltage is not proportional to the absolute deflection, but to the rate of deflection, i. e. to the deflection differentiated with respect to time.

c) Similar principles may also be used in a mechanical stimulus mark. For instance a telephone is fixed to the neurological hammer. The elastic button of the latter is attached to the membrane.

2) *Piezoelectric transformation.* Isometric myographs may be constructed in such a way that the muscle bends a piezoelectric crystal, e. g. a crystal gramophone pickup. When using an electrometric amplifier, e. g. a cathode follower, the voltage in the piezoelectric crystal itself is proportional to the deflection, provided insulation is good. If the deflection continues, the voltage disappears only slowly with a time constant of several seconds (given by the total leak).

3) *Resistance transformation*

a) Transformation of movement. A change in position is transformed into a change in resistance either by a variable wire resistor with a very fine and light sliding contact, which can be bought or improvised, or with an electrolytic potentiometer. A diagrammatic representation of the equipment for recording respiration in rabbit is shown in Fig. 128.

b) Transformation of tension. For this resistance wires may be used. Within the limits of Hook's law, these are prolonged slightly with increasing tension, while their diameter decreases. Thus their resistance increases. They are chiefly used for technical purposes (strain gauge) but may also be utilised as an isometric myograph. A bridge connection must be used, since the change in resistance is small.

4) *Current transformation* using a valve with a movable electrode is somewhat similar (Klensch 1954).

5) *Capacitance transformation.* This utilises the relation between the voltage, capacitance and charge $V = \frac{Q}{C}$. An air or liquid condenser is connected with the source of mechanical movement. The latter is measured using the above equation. For small deflections flat condensers with a very small inter-electrode distance are suitable (condenser microphones, capacitance recorders of vibration, etc.). For large deflections variable condensers with very small friction in the bearings or differential condensers may often be used.

Let us determine the sensitivity: By differentiating the above equation with respect to C

$$dV = - C^{-2} Q \, dC$$

is obtained, and by substituting for Q from the above equation

$$dV = - V \frac{dC}{C}$$

Hence the voltage changes are directly proportional to the voltage and the relative capacitance decrease.*)

*) It is evident from the above equation that this principle may also be applied for measuring D. C. voltage by transforming it to A. C. voltage (see p. 123), since

$$V = - \frac{dV}{dC} C$$

If C is always changed by exactly the same amount, then dV (changes in V), as measured by an A. C. amplifier, is proportional to the voltage V. This exact periodic change in capacitance is realised by either a motor or a dynamic system (as in a loudspeaker) supplied with a regulated A. C. voltage. Such device is termed a dynamic condenser.

The usual circuit is shown in Fig. 53, where R_n is the charging resistance, R_e the leak resistance of the measuring condenser, R_i the leak of the measuring instrument, M the electrometer (electronic voltmeter, cathode follower or other electrometric instrument). The time constant of this apparatus is again $\tau = RC$, where R is the resistance of all leaks, i. e.

$$\frac{1}{R} = \frac{1}{R_n} + \frac{1}{R_e} + \frac{1}{R_i}.$$

If the time constant is not sufficiently long and R cannot be increased, C may be raised, i. e. the capacitance of the measuring condenser (or without changing the measuring condenser itself, by connecting in parallel to it a constant condenser without dielectric losses, e. g. with styroflex dielectric and the sensitivity decrement is made up for by proportionally increasing the sensitivity of the measuring instrument).

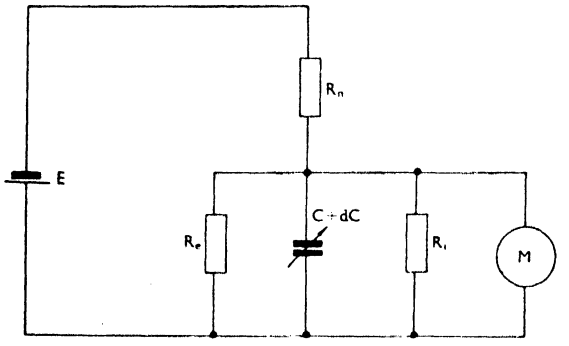


Fig. 53. Capacitance recorder — basic circuit.

Capacitance transformation may also be achieved by connecting the measuring condenser into the circuit of an A. C. bridge and by measuring changes in its capcitance with a low or high frequency. Here it is necessary for the frequency to be about ten times higher than that of the most rapid component of the movement studied.

6. *Photoelectric transformation.* Mechanical movement controls the flow of light illuminating a photo tube, a photovoltaic or photoconductive cell. The voltage or current (or the drop across the anode or cathode load) is amplified and recorded in a single or double beam oscilloscope.

Simultaneous recording with cathode-ray oscilloscope and mirror instrument

The cathode-ray tube tracing may also be combined with one or several other recordings, e. g. mechanical phenomena using the mirror method. The procedure is as follows:

A piece of white paper is glued above or below the cathode-ray tube screen and the spot of the accessory recording system is projected onto the paper as in an optical kymograph. The lamp, however, has not a slit but a hole. The

camera (optical kymograph) must be moved a little further away and again focused, so that all tracings can be projected upon film. When recording in oblique coordinates across the film, the paper must be placed at the sides of the screen and the mirror spot must oscillate in a direction perpendicular to the movement of the film, i. e. horizontally in this case. Here, of course, it is more difficult to find corresponding points in both recordings. The advantage of this method rests in the fact that the oscillograph is not crowded with tracings for which the screen is not absolutely necessary and that, on the contrary, equipment transforming non-electric to electric functions is often saved (Petráň 1955).

Location of faults and interferences

Improper function of an electrophysiological apparatus may reveal itself as follows

1) the complete apparatus is not working,
2) it is working unreliably, occasionally its function changes, e. g. with vibration etc.,
3) its function is changed (e. g. the stimulator stimulates with a frequency that was not set, the registering arrangement only records excessive noise or hum),
4) the function is changed in such a way that the fault cannot be detected without special tests. For example the recording equipment registers artifacts very similar to action potentials.

Faults

1) Persistent faults

This group of faults is most easily repaired. The fault, however, must be sought systematically.

a) First one checks whether all components receive the necessary voltages (with voltmeter, according to the valve temperature, lighting of the valve cathodes, etc.).

b) All external connections are tested with an ohmmeter or a bulb tester for short circuits, faulty connections, correct connections, etc. The most frequent site of faulty connections is that between the conductor and the plug.

c) If the fault does not lie in a) or b), an attempt is made to localise it at a certain part of the apparatus. One tests, for instance, whether the fault

is in the preamplifier or the oscilloscope by placing a short antenna into the oscilloscope input and observing whether the main A. C. frequency is of the usual size. If that is the case, the fault is in the preamplifier. When testing the stimulator the oscillograph is used to test the shape and amplitude of pulses for each stage of the stimulator, and thus the faulty stage is found. After localising the fault, further search is continued:

d) The corresponding part is checked mechanically (i. e. visually, with a forceps) — for satisfactory joint soldering. This is the first thing done in electrotechnical factories. A drop of an alcoholic solution of an aniline dye is placed on each point as tested so as not to miss any. All suspected places are repaired (dry joints and loose connections are cleaned and resoldered etc.). If this does not work, then

e) the voltage marked at important points in the diagrams is checked. If there are no voltage values in the diagram, the following voltages must be checked: first, the voltage of the heating, anode and bias supply and the voltage at the electrodes during operation (cathode, anode, screen grid). The lighting of the valve is observed. If that is in order, the voltage at the socket contacts for the cathode, anode and screen grid are tested after disconnecting the heating voltage or after removing the valve. The results are critically evaluated. If the anode voltage with the heating on and off is high and does not change, no anode current is flowing (provided the valve is not operating in an impulse circuit with very short conducting periods). The resistance of the voltmeter must be much larger than the anode load. The causes may be the following:

A large grid bias (broken grid lead, interrupted grid leak). Screen grid without voltage (broken lead, burned load, short circuit, e. g. in blocking condenser). Large suppressor grid bias (rare). Opencircuited cathode (burned cathode resistor) or anode (in the valve). If, on the other hand, the anode voltage drops considerably on heating (i. e. the current rises considerably), this may be due to a positive grid voltage (leaky grid condenser), a short circuit between the cathode and the frame, bad vacuum, etc. Sometimes it is worth while testing not only the electrode voltages but also their currents. The voltage across electrodes connected through large resistances, especially the grid, can be measured only by using electrostatic or electronic voltmeters or a cathode-ray oscilloscope. Their voltage can, however, easily be calculated from the known or measured resistance and current. Usually the fault can be thus localised more accurately. Then

f) components at the localised site are tested. First the resistance values for resistors are checked with an ohmmeter. Then condensers are checked for short circuit and open circuit. The valves are tested by either measuring their properties in special bridges or by replacement. In sensitive instruments, only valves already aged for 50—100 hours can be used, in D. C. amplifiers both valves of the same pair must be replaced by a suitable pair. If no spare

valves are available, voltage and current are tested under working conditions. The suspicious valve may also be tested in another reliable apparatus, when it is connected in the same way.

g) If the above did not give the expected result, we test

α) the amplifier using the oscilloscope and a tone or pulse generator. The voltage from the tone generator is directed through a small condenser between the grid and the frame or between both grids, depending on the construction. With the oscilloscope connected also through a condenser to the anode (anode-frame or both anodes), the amplification and distortion are measured. Insufficient amplification may be due to an interruption or short circuit along the path of the signal. Distortion is best tested with a double beam oscilloscope in such a way that the upward deflection is produced by a signal of the same and known polarity in both beams. Input voltage is recorded on one beam and output voltage on the other. The following voltages are measured: at the tone generator output, at the grid of the amplifier input, at the cathode, at the screen grid, at the anode. The grid voltage (which may be calculated from grid condenser and leak) should be somewhat smaller than the tone generator output voltage. If its positive halfwave is limited, the fault is in the bias (or a short circuit at the cathode). No A. C. voltage should appear at the cathode blocked by a condenser. If there is an A. C. voltage, the condenser is faulty (it has lost its capacitance, is interrupted). Only a fraction of the A. C. input voltage can be found between the joint of cathodes of balanced A. C. and D. C. amplifiers and the frame. If it is large, the circuit has become asymmetrical either because of an interruption of the grid, cathode or screen circuit or because of changed characteristics of one valve. The same is valid for A. C. voltage at the blocked screen grid and the joined screen grids of a balanced amplifier. The A. C. voltage at the anode (or between the anodes) must be amplified and undistorted. If amplification is small, the fault may lie in the formation of negative feedback (faulty blocking or asymmetry in the cathode or screen grid circuit), decreased voltages at the anode or screen grid, large grid bias in balanced amplifiers. Reduction of negative halfwaves at the anode is due either to grid limiting by grid current (small grid bias, see above) or to low anode voltage or high anode load. Suppression of positive halfwaves occurs if the grid bias is increased. Symmetrical limiting is due to the input signal being too large.

Time constants are tested with the pulse generator. Decreased time constants produce a larger drop in the terminal portion of the rectangular pulse top voltage (see p. 131).

The amplifier oscillates with its own freqency. This is due to feedback which sometimes may be the result of connecting the tone generator and oscilloscope to the amplifier. On excluding this possibility, the cause must

be sought in interruption of a resistor, disconnected blocking condenser, internal short circuits in the valves or between other components, etc.

β) Stimulators are tested in a similar way by making sure that correct limiting and amplification occur at the proper sites.

2) *Intermittent faults*

The second group is more difficult to mend. We either

a) wait until the fault occurs permanently and then proceed as under 1) or

b) accelerate its occurrence and stabilise it by mechanical shaking, and then proceed as under 1) or

c) we attempt to localise the fault by gently knocking different parts of the instrument and then individual components and joints. After attaining maximum localisation, this part is tested mechanically or the suspected component is exchanged. Switch contacts and the valve sockets etc. are cleaned with tetrachlormethane and the finest polishing paper. Electrical testing only rarely gives any results here.

3) *Faults distorting the function of the apparatus*

This heterogenous group is repaired either as under 1) or sub 2), if it belongs here, or some phenomena of these types are repaired specifically.

a) Incorrect stimulus frequency (double the frequency set, 50 c/sec., 100 c/sec.) may occur if the frames of various parts of the apparatus are not connected together. It may be due to faulty earthing, to disconnection of inputs and outputs, to "antennas" ("receiving" and "transmitting") in different stages.

b) Large hum has the same causes as in a). For elimination of residual hum see below. Sometimes phenomena a) or b) are due to complete insulation of heating from earth and frame and may appear only after some time, when the filament-cathode resistances change. The origin of hum is often ascertainable from the frequency (or phase shift). 50 c/sec. is hum from the mains. 100 c/sec. is hum either from the anode supply, the valve heating or from the mains. In a halfwave rectifier for anode voltage a hum of 50 c/sec. may also occur. In USA the mains frequency is 60 c/sec. and the corresponding frequencies are 60 or 120 c/sec.

c) Large noise is either due to interruption of some resistor or bad contact of its leads, dirty contacts, "dry joint", inoperative valve. Sometimes the

cause lies in a faulty anode battery, although the full voltage is still indicated by the voltmeter.

Faults belonging to this group can also be elicited by voltage changes of the regulated supplies.

4) Inconspicuous faults

The faults in this group must be systematically eliminated. They are usually not due to the electrical apparatus but lie in the experimental design, the preparation, chamber, electrodes, the mains, the electromagnetic field in the laboratory, and often it is very difficult to find their exact cause. In the description below it is assumed that the individual apparatus themselves are in order.

a) *Stimulation.* This is best tested in a nerve-muscle preparation, i. e. without electrical recording.

α) The stimulator does not stimulate. The excitability of the preparation is tested with a galvanic forceps. If that is good, the fault is searched for elsewhere. The supply of the stimulus may be interrupted or there is a short circuit between the electrodes or the leads. This short circuit is often due to an improper connection of wires: The directly earthed electrode is connected to the active output terminal and the earthed output terminal to the other, unearthed electrode.

β) The stimulator stimulates even when the amplitude of the stimulus is decreased to zero or when switched off. Either a large parasitic voltage enters the output (e. g. capacitance transmitted impulses from the preceding stage, hum, etc.) or various external voltages - e. g. from sparking electromotors, X-ray instruments, etc. — are induced in the stimulating lead because of bad screening and a large impedance of the stimulator output.

b) *Registration.* When registering action potentials the danger of errors, interference and artifacts is even larger. Much thorough knowledge and experience are needed for awareness of these posibilities. Repeated testing of the experimental set-up and continuous checking of all variables are necessary.

Interferences

Stimulation artifacts

A stimulus artifact occurs when the stimulating pulse is transmitted to the recording apparatus. This transmission leads along several pathways and as the relative participation of these paths changes, the amplitude and shape

of the stimulus artifact may change considerably even during a single experiment.

a) The resistive component. The current flowing through the tissue between the stimulating electrodes during the application of the pulse passes through a volume conductor. An electric field thus produced is characterised by two systems of surfaces: tubes of electrical flux and equipotential surfaces. No potential difference is obtained at the recording electrodes only if both electrodes are in the same equipotential plane.

In addition to the connection of both pairs of electrodes across the tissue, however, they are also directly connected by external resistances, especially by different resistances of each electrode against the frame (earth). Part of the balancing current flows through them and produces a drop across them. The drop across the recording electrode resistance is then also recorded as an artifact. On the other hand, a stimulating voltage reaches the recording electrodes through the external resistances. Thus, the stimulating voltage can produce excitation or at least subthreshold changes in the substrate also at the recording electrodes. This, of course, is valid not only for the recording electrodes but for all other electrodes or metal contacts, especially earthing leads. There is only one necessary, even if not sufficient, condition under which this cannot occur: if the resistance of all electrodes against each other is infinitely large (excepting the resistance between stimulating electrodes of the same pair).

From this condition follows another one: If n electrodes are connected to the tissue, at least $n - 1$ electrodes must have an infinitely large resistance against the frame (earth). Evidently it is most important that no electrode save one is ever directly earthed, for otherwise stimulation might occur below any or even every earthed electrode.

b) The rapid capacitance component. As far as the volume conductor itself is concerned, the capacitance currents may be neglected in the first approximation. Capacitance currents between the electrodes, however, are all the more important. As in the case of the resistive component, capacitative connection of the electrodes also produces a voltage drop across the recording electrodes and this has a time course different from that across the ohmic resistances. Since the capacitances between the electrodes are small, often only potentials corresponding to the differentiated stimulating pulse are transmitted capacitatively.

c) The slow capacitance component. Schäfer (1936) explains the slow disappearance of the artifact by charging and discharging of the common capacitance of the electrodes against earth (Fig. 54). When switching on the stimulus, the stimulator-earth capacitance is charged. The charging current flows to the other plate of the condenser through earth via stimulating elec-

trodes — recording electrodes (interrupted line in Fig. 54). A voltage drop is thus formed across the leak resistances of the recording electrodes.

After the stimulus is over, this capacitance is discharged along the same path and an opposite voltage drop is produced. Since the longitudinal resistance of a nerve can reach a maximum value of about $10^7\ \Omega$, the capacitance against

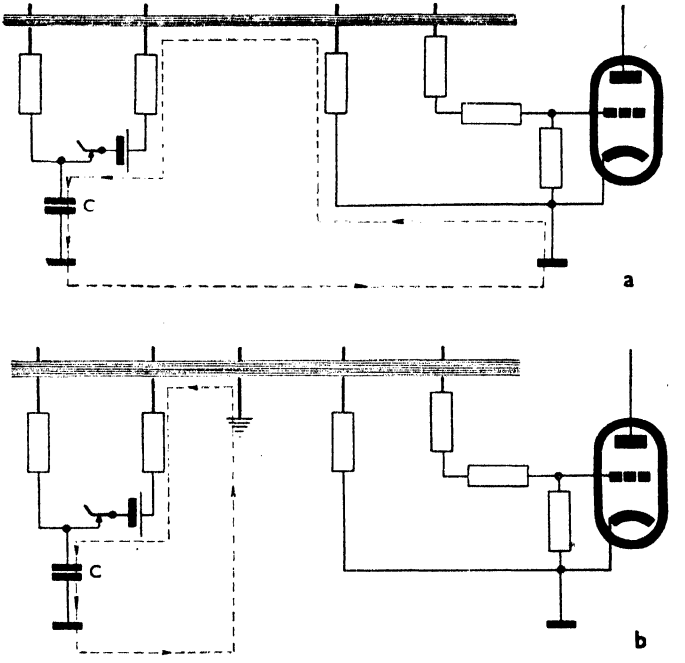


Fig. 54. a) The origin of a stimulus artifact (Schäfer, 1940). *C* — capacitance of stimulator against earth. b) removal of artifact by earthing between the stimulator and amplifier.

earth maximum 1000 pF, $\tau = 10^7 \cdot 10^{-9} = 10^{-2}$ sec., which is of the same order as the measured values.

Today, electronic stimulators with a small output resistance against earth are usually used. The prolonged tail of the stimulus artifact, however, although smaller, is still present. The whole artifact, including the tail, may be considerably reduced by direct earthing of one stimulating electrode or by earthing the object (see p. 326). A number of further problems, however, arises if this is done (see above). The stimulus artifact may be nearly eliminated by completely separating the stimulating electrodes from earth (resistive and capacitative insulation — see page 89). This shows that the causes of the artifact are those described above and that the slow capacitance component (tail) of the artifact is due to current loops reaching far from the stimulating electrodes.

d) The polarization component: The current coming from an unearthed stimulating electrode divides into a larger part flowing to the second stimulating electrode and a smaller one flowing to all electrodes through their earth leaks. These currents (D. C. pulses) produce polarisation of all electrodes accordingly to the current. The polarising voltage at the recording electrodes remains there for a long time, since the equivalent capacitance which could be substituted for the polarising cell is enormous and the discharging resistances are fairly large. It is clear that, all other things being equal, the polarising component is largest for earthed electrodes. This is effectively reduced by the same means as used for the capacitative component (especially the slow one from which the polarising component can be distinguished only with difficulty).

The last traces of the stimulus artifact are usually not eliminated, but serve as the zero-point of the time axis. If they are to be eliminated completely, again a radiofrequency transformer at the stimulator output (see p. 89) or, in addition, radiofrequency recording (see p. 122) is used rather than bridge equilibration of the artifact transmission (Bishop 1927, 1928, 1929). For other techniques of artifact elimination see p. 323.

Artifacts due to improper leading off of bioelectric potentials

a) The single ended amplifier. This requires one recording electrode to be connected with the input valve cathode and thus also the frame of the amplifier and earth. The grid connected with the recording electrode then records the potential difference between this electrodes and earth, i. e. between this electrode and all points connected to earth, even across rather large resistances. This gives rise to the long known mutual influence of several bipolar leads, since all leads, though having different recording electrodes and amplifiers, are connected through earth. Thus it is impossible to define the contribution of individual electrode to the recording, which may lead to erroneous conclusions. (For example, when recording action potentials in isolated nerves, polyphasic curves are obtained simulating different conduction velocities.) Several leads with asymmetrical amplifiers may only be used for monopolar recordings, the common earth of all amplifiers being connected to only a single common reference electrode or to an artificial average electrode analogous to the Wilson electrode used in ECG (see p. 323).

b) A symmetrical amplifier records the potential difference between the grids, which in the apparatus usually have a resistance of 0·5—5 M against earth. Nervous tissue, however, to which the grids are connected, often has only a small resistance, and thus not only the grid-to-grid resistance of the same amplifier, but also the resistance to neighbouring amplifier grids may be

decreased considerably. In addition, and this is of particular importance, the grid resistance towards earth, mainly if some part of the object is earthed (indifferent electrode of another recording system, stimulating electrode, etc.) may also be decreased. From this aspect, the earthing lead used to eliminate the stimulus artifact is dangerous, since it is usually near to electrodes. This then produces a situation identical to that in an asymmetrical amplifier (see above): again, in one channel, potentials from several sites are recorded simultaneously (e. g. from the earthing plate and the recording electrode nearer to it against the other recording electrode). Mutual dependence (interaction) of the leads is thus produced. Potentials of extremely small amplitude such as synaptic potentials, extrinsic potentials, local potentials, etc. are interfered with by artifacts or even simulated by them the more the smaller they are and the more complex the total layout of the experiment (a larger number of stimulating and recording systems, small interelectrode distances etc.) See e. g. Zachar 1955 a, b, c.

The source of such artifacts is most frequently in the recording of potentials at other points or of another kind (those having a higher amplitude, e. g. of the stimulus or action potential), which are distorted and reduced during transmission and even may simulate the phenomenon looked for.

Determination of artifacts

There is no universal prescription for detecting artifacts. If often happens that phenomena are found which may either be genuine or artifacts, and it may take days or even weeks to decide which is the case. It is therefore necessary:

1) To prevent artifacts by using appropriate equipment: radio frequency stimulus transmission, symmetrical amplifiers with a large discrimination factor, radiofrequency transmission of the input signal, resistive and capacitative insulation of all electrodes from earth.

2) To test systematically the character of all parts of the recorded curve, using physical and electrotechnical means:

a) To observe changes in the curve on changing the amplitude and duration of the stimulus:

α) Action potentials. Preparations with a small number (up to ten) of fibres change the shape of their action potentials in steps, and those with more fibres (the whole nerve) gradually, but the amplitude increases only up to a certain value. On further increasing the stimulus, the action potential amplitude remains either unchanged or even decreases.

β) Local potentials immediately caused by the electrical stimulus increase continuously with the stimulus as a "smooth", usually progressive

function of the latter. A step-like change occurs for strong stimuli (the onset of an impulse).

γ) Local potentials due to nerve impulses (synaptic, extrinsic potentials, etc.) increase with the number of conducting fibres. They cannot be further increased after the maximum stimulus is reached.

δ) The stimulus artifact is directly proportional to the amplitude of the stimulus.

b) To determine the behaviour of all parts of the curve when interchanging the recording electrodes:

α) If the curve (wave) studied does not change its polarity, its source may either be in the amplifier itself (disturbances acting on it, etc.) or the potential is recorded from a very distant electrode somehow connected to any of the recording electrodes. Of course, this is so only if the connection is realised directly in the amplifier (e. g. in an asymmetrical amplifier).

β) The wave changes its polarity. In this case, either the actual potential difference between recording electrodes is measured, or that between a distant electrode connected with one recording electrode, if this connection is made directly in the electrode circuit. This, however, occurs only rarely.

As far as the stimulating artifact is concerned, it can be seen that it may, but need not, change its polarity on commutation of recording electrodes. This depends upon the way and point at which it enters the recording arrangement.

c) To determine analogous changes in the curve when changing the polarity of the stimulus. Let us first assume that the nerve impulse arises at least approximately at the same point as before.

α) The recorded wave does not change its polarity. In that case, either a real bioelectric potential is recorded (either between the recording electrodes or from another point, e. g. the earthing plate) or a stimulus artifact entering the recording arrangement not through the preparation or capacitive or resistive leakage between the leads, but directly from the stimulating apparatus.

β) The wave changes its polarity. This is a stimulus artifact passing along the usual paths to the recording electrodes.

γ) The reversal of the stimulus polarity produces changes in the amplitude and time relations of the individual parts of the curve.

The amplitude of the stimulus artifact increases (decreases) if the earthed stimulating electrode is nearer to (further away from) the recording electrodes.

The distance of the wave from the stimulus artifact decreases (increases) slightly if the impulse arises below the nearer (more distant) stimulating electrode. The distance changes considerably if the stimulus arises below one stimulating electrode and after commutation at another distant point, which is usually earthed. The conduction times must then be calculated for more

accurate orientation. At the same time, the amplitude and shape of the response may also change considerably. At first it often suffices to disconnect the earthing lead; the changed time relations indicate that the point of stimulation or recording has changed.

d) Changes in the recorded waves during changes in the physical and physico-chemical state of the preparation and electrodes may be determined.

α) The resistance between the stimulating electrodes may be changed by either wetting them with a drop of Ringer solution or drying them with a piece of filter paper. This changes the current passing through the tissue (especially in stimulators with a "constant current" output) and the current density at those points at which an impulse is evoked. The stimulation threshold is thus changed. This is true only if the stimulation occurs in the region of the stimulating electrodes.

β) The resistance between the recording electrodes can be changed in an analogous manner. This changes the amplitude of all potential waves in recording of which both grids participate. Only potential changes entering the recording apparatus otherwise than through grids of the first stage do not become altered.

γ) It is also possible to change in this manner the nerve resistance between the stimulating and recording electrode pairs. If excitability changes on decreasing the longitudinal resistance, it is probable that excitation is produced not at the stimulating electrodes but elsewhere. If the wave amplitude is increased, probably this wave is due to the conductance or polarisation component of the stimulus artifact. (The capacitance, resistance or polarisation artifact component may, however, also be decreased by reduced shunting resistance.) In a similar way, the amplitude of potentials not due to impulses may be changed. Each concrete case, however, must be analysed individually. The amplitude of impulse potentials, however, hardly changes at all if actually recorded from between the recording electrodes.

e) Changes in the recording due to impairment of the normal function of the preparation:

α) Blocks due to ions, drugs, cold, etc. (see p. 221) are included here. Only the stimulus artifact remains unchanged. By suitably choosing the site of the block, an attempt is made to obtain an answer to questions such as: Is the registered potential in fact recorded from the recording electrodes?

β) Interruption of transmission in the nerve due to mechanical injury (squeezing) provide the answer to the question mentioned above. The action potentials evoked by the mechanical stimulus, if a forceps of nonconductive material is used, may also provide a clue.

γ) Severing the nerve interrupts the nerve not only as a physiological, but also as an electrical conductor.

δ) More gently (e. g. without moving the nerve along the electrodes) the blocking of transmission and interruption of the nerve as a conductor may be brought about using local rapid cooling (solid CO_2, application of methyl- or ethyl-chloride). The ice, even though containing salt impurities, has a specific resistance (depending upon the rate of freezing and the temperature) of 10^5 to 10^6 Ωcm, i. e. about 1000 to 10 000 times higher than nervous tissue.

By local freezing, the nerve may also be insulated from some of the electrodes and the unipolar, apolar and other aberrant stimuli may be determined. The entry of the stimulus into the surrounding solution, interrupted insulation of the electrode leads, recording of potentials coming through the faulty insulation inside the chamber, etc., may be detected in the same way.

ε) The whole nerve is killed, as far as possible without impairing its passive electrical characteristics, using ammonia, ammonia fumes, formalin, formaldehyde fumes, heat etc. It is then necessary to maintain or renew normal moisture of the preparation. Only the stimulus and other artifacts are preserved.

ζ) A physical conductor (wick thread, band of filter paper) soaked in Ringer solution is substituted for the nerve.

η) The behaviour of the apparatus is determined without any object at the electrodes.

Hum and other interferences limiting the recording of low amplitude signals

Hum is an A. C. artificial voltage of a certain frequency entering the recording apparatus from outside or inside either directly in the form of an electromagnetic field or as mechanical oscillations transformed to electrical ones in the recording apparatus. Hum in the narrower sense of the term is the basic frequency of the mains (50, 60 or 40 c/sec.) and its second and perhaps also higher harmonics. Low frequency hum is often due to electric motors, from which it spreads electromagnetically or mechanically. High frequency hum, especially if not continuous, is often due to bouncing of the contacts, sparking of the motor brushes, etc.

Hum is usually reduced in two ways: at the source by limiting the spread of the hum and in the recording apparatus by protecting it from the effects of hum. Protection of the recording apparatus never eliminates hum completely, but only reduces it to a certain extent, and further reduction becomes more and more difficult. Hence it is necessary to determine exactly the part played by the individual sources of hum in the formation of its final amplitude in the recording apparatus.

A. Internal hum is not affected by changing the position of the apparatus and persists on disconnecting the input. It is due to bad filtration of the anode voltage supply (50 c/sec. for halfwave, 100 c/sec. for fullwave rectifiers), to heating of the valves with A. C. current (100 c/sec.), to the stray field of the mains transformer (50 c/sec., 100 c/sec.), to mechanical oscillations of loose core-laminations of the transformer (100 c/sec.).

B. External hum increases with the size of the "antenna" connected to the input and with the resistance between the electrodes (electrostatic hum) or with the number of turns, diameter and core permeability of the coil connected to the input electrodes. In both cases its size depends on the orientation of the antenna or coil. External static hum usually has a frequency of 50 c/sec., magnetic 50, 100, 150, 300 c/sec. The size of static hum depends on the intensity of the electrostatic field, i. e. for the same distance of the source of the disturbance, it is proportional to the voltage. The most important sources of static hum thus are high voltage lines, high voltage transformers, X-ray apparatus, neon lights, etc. With the source of the disturbance remaining at the same distance, the size of magnetic hum is proportional to the current and length of the conductor or the diameter and number of turns of the coil and considerably depends on the shape of the core. It is much larger for an open core than for a closed one. The largest sources of magnetic hum thus are high power electric equipment for low voltages (apparatus for charging large accumulators or for electroplating, large motors, electric trains using A. C. current, electric furnaces).

Parts of the recording apparatus itself may also be a source of external hum if their field can affect the input electrodes.

Mechanical oscillations are a special source of external hum. If not originating within the recording apparatus, they are easily identified by testing the effect of placing the apparatus on a soft base (sand, felt, foam-rubber).

After determining the hum source and its propagation (by an electric or magnetic field) it is reduced:

1) At the source. Static sources are screened. Conductors (at a safe distance) are covered with an earthed conductive screen. Sparking contacts and brushes are connected to *LC* filters and the whole apparatus is again screened by earthing the metal box.

Magnetic sources may be screened only by using one or more solid, soldered covers of soft iron or alloys. Since such a cover may have considerable dimensions, weight and price, it is often not used and the effect of the source is reduced by placing it further away and by suitable orientation of the recording apparatus.

Sources of mechanical vibrations are eliminated by resilient mounting of movable elements, addition of antiresonant weights, introduction of mech-

anical and acoustic isolation (dilating slits), pads and partitions made of foam glass or foam plastics, by placing rubber, felt or sand pads under the apparatus, etc.

2) The recording apparatus is then treated so as to decrease hum at least to a size that does not prevent the performance of the necessary electrophysiological measurements. Fundamentally the same means are used as under 1).

a) The propagation of the disturbing voltage along mains lines and earthing leads is reduced. Mains lines are supplied with *LC* filters and enclosed in strongwalled earthed tubing (so-called screwed tubing).

In the mains-supplied transformers, static screening is applied using one (open!) winding of copper plate between the primary and secondary winding (this only reduces the H. F. components, especially discharges and sparking brushes, X-ray interference, diathermy, etc.). The earthing connection must be as short as possible, using a thick wire (about 5 mm in diameter) leading directly to earth, where it ends at a depth of 1—2 m in a large copper plate (1—3 m^2). The earth lead must never be loaded with current. This might produce a voltage drop acting as a source of static interference. The current source might cause magnetic interference and the life span of the earthing plate would also be shortened, since it would be corroded by electrolytic processes. Consequently, the earthing wire must never be connected to the neutral conductor of the mains. This, it is true, is also earthed somewhere, but since usually a large current is passing through it (and in towns also through earth) a certain, even if small, voltage drop arises across it. In view of the very low resistances of the neutral conductor and the laboratory earthing, this small difference might initiate very large currents. At our institute, a potential difference of 0·2 V was found between the laboratory earth and the neutral conductor of the mains. Thus a current of 5 A arose when connecting these both grounds across a meter with a small resistance. In view of the fact that the internal resistance of the ammeter formed the larger part of the inserted resistance, the current on direct connection of the two earths would be still larger. It follows that it is always necessary to check whether such a direct short-circuit is not present in the system. It is most easily formed as follows: A mains-supplied apparatus is connected by a three pronged plug to the mains, and its box is regularly connected directly to the neutral mains conductor. If the cover is now earthed unintentionally (directly or through other parts of the apparatus) to the special laboratory earth, the short circuit is formed. This may be avoided in several ways: most effectively by simple adaptation of the mains cable (which is usually against safety regulation). Either the earthing conductor is disconnected in the outlet (care must be taken not to exchange the mains lines) or in the mains three point cable plug the connection to the earth point is disconnected. The cover

and frame of the apparatus are connected to laboratory earth permanently, so as to comply with safety regulations. The above case is a special and malicious example of an earth loop caused by earthing various parts of the circuit at several points. Such earth loops must be avoided for reasons mentioned here and sub b) and c).

b) The least sensitive parts of the apparatus are screened electrically. Care must be taken to prevent sizable currents from flowing through the screening covers. All covers must have exactly the same potential (this is achieved by connecting them at one point). Often the experimental animal is screened together with the preamplifier, and sometimes also with the experimenter, in a metal box called Faraday's cage. If it has double walls, the two layers, separated by a distance of several cm, are connected only in one corner from which the earthing wire is led. All screening cores are connected to this corner. The inside of the box is connected to the apparatus outside by screened cables, the screening of which is also connected to the common earthing point. The frames of these apparatus are earthed at the same point. The screened cable passing through the box must be insulated from it and as short as possible. If these principles are not adhered to, interference voltages may arise, due to currents flowing in the earthing conductors or to magnetic induction.

c) Magnetic screening. Usually the voltage induced in conductors by the A. C. magnetic field of the interfering source are so small that magnetic screening is not necessary. It is sufficient to prevent conductors from forming loops with a large cross section to make them as short as possible and to lead them in the direction of the lines of force. Magnetic hum is of greater significance for sensitive apparatus working with larger currents (galvanometers, loop oscillographs, string oscillographs), particularly if they have small internal and external resistances. Sometimes the cathode-ray oscilloscope may also be affected. If magnetic screening is necessary, this is done by using a closed box of soft iron or iron alloys, in several layers if possible. Usually combined static and magnetic screening is used: the covers of the apparatus are made of solid iron plates soldered at the corners and edges. Faraday's cages are made in a similar way, but here dense rivetting is sufficient. They nearly always have two layers.

d) Finally, hum may be compensated. Only primitive compensation using interfering voltages themselves is used: we test whether the interfering voltage decreases at the amplifier input when applying and earthing conductors, when changing the position of the loops when changing main plugs (turning them), when changing the phases (R, S, or T) which supply this or other apparatus, etc.

e) mechanical vibrations of the recording apparatus are eliminated as described under 1).

f) If interference becomes worse in dry weather the soil in which the earthing plates are buried is moistened.

g) In exceptional cases, when recording D. C. and very slow potentials, hum is eliminated by cutting off frequencies higher than about 30 c/sec. using *LC* or, more rarely, *RC* filters.

Electrodes

Different types of electrodes are used in electrophysiology, depending upon the requirements for accuracy, reproducibility of results, etc.

Often simple metal electrodes are used: wires and plates of silver, platinum, nickel, stainless steel, tungsten, etc. They are simple and usually have a small resistance. They are disadvantageous in chronic experiments with long stimulation periods. Ions from the electrodes pass into the tissue under the action of current and may have a toxic effect. Other difficulties occur when recording D. C. potentials. They are due to the formation of electrochemical cells. Consequently, nonpolarisable electrodes must be used for exact measurements.

Calomel electrodes

The metal is mercury covered by a layer of calomel (Hg_2Cl_2), suspended in saturated or dilute (0·9%) KCl or NaCl. If the electrode forms the anode, anions from the tissue accumulate on it, especially Cl^-. This forms Hg_2Cl_2 at the electrode and thus the latter's quality does not change. If the electrode acts as the cathode, tissue cations react with the chloride ion of the calomel and thus release mercury. Again the electrode remains qualitatively unchanged.

Fig. 55 a, b, c shows how to prepare a calomel electrode. Distilled mercury is covered with calomel, thoroughly washed with 0·9% NaCl. Two such electrodes are connected by a 0·9% NaCl bridge. A small D. C. current (up to 1 mA) is permitted to pass through the electrodes alternately in one and then the other direction. After this the electrodes have no voltage between them. The electrode pairs are always stored shortcircuited. When measuring, they are connected to the tissue via a liquid or agar bridge: about 2% agar is added to a NaCl or KCl solution. After swelling the solution is boiled and poured into a bent tube (avoiding bubbles). After it has cooled, the electrode is connected to the tissue through this tube containing the agar gel. The electrode may also be separated from the tissue by cellophane. This has a low resistance and takes up little space.

Silver chloride electrodes

In principle the Ag-AgCl electrode is similar to the calomel electrode. A thin layer of AgCl is formed electrochemically on the silver electrode surface. Its preparation is less difficult, it takes up less space and is easier to handle than calomel electrodes. The electrode must be protected from light, drying and friction. It is, therefore, best to store it in a special dark vessel in 0·9% NaCl and to connect this to the tissue via a liquid or agar bridge or with the aid of celophane. If there is little space, the electrode may be placed directly in the tissue or connected to it with a thin silk or cotton wick. Great care must be taken to prevent drying and mechanical injury to the AgCl layer.

Electrolytic chlorination is carried out with a very weak current density (about 0·1 to 10 A/m²) overnight if possible, and always in the dark. Both electrodes of the same pair are connected in parallel as the anode, a piece of

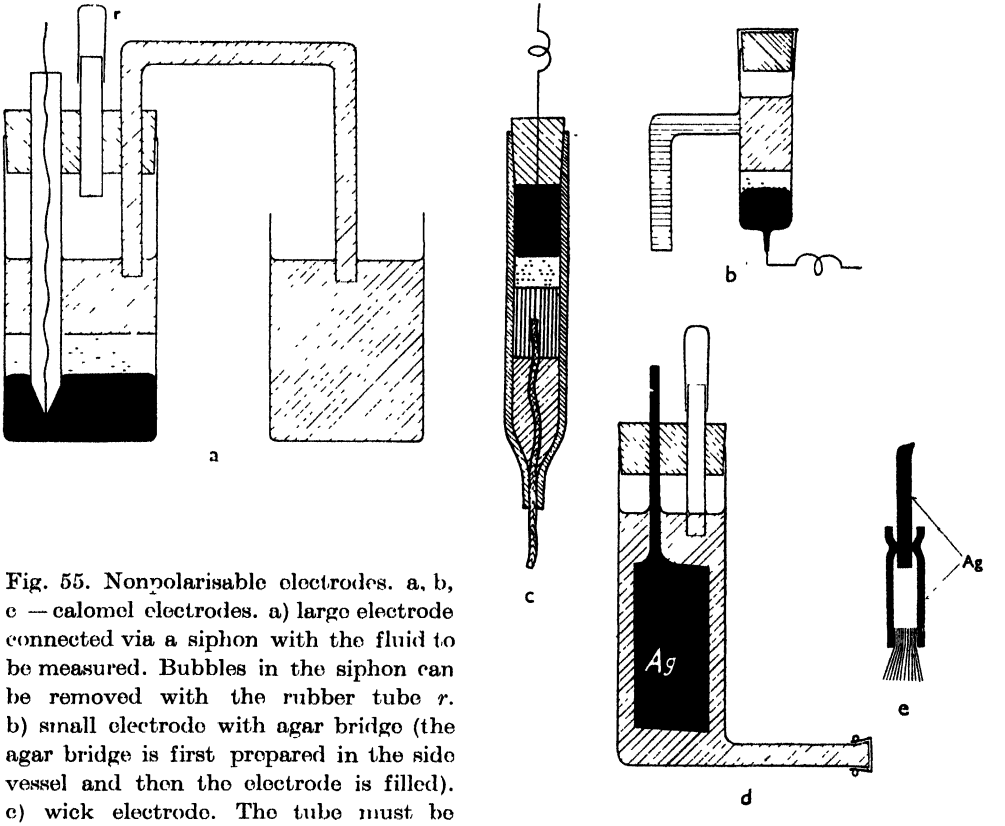


Fig. 55. Nonpolarisable electrodes. a, b, c — calomel electrodes. a) large electrode connected via a siphon with the fluid to be measured. Bubbles in the siphon can be removed with the rubber tube *r*. b) small electrode with agar bridge (the agar bridge is first prepared in the side vessel and then the electrode is filled). c) wick electrode. The tube must be narrow so that cohesive forces can maintain a mercury drop in the upper end of the tube. Below the mercury is calomel and a cotton wool stopper through which the wick passes. The lower part is filled with 0·9% NaCl, d,e) Ag-AgCl electrodes, d) large, e) small wick electrode (magnified).

silver plate being the cathode. NaCl (0·9%) is the electrolyte. The lower the current density used, the lower will be the final electrode resistance. The quality of the electrodes should be tested. During electrolysis the electrode resistance rises rapidly at first and after several minutes to hours drops considerably and attains a purely ohmic character. For measuring this resistance see Vogel and Kryšpín (1956). Two types of Ag-AgCl electrodes are shown in Fig. 55 d and e.

Metal microelectrodes

Use: These are suitable for extracellular recording of action potentials or synaptic activity. They cannot be used for recording D. C. potential differences (polarisation, contact potentials).

Material: Stainless steel or tungsten wire, diameter 0·15—0·3 mm. The wire is gradually heated to red and straightened by slight stretching. The surface is thoroughly cleaned with sand paper. Pieces 5—6 cm in length are cut from the wire. These are bent to an angle of 45° one cm from their end and half their length is inserted into injection needles (diameter 0·4 mm). The bend ensures a good electrical contact with the wall of the needle. Finally, fat is removed from the wire by immersing it in carbon tetrachloride.

Electrolytic treatment. The carrier needle is fixed into a vertically movable holder in such a way that the wire is well visible in a microscope horizontally-placed (Fig. 56). A U-shaped vessel filled with 10—20% HCl is placed below the end of the wire. The holder of this vessel can also be moved vertically. By alternately lifting and lowering the vessel, the wire is immersed and pulled out of the acid. A positive pole (1·5—3·0 V) of a dry cell is connected to the electrode, the negative pole (silver foil) to the electrolyte. First 10—15 mm of wire are immersed into the acid and current is permitted to flow for 30—60 sec. The wire is then pulled out and carefully cleaned with a brush soaked in distilled water. Narrowing of the tip is observed under the microscope. The electrode is again immersed for a shorter period and to a lesser depth and the whole procedure is repeated until the tip is sufficiently narrow (about 2—5 μ). The length of the tip is also important. 100 μ from the tip the electrode should not be less than 15 μ nor more than 20 μ in diameter. During the final stages

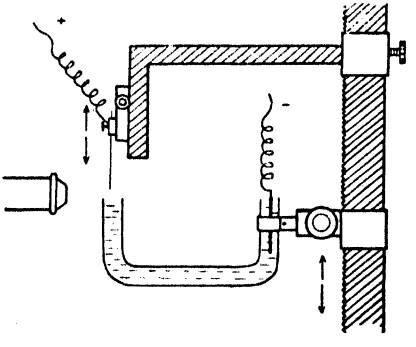


Fig. 56. Electrolytic sharpening of metal semi-microelectrodes (see text).

only the tip is immersed, for only 1—2 sec. When the desired shape is attained, the electrode is removed from the holder, washed with water and thorougly dried in an air stream. A. C. current may also by used for electrolysis (6 V).

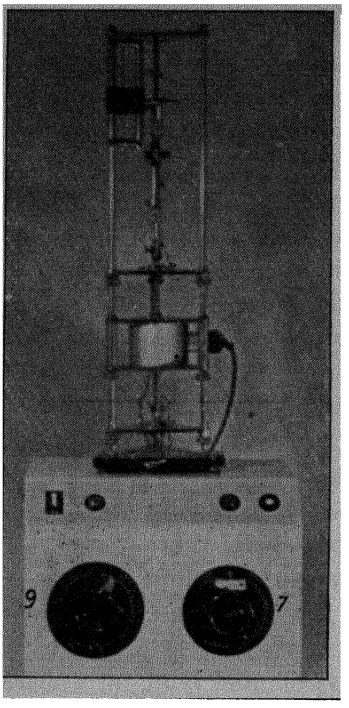
Insulation. Various substances may be used for insulation. Their choice, however, is not decisive. They vary from ordinary nail varnish to special varnishes with exact descriptions for use. The whole electrode, together with several cm of the needle, is immersed in the varnish and then slowly pulled out. The slow backward movement prevents formation of drops along the electrode. It is best to use a mechanical device pulling the electrode continuously out of the varnish (e. g. an electromotor driving a screw). In another method of insulation, the needle tip leaves the varnish first. The electrode is completely submerged. Just before pulling it out air is blown on the surface of the varnish and thus a fine layer contributes to better insulation of the tip, which due to surface tension remains uninsulated. Depending on the type of varnish used the electrode is dried in air or in an oven, usually with its tip facing upwards. If necessary varnishing is repeated several times.

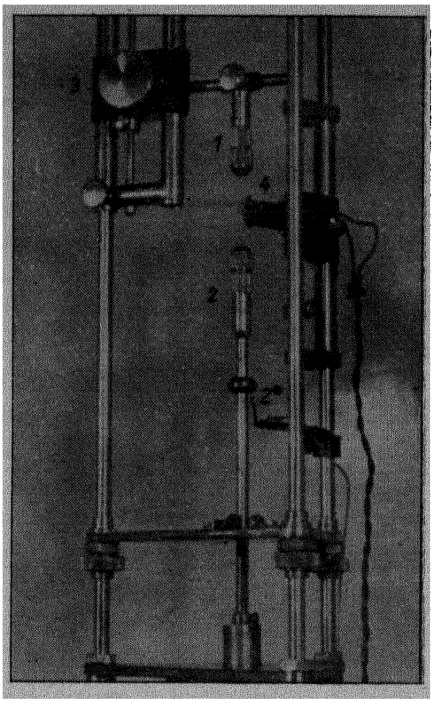
Checking insulation. Before use it is necessary to determine the size of the uninsulated tip and flaws in insulation along the electrode. This is done most simply by connecting the electrode to the negative (!) pole of a 1·5 V battery and moving it in a Petri dish filled with saline and lying on a black bottom. Where insulation is incomplete, small chains of bubbles are formed, which are easily visible under direct illumination. An electrode forming bubbles only at its tip is satisfactory. Its resistance is less than 50—200 KΩ for a diameter of 3—6 μ.

Metal ultramicroelectrodes (tip about 1 μ) cannot be tested in such a way, since their resistance is too large. For them it is sufficient to measure their resistance. Only those electrodes, whose shape is satisfactory under the microscope and whose resistance is between 5—15 MΩ are used. The resistance is measured with an electrometric apparatus (a cathode-ray oscilloscope is best) as the drop across the resistance connected in series with the source and the micro-electrode.

In larger electrodes it is possible to check the insulation along the needle directly by connecting a sine wave voltage from a tone generator (0·1—0·5 V, 300 c/sec.) to the electrode, the other terminal being connected through an ear phone to a strip of moist filter paper. The electrode is gradually pushed through the paper and the intensity of sound in the ear phone directly reflects even very fine disturbances in insulation.

Storage of electrodes. It is best to prepare a supply of electrodes and then to use them as needed. They must be protected from mechanical damage and chemical influences. It is best to store them, tip upward, in a layer of plasticine or soft wax in large desiccators. Before use electrodes should be examined under the microscope and their resistance tested.





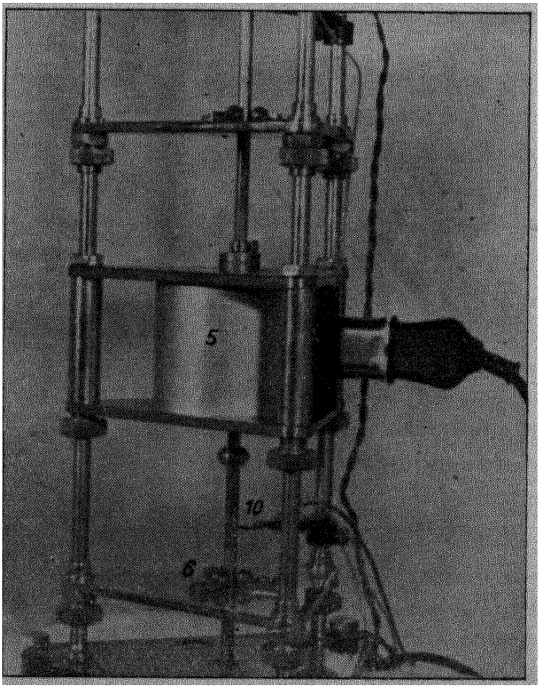


Fig. 57. Apparatus for producing glass microelectrodes (for detailed description see text).

Glass microelectrodes (or ultramicroelectrodes)

These are glass tubes with a conical capillary tip smaller than 1 μ in outer diameter. They are filled with salt solutions (usually 3M-KCl). The ions form an internal conductive medium.

Use: They are suitable for intracellular recording of impulse activity and for measuring membrane potentials. If filled with suitable solution they can also be used for intracellular injections.

Production: They are made from hard (e. g. Pyrex) glass tubing, usually 1—7 mm in diameter. Techniques differ, from pulling by hand to automatic devices. Pulling, however, always proceeds in several (2—3) stages. First the tube is narrowed to 50—100 μ, then to about 10—20 μ and finally to 1 μ or less. The second stage is often omitted.

At first microelectrodes were extended manually (Ling and Gerard 1949, Nastuk and Hodgkin 1950). This is time-consuming, and the size and shape of the tips tend to vary. Production then became more automatic. A heated platinum wire was used instead of an open flame and weights were used. Different types of laboratory microforges were described for this purpose (Fonbrune 1932, Kurella 1958). Finally production was made fully automatic (Du Bois 1931, Alexander and Nastuk 1953, Winsburry 1955, Beránek and Vít 1958).

The pulling machine, similar in principle to that of Winsburry, is shown in Fig. 57.

A pyrex glass tube, diameter 3 mm (wall strength about 0·5 mm), 8 cm long, is fixed to holders (1, 2) and passes through a platinum spiral (3—5 turns of platinum wire, 0·5 mm). One holder is fixed to a rack and pinion (3). The spiral, fixed in a ceramic holder (4), can be moved vertically by another rack and pinion.

The second, lower holder is on a stainless steel shaft and has a core attached to it which is pulled into the solenoid (5) after the current is switched on. The lower shaft moves on two sets of ball bearings, each of which consists of three bearings 120° apart (6).

After mounting the glass tube, the platinum is heated to a bright orange colour. The current is regulated by rheostat (7). Suitable heating has to be found empirically. When the glass begins to melt, it is pulled by the weight of the lower shaft and the core. The lower shaft has a small elevation which presses on a contact (2·) when the shaft moves downward. This pull decreases the tube diameter to 0·1—0·2 mm. The length of this sector is given by the distance between the elevation on the lower shaft and the contact, as measured before heating is started. The contact (2·) permits a current to pass into the solenoid which can be changed by an autotransformer (9). This pull extends

the narrowed tube to the required diameter. The necessary pull for the required diameter and length of the capillary must also be found experimentally.

Finally, an elevation (10) on the lower shaft breaks the current in the solenoid and the heating circuit is switched off. It is evident from the description that the pull is fully automatised. Once the values for heating, solenoid current and contact distances are found, production is rapid and electrodes are uniform. Two electrodes can be made from one piece of standard glass within 1—2 minutes.

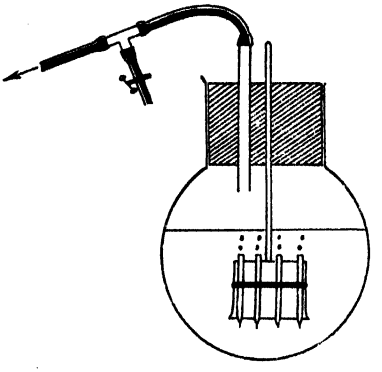


Fig. 58. Filling of glass microelectrodes.

Filling the electrodes: The microelectrodes are fixed to the wall of a glass beaker with their tips facing downward (Fig. 58). A glass rod is fused to the bottom of the beaker and pushed into the rubber stopper of the vessel. The stopper contains a glass tube connected to a water suction pump. The vessel is filled with 3M-KCl so that the electrodes are completely immersed. The solution is heated to boiling point and steam is slowly sucked off. Small bubbles begin to leave the wide ends of the electrodes indicating that these are being filled. When no more bubbles emerge, the electrodes are full. The whole procedure lasts 10—15 min.

Some workers prefer to fill the electrodes by boiling in distilled water or methylalcohol and exchanging these solutions for that with which the electrode is to be filled. The electrodes are simply placed into the required solution for several weeks. This is a good procedure if electrodes are used for microinjections. Filling, however, lasts a longer time.

The electrodes are filled without boiling as follows (Caldwell and Downing 1955, Kurella 1958): The electrode tip is immersed in the electrolyte. Capillary forces enable the solution to attain a certain height. Then filling proceeds more slowly. To speed up this process, distilled water is poured into the wide end. An air bubble remains between both solutions. Because of the difference in vapour pressure water distils across the air bubble to the 3M-KCl. The air bubble reaches the wide part of the electrode within 1—3 days. The electrode is then easily filled with the electrolyte.

Storing and measuring: Electrodes are stored in Petri dishes in a 3M-KCl solution. They cannot be kept for too long, since crystals of KCl begin to form. It is best to prepare the electrodes just before the experiment.

Before starting to work with a microelectrode it is best to check its shape and size under the microscope. If production is automatised, the resistance of the electrode is a good indicator of the size of its tip (cf. p. 161).

Remarks: Microelectrodes with a diameter of about 0·5 μ have a resistance of about 20—40 MΩ. This increases with decreasing diameter. A sudden drop usually indicates breaking of the tip.

In addition to simple microelectrodes, double barreled ones are used either for simultaneous recording and stimulation or for simultaneous recording and intracellular injection using suitably directed electric currents (Eccles 1957, Castillo and Katz 1957).

For recording from moving organs, microelectrode tips connected to the amplifying apparatus by a fine flexible wire are used (Woodbury and Brady 1956).

Metals can be used instead of electrolytes for filling capillary electrodes (Svaetichin 1951). Their resistance is low, but they cannot be used for measuring D. C. potential differences.

III

General electrophysiology of cells and tissues

A. Electric potentials of cells

Electric potential differences in living matter are related to the physicochemical characteristics of its fundamental components — amino acids and proteins. Since it would be beyond the scope of this book to consider bioelectric phenomena at this level, however, we shall commence with the electrophysiology of the cell.

For didactic purposes the following is based on the membrane theory (cf. p. 23), according to which the inside of a cell is negative with respect to the surface because the surface structures of the cell (the cell membrane) have the properties of an ionic sieve, selectively permeable to different cations and anions. The resulting membrane potential can be measured if one electrode is inside the cell and the other in contact with its surface. Several examples will show the different ways in which this membrane potential can be demonstrated experimentally.

a) The membrane potential of large plant cells (Nitella, Chara)

Problem: Determine the potential difference between killed and intact ends of the internodal cell of *Nitella* or *Chara* and its dependence on the ionic composition of the external medium.

Principle: The large cells of fresh water algae sometimes attain a length of several centimeters and a diameter of 1 mm. The individual internodes can easily be isolated and, because of their size, are particularly suitable for studying membrane polarity (Osterhout 1936, 1958, Kümmel 1929). The inside of the cell can be reached either by a penetrating microelectrode (see below) or by injuring one end of the cell and thus destroying the membrane integrity at this point. In both cases the real membrane potential will only be measured if the other parts of the cell remain intact and maintain their normal

polarity. The second method is, of course, more convenient than the first, provided that the resistance of the external medium between the injured and intact parts of the cell is high. The e. m. f. of the membrane (E_m), measured between the normal and depolarised parts, is usually decreased by the so called short-circuiting factor, so that the potential recorded (E_a) is only a fraction of the actual E_m as follows:

$$E_a = \frac{R_1}{R_1 + R_2} \cdot E_m$$

where R_1 is the resistance of the external fluid and R_2 the resistance of the axoplasma between the electrodes. The full potential difference ($E_a = E_m$) is measured only when

$$\frac{R_1}{R_1 + R_2} = 1$$

This ideal value can be approached by increasing R_1 and decreasing R_2. We therefore attempt to choose rather thick fibres (low R_2) and to increase R_1 by interrupting the external communication between the normal and injured ends of the internode with vaseline.

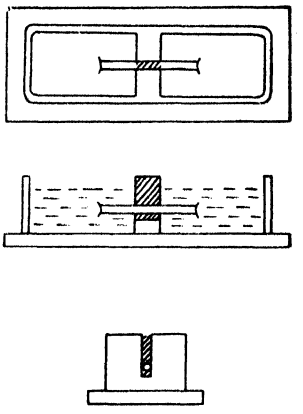


Fig. 59. A paraffin chamber on a slide for work with the isolated internode of *Nitella*. Shaded: vaseline seal.

Object: The isolated internodes of *Nitella* or *Chara* at least 3 cm long and 0·3—0·5 mm in diameter. Protoplasma streaming must be well evident under the microscope.

Apparatus: A cathode follower with a galvanometer or a cathode-ray oscilloscope with a D. C. amplifier, overall sensitivity of at least 5 mV/cm. Calibrator. Compensator.

Other requirements: Wick calomel cell electrodes. A paraffin frame, mounted on a slide, with two chambers separated by a vaseline seal of 2 mm width as shown in Fig. 59. 20% alcohol, 0·1, 0·01, 0·001, 0·0001M-KCl. Glass dissecting needles and scalpels for isolating and mounting the internodes into the chamber. An electrocautery for thermocoagulation. A low-power microscope.

Procedure: Leaves are carefully removed from several young internodes. The cells are isolated and placed into a vessel with water for one or more hours in order to permit the injury potentials to disappear. Carefully cleaned glass instruments are used for dissection, as plant cells are very sensitive to even slight contact with metals. The functional state of the isolated cells is followed under the microscope. Only internodes showing active protoplasmic streaming are suitable for experimentation. One cell is chosen, carefully fixed to a bent glass rod and with the latter's aid pressed to the bottom of the vaseline filled

slit separating the two chambers. Leakage is checked by filling one compartment with water, which must not penetrate into the other compartment along the internode. Only if the seal is watertight is the second chamber also filled with water. The slide is then set on the stage of a microscope allowing observation of the protoplasmic streaming in both parts of the cell. Using calomel electrodes, the potential difference between the two compartments is measured. After recording the potential difference for several minutes, the water in one chamber is replaced by 20% alcohol and measurements are continued.

In another experiment, after first determining the initial potential difference, one end of the internode is coagulated with an electrocautery and the potential difference is again measured.

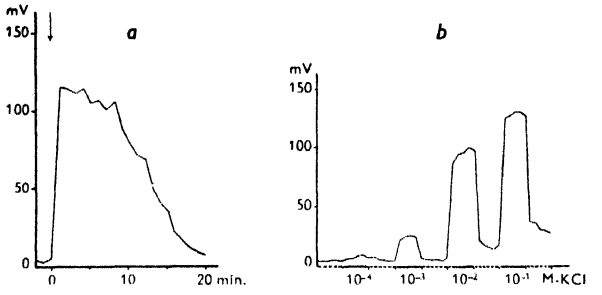


Fig. 60. a) Gradual decrease in demarcation potential produced by thermocoagulation of one end of the internode of *Nitella*. b) Depolarisation of the membrane potential of the internode of *Nitella* by different concentrations of KCl. One half of the internode is permanently washed by water, the other alternately with water (interrupted abscissa) or the test solution (full abscissa).

In both the above experiments the cell membrane is injured irreversibly and thus is completely depolarised. The membrane potential can be changed reversibly, however, by application of solutions of KCl to one end of the internode, the other being in water. We start with 0·0001M-KCl applied for 5 minutes. The chamber is then filled with water for 5 minutes and only then are the more concentrated KCl solutions used (0·001M, etc.).

Results: The potential difference between the ends of the internode in water is usually zero (values from +10 to −10 mV can be explained by persistence of the injury potential at one end of the internode). After thermocoagulation or 20% alcohol, the killed end of the cell becomes 110 to 130 mV negative. Protoplasmic streaming immediately ceases in the injured part of the cell while it continues undisturbed in the other parts. The potential difference, however, gradually decreases down to zero (Fig. 60a) within several tens of minutes, evidently because the cell dies. Also the protoplasmic streaming ceases gradually.

Fig. 60b illustrates a typical experiment in which different concentrations of KCl were used. 0·0001M-KCl does not influence the potential difference, while 0·001M-KCl shows an evident depolarising effect. The change caused by potassium ions is entirely reversible and confirms the assumption that the potential difference between the inside and the outside of a cell is mainly

due to different concentrations of potassium ions inside and outside the cell. The relationship may be expressed by the equation

$$E_m = 0{\cdot}058 \log \frac{[K_i]}{[K_0]}$$

(cf. p. 24).

If alcohol is added to the part of the cell which was maximally depolarized by raising the external [K^+], no further increase in negativity occurs.

Conclusion: Direct measurement of injury potential (in this case identical with membrane potential) in the large cells of *Chara* or *Nitella* shows high potential differences (up to 150 mV) between the negative interior and positive surface of the cell. The electromotive force is due to selective membrane permeability and to different concentration of ions, particularly of potassium, inside and outside the cell.

b) The membrane potential of nerves

Problem: Determine the membrane potential of myelinated nerve fibres of the frog and its dependence in the composition of the external medium.

Principle: It is possible to use the method described in the previous experiment (increasing the external resistance with vaseline) in the case of single cells or isolated nerve or muscle fibres (e. g. Tasaki and Frankenhäuser 1952). For a whole bundle of nerve fibres this method would not be effective, however, since vaseline or mineral oil cannot penetrate into the capillary spaces between individual fibres of the bundle. These spaces form a considerable shunt even after the external resistance has been raised by vaseline. Stämpfli (1954), therefore, suggested a simple method to increase R_1, making it possible to approximate the true membrane potential in a bundle of nerve fibres. The R_1 is increased by threading the bundle of nerve fibres through a thin glass tube with an appropriate diameter. Isotonic sucrose flows continuously from the centre of the tube to both ends, thus separating the ends of the nerve, immersed in normal Ringer solution or the test solution. Sucrose rapidly enters the extracellular spaces of the nerve bundle and thus effectively increases R_1.

Object: Frog nerve bundles freed of connective tissue and dissected for a length of at least 5 cm.

Apparatus: A cathode follower with a galvanometer or a D. C. amplifier with a cathode oscilloscope (total amplification 5 mV/cm). Compensator and calibrator.

Other requirements: Nonpolarisable calomel electrodes. A special apparatus, as in Fig. 61, consisting of a glass T-shaped tube (diameter of the horizontal arm 0·8, 1·2 or 1·6 mm, length 14 mm) and two side tubes (diameter 2 mm, length 20 mm) joining the horizontal part of the T tube. The side tubes can be rotated, so that they can assume either a horizontal or vertical position. The vertical part of the T tube is joined by rubber tubing to a Mariotte's bottle with a supply of isotonic sucrose. The side tubes are connected to Marriotte's bottles filled with Ringer solution. One of the side tubes is further connected via a two-way stop-cock to a burette containing the test solution.

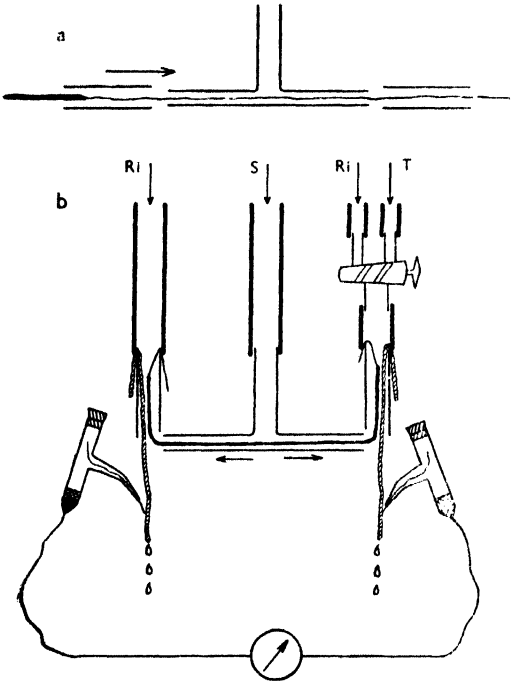


Fig. 61. Diagram of apparatus for measuring membrane potentials of a nerve bundle (Stämpfli, 1954). Above: Introduction of the nerve bundle into the apparatus. Below: The apparatus during measurement. Ri — Ringer solution, S — sucrose, T — test solution.

Isotonic sucrose (7·3%). The sucrose must be as pure as possible, the solution must be prepared in distilled water and its specific resistance must be greater than 100 KΩ.cm. If not, it must be cleaned in an ion exchanger. Several litres of Frog Ringer solution. Solutions of KCl (0·1, 0·03, 0·01, 0·003, 0·001M) 100 ml. each, 100 ml. of 0·02M KCl in 0·02M $CaCl_2$. 95% O_2 + 5% CO_2.

Procedure: The frog sciatic nerve is prepared in the usual way (p. 202, Fig. 80). Connective tissue is carefully dissected away and a branch 5—6 cm long is isolated. A thread is tied to both ends of the isolated nerve bundle. The preparation is left in Ringer solution for 1—2 hours so that the injury potentials can pass off. Several preparations can thus be made from one sciatic nerve and its branches. A T-tube with an appropriate horizontal diameter is inserted into the apparatus. The lateral tubes are moved into a horizontal position during this phase of work (Fig. 61a). This makes it easier to pull the nerve bundle through the system of tubes. The lateral tubes are then rotated by 90° (Fig. 61b) and the threads holding the preparation are fixed with rubber tubing. In addition a cotton wick is fixed in the lateral tube extending by 1—2 cm below the lower end of the tube. The rubber tubings are fitted

to the Mariotte's bottles filled with sucrose or Ringer solution and the flow of sucrose through the T tube and of Ringer solution through the lateral tubes is regulated with clamps. A two-way stop-cock is inserted on one side allowing rapid exchange of the Ringer for the test solution prepared previously in a 20 ml burette. The latency of exchange of solutions depends on the rate of flow and the volume of the rubber tubing between the cock and the preparation (with a rate of flow 2 ml./min. and a volume of the system 0·35 ml. this latency will be about 10 sec.). The test solution is usually permitted to act for 1 minute, after which the stopcock is switched back to Ringer solution. The calomel electrodes make contact with the cotton threads in the lateral tubes. In view of the fact that continuously outflowing solutions exclude diffusion from the electrodes to the preparation, calomel electrodes filled with saturated KCl and having high stability may be used.

Results: Usually only small potential differences (up to 5 mV) are registered between both ends of the nerve bundle separated by the sucrose gap. Fig. 62 shows a recording of the change due to application of 20 mM-KCl to one end of the nerve bundle. Results are similar to those obtained with *Nitella*. Maximum potentials attain values ranging from 50 to 80 mV. When 0·02M-$CaCl_2$ is added to 0·02M-KCl no depolarisation is produced. This is a typical example of the antagonism between potassium and calcium (Woronzow 1924, Fleckenstein, Hille and Adam 1951. It is possible to find for different potassium concentrations the ratio $\frac{[Ca^{++}]}{[K^{+}]}$ that will suppress the effect of potassium ions either completely or reduce depolarisation by 50%.

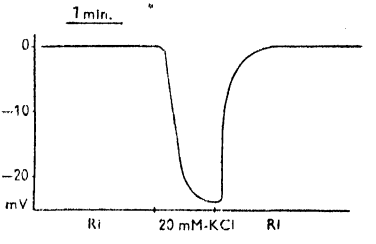


Fig. 62. Depolarisation evoked by applying 20M-KCl to a nerve bundle of the frog tibial nerve. The sucrose gap technique.

In addition to various depolarising agents an increase in membrane potential may be demonstrated with this method. Thus Ringer solution aerated with a mixture of 95% O_2 and 5% CO_2 after several minutes produces hyperpolarisation in the treated part of the nerve (Lorente de Nó 1947).

Conclusion: The sucrose gap technique permits measurements of membrane potential in a bundle of nerve fibres by increasing the resistance of the extracellular fluid between the external electrodes. In this way it is possible to approach values of membrane potential recorded with microelectrodes in the giant nerve fibres (Hodgkin, 1951), or in single myelinated fibres (Woodbury 1952).

c) The membrane potential of a muscle fibre

Problem: Determine the membrane potential of the frog sartorius muscle using ultramicroelectrodes.

Principle: Microelectrodes have to be used in order to obtain accurate values of the membrane potentials of striated muscle fibres.

These electrodes, having a high resistance, make great demands on the input stage of the amplifying apparatus. In addition to having to use a cathode follower (cf. p. 109) at the amplifier input the following parameters have to be controlled during measuring:

1) Electrode resistance. This is tested several times during the experiment. It shows whether the tip of the electrode is not broken or whether it is plugged. It also informs us of the time constant of the input circuit.

2) The time constant. Since the electrical resistance is very large, any slight change in capacitance at the cathode follower input (even a few pF only) results in large time constant changes (RC) and may thus distort the shape and amplitude of the recorded response.

3) Grid current (cf. p. 110) may polarise the object and produce a large potential drop across the electrode. These changes have also to be checked during the experiment.

The microelectrode is introduced with a micromanipulator. Many types have been described (Barer and Sanders-Singer 1948, 1951, Békésy 1952, Bishop and Tharaldson 1921, Browaeys 1943, Buchthal and Persson 1936, Bush et al. 1953, Cailloux 1943, Chambers 1921, Florian 1928, Fonbrune 1932, Hansen 1938, Kopac 1929, McNeil and Gullberg 1931, Ordway 1952, Reinert 1939, Schouten 1934, Tschachotin 1912, Watanabe 1926) some of which may be bought. They can also be improvised from the micro-drive of the microscope stage or tubus, from micrometers etc.

It is not very difficult to introduce a microelectrode into a muscle fibre for determining its membrane potential. If, however, the action potential is also to be recorded then difficulties arise due to muscular contraction which may break the electrodes. Different means are used to minimise this danger such as: stimulation of small bundles of fibres, very firm fixation of the muscle, stimulation of the same fibre with the microelectrode.

Object: The sartorius muscle of Rana temporaria.

Apparatus: Cathode follower and cathode-ray oscilloscope with a D. C. amplifier. Total sensitivity at least 5 mV/cm. Calibrator with 10 mV and 1 mV steps. A simple stimulator giving rectangular pulses (lasting for about 0·2 msec) synchronised with the time base. A micromanipulator and microscope.

Other requirements: Arrangement for fixing the preparation. Capillary glass microelectrodes, tip diameter about 0·5 μ filled with 3M-KCl (for pro-

duction see p. 163). Ringer solution (Na^+ : 116·55, K^+ : 2·5, Ca^{++} : 18, Cl^- : 117·1, $H_2PO_4^-$: 0·40, HPO_4^{--} : 2·55 mmol/l). Dissecting instruments.

Procedure: The sartorius muscle is prepared in the usual way (cf. p. 257) and fixed to the fixing arrangement with its internal side upward as shown in fig. 63. The muscle is extended to about 1·2 to 1·4 its original length between two forceps that are regulated by screws. The nerve passing to the muscle is placed on the stimulating electrodes (s). The muscle is in a Petri dish filled with Ringer solution. The microelectrode is mounted to the holder on the arm of the micromanipulator and a Ag-AgCl wire connected with a cable to the cathode follower grid, is inserted into its broader end. The cathode follower is placed as near as possible so that the connection to the grid is short and thus its capacitance small. The reference electrode is immersed in the Ringer solution and connected through the calibrator to the grid of the second valve of the cathode follower (if a symmetrical follower is used) or to earth (if an asymmetrical one is used).

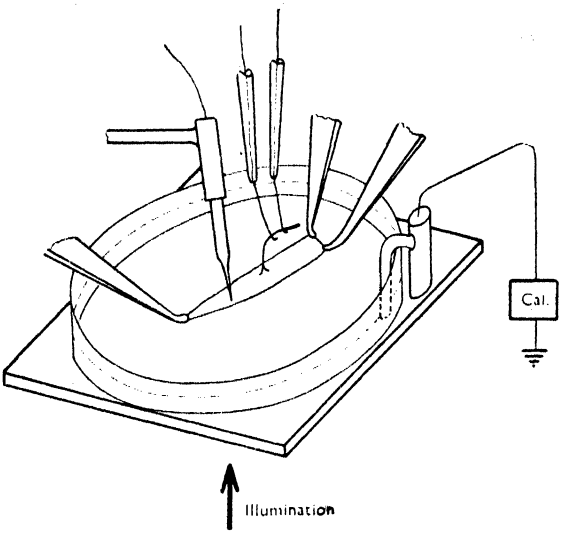


Fig. 63. Experimental arrangement for studying membrane and action potentials of the frog sartorius muscle using capillary microelectrodes.

Before inserting the microelectrode into the muscle fibre, the amplification of the whole recording arrangement and the microelectrode resistance are measured.

By switching over the calibration voltage in 10 mV steps, an amplification is found for which 50 mV produce a deflection across half the screen.

The microelectrode resistance is determined with an accurate shunt (e. g. 50 MΩ) connected in parallel to the electrode. The beam deflection in mm is measured after switching on the calibration pulse with the calibration resistance (R) connected (N_2) or disconnected (N_1). Then the resistance of the microelectrode (R_m) can be calculated from

$$R_m = R \cdot \frac{N_1}{N_2} = 1$$

The time constant is determined by applying a rectangular pulse to the microelectrode tip and measuring the time increment of the pulse to 63% of the final value thus determining the capacitance of the input stage. This is

only an approximate value since for complete calculation the capacitance of the electrode tip immersed in the solution (which increases with immersion) must be taken into account (Alexander and Nastuk 1953).

The size of the grid current must be controlled before introducing the microelectrode. Different cathode followers even though they have selected valves with a minimal grid current (less than 10^{-11} A) have an arrangement for decreasing this current (cf. p. 110). The size of the grid current is found quantitatively by observing the voltage drop caused by grid current across the resistance. The resistance is chosen in such a way that the grid current can be rapidly calculated, i. e. that the voltage step is not higher than a certain given value corresponding to the permissible level of grid current. This is done by following the beam of the oscilloscope with the calibration resistance connected or disconnected.

Then the experiment is commenced.

The muscle is illuminated from below and the microelectrode is observed under the microscope as it approaches the muscle fibre. It is vertical to the fibre. Then the microelectrode is moved vertically towards the fibre with the aid of the micromanipulator. If the electrode tip is not too small, dimpling of the fibre may be seen under the microscope as the electrode enters the fibre. On the oscilloscope this moment is seen as an abrupt drop of the spot (if negativity is downward). On further moving the electrode vertically, the beam position does not change as long as the electrode remains within the fibre. When the fibre membrane is pierced on the other side, the beam returns to its original position. The same is obtained when pulling the electrode out of the fibre. The return to the original level is not always exact and differs by 1—2 mV. The mean between the original level and the level on pulling out the electrode is used for determining the membrane potential.

On filming the whole process it can be seen that the entry into the fibre is very abrupt and occurs within fractions of a msec.

In such a way the membrane potentials of several fibres from the same muscle can be determined.

Finally the size of the membrane potential is compared with that of the intracellularly recorded muscle spike.

The microelectrode is introduced into the muscle and the nerve stimulated. The spike is recorded, the microelectrode, however, is either pulled out of the fibre or broken as a result of the contraction. This is seen as a sudden return of the beam to the level before piercing the fibre (Fig. 64).

By exchanging the Ringer solution for solutions with different K and Na contents, the effect of those ions on the membrane and action potentials of a muscle may be studied.

Results: In the experiment shown in Fig. 64 the membrane potential is 56 mV, the action potential 79 mV. The spike overshoot is thus 23 mV on the

average. The existence of overshoot resulted in a revision of Bernstein's original conception. This author assumed that the membrane and action potentials were of equal size. Several hypotheses attempted to explain this discrepance (Curtis and Cole 1942, Hodgkin and Huxley 1945, Höber 1946, Grundfest 1947). Today the ionic hypothesis of nerve impulses is generally accepted (Hodgkin, Huxley and Katz 1952, cf. p. 23).

Conclusion: Membrane and action potentials may also be measured in other structures. The sudden potential increase on piercing the membrane has been utilised to record intracellular potentials from structures that are not accessible to direct visual control (motoneurones — Brock et al. 1951, Betz cells in the cerebral cortex — Phillips 1956 etc.). The technique used in other structures is principally the same. Differences are found in preparations or auxiliary means (e. g. suppression of brain pulsations, recording from the living heart, etc.). Intracellular recording may be combined with microinjections into cells (Castillo and Katz 1956), using either two electrodes or a double barrelled microelectrode. Finally intracellular stimulation or artificial changes in membrane potential (so called "voltage clamp") may also be used.

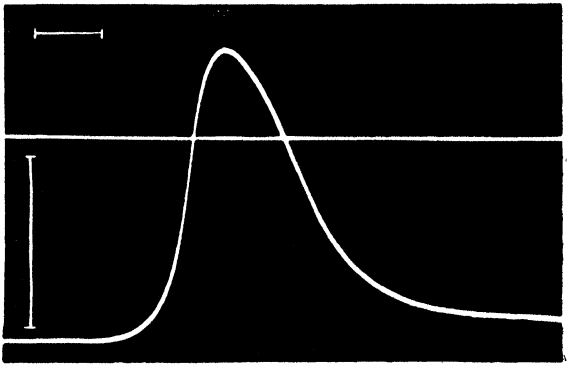


Fig. 64. Membrane and action potentials of a muscle fibre. Calibration: 50 mV; time mark: 1 msec.

Microelectrodes may be introduced longitudinally into some invertebrate nerve fibres of large diameter (*Loligo*, squid). This was the first method used for intracellular recording of membrane potentials (Hodgkin, Huxley 1939). Its great advantage lies in the fact that the membrane is not injured and that several intracellular electrodes can be used having a much larger diameter than when recording through the membrane. These larger electrodes of course also have smaller resistances.

B. Electrical potentials of tissues

In metazoa, cells are organised into tissues and organs. An explanation of the electrical characteristics of tissues based on the electrical properties of individual cells is rendered very difficult by variability in cellular shape and function, by innumerable variations in their spatial organisation, and by

different properties of intercellular substances. The interpretation of potentials registered in a complex volume conductor is also complex. Even here, however, at least some simple fundamental principles can be applied (cf. also p. 386).

No potential difference can be detected between two intact sites of a tissue composed of apolar cells, the membranes of which have the same surface polarity everywhere (Fig. 65).

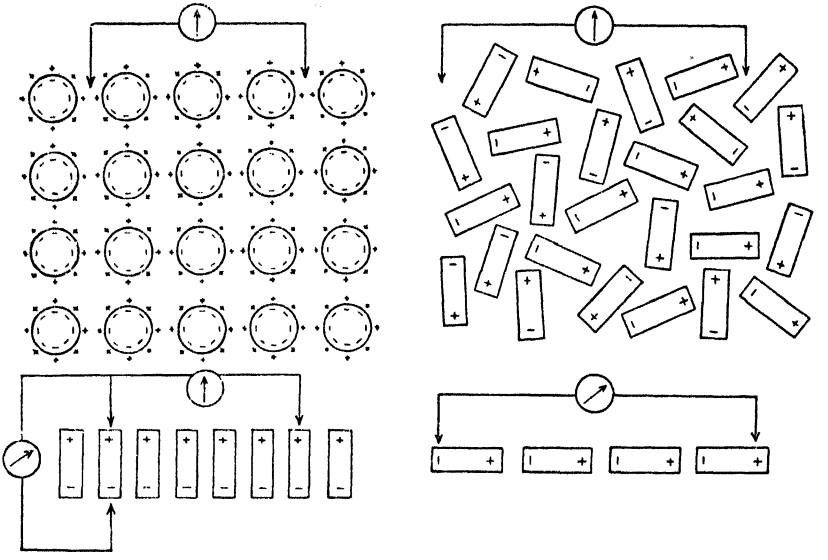


Fig. 65. Potential difference between two different points in a tissue composed of apolar cells (top left), polar cells arranged irregularly (top right), in parallel (below left) or in series (below right).

In tissues composed of polar cells, the membrane of which has, for morphological or metabolic reasons, a higher (lower) potential at one site than at another, either no potential can be registered between two uninjured sites if the cells are irregularly spaced and oriented so that their potentials cancel one another (Fig. 65) or a certain polarity is present given by the vectorial addition of elementary cellular dipoles and by resistance conditions (Lund 1928). If the anatomic structure of the tissue is very regular, the arrangement of cellular dipoles may, from an electrical point of view, approach a connection in parallel, in series or a combination of both (Fig. 65). Finally, semipermeable membranes of noncellular nature may also be a source of electrical potentials.

The demarcation potential of a tissue — the potential difference arising between an injured and a normal area of the tissue — is produced in most cases by destruction of the inherent tissue polarity at the site of injury (Fig. 66). A certain demarcation potential, however, also occurs in tissues composed of

nonpolar cells as the result of a diffusion potential arising between the normal region and the injured site, where outflowing intracellular ions accumulate. The formation of a layer of injured cells at the border between the destroyed and normal tissue seems even more important.

The fundamental examples of the possibilities mentioned above will be illustrated by the following experiments.

a) Polarity of frog skin

Problem: Determine the potential difference between the inner and outer surface of isolated frog skin under various conditions.

Principle: Frog skin which, according to the theory of Koefoed-Johnsen and Ussing (1956), contains in the epidermal layer bordering on the corium polar cells with different permeability of the cell membrane facing the inner and outer skin surfaces, represents a cellular membrane with parallelly-arranged dipoles. In addition to the physico-chemical causes of the membrane potential described in more detail in chapter I., so-called active transport of ions is of prime importance in the frog skin. This is a metabolically maintained ability of the cell to transfer a certain ion (ions), even against a concentration or electrical gradient, from one side of the membrane to the other. If the solutions on both sides of the skin have the same composition, the potential on the membrane or the current passing through the membrane is an expression of active ion transport only, which can be demonstrated by chemical analysis of both solutions or, even better, by the use of radioactive tracers (Ussing 1949, Kirschner 1955a).

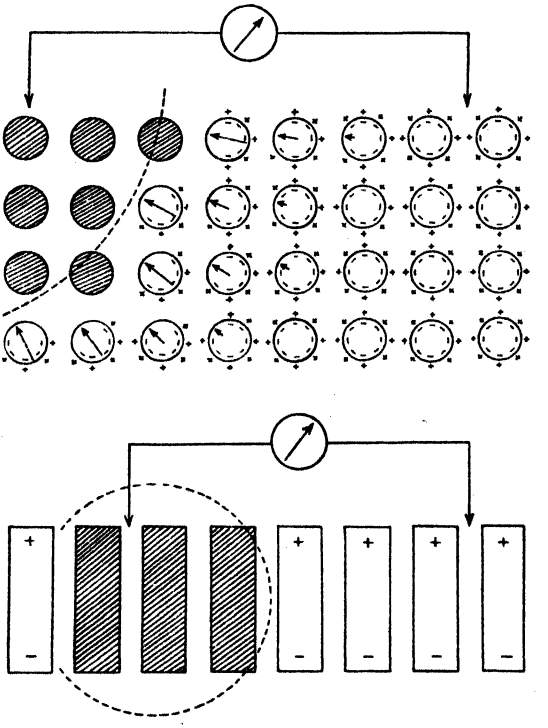


Fig. 66. Demarcation potential in tissue composed of apolar (top) and polar, regularly arranged cells (below).

Object: Rana temporia or *esculenta*, weighing about 50 g. A fresh specimen as far as possible.

Apparatus: A galvanometer with a sensitivity of 10^{-8}—10^{-9} A/mm/m and an internal resistance of 500—1000 Ω. A cathode follower with a galvano-

meter (sensitivity of 10^{-7} A/mm/m or more), or a cathode-ray oscillograph with D. C. amplifier (overall sensitivity at least 5 mV/cm). A calibrator and compensator.

Further requirements: Nonpolarisable Ag-AgCl electrodes with Ringer-agar bridges, preparation dishes, dissecting instruments. A special chamber for fixing the frog skin (according to Ussing and Zerahn 1951 and Linderholm 1952): two tubes with an internal diameter of 3 cm and a length of 5 cm each glued to a plate of plexiglass which can be connected to the other one with screws (cf. Fig. 67). The plexiglass plates are fitted with rubber packing rings on the inside for fixation of the isolated skin. The free ends of the tubes are closed with rubber stoppers having an opening for a 5 mm glass tube with a Ringer-agar bridge of the Ag-AgCl electrodes (E_3, E_4). A capillary electrode (E_1, E_2), diameter 0·5 mm, is introduced into the inside of each tube through a vertical opening in the plexiglass plate. The electrode tip is in the centre of the tube about 1 mm from the skin surface. The apparatus has further openings for admission of air or oxygen. These may also serve for exchange of solutions.

Solutions:	Ringer	NaCl	KCl	$CaCl_2$	$NaHCO_3$
		112 mM	2·0 mM	1·0 mM	2·5 mM
	Ringer without Na	choline chloride	$KHCO_3$	$CaCl_2$	
		115 mM	2·3 mM	1·0 mM	
	Ringer without Cl	Na_2SO_4	$KHCO_3$	$CaSO_4$	
		75 mM	2·6 mM	1·0 mM	

5% CO_2 in O_2, oxygen or compressed air. Adrenaline 1/1000. Atropine.

Procedure: The frog is killed in the usual way, the skin is carefully removed from the trunk and spread on the preparation dish filled with Ringer solution. Depending upon the size of the frog, one or two circles about 4 cm in diameter are cut from the lateral or abdominal skin. The skin is carefully mounted between the two parts of the apparatus which are then firmly screwed together. The tube in contact with the internal surface of the skin is filled with Ringer solution to the level shown in Fig. 67. The other half of the apparatus is filled in a similar way with the test solution. The solutions are aerated, thus also bringing about a slow circulation of the solutions in each half of the apparatus.

1) The dependence of the skin potential on the composition of the external solution.

The half of the apparatus in contact with the external surface of the skin is also filled with Ringer solution and the potential difference measured for 20 minutes with capillary electrodes E_1 E_2. This solution is then exchanged for 10% Ringer (obtained by mixing 9 parts of Ringer solution without Na with one part of normal Ringer solution) and potential differences are again registered for 20 minutes. Finally, this solution is replaced by Ringer without Na and recordings are taken for a further 20 minutes.

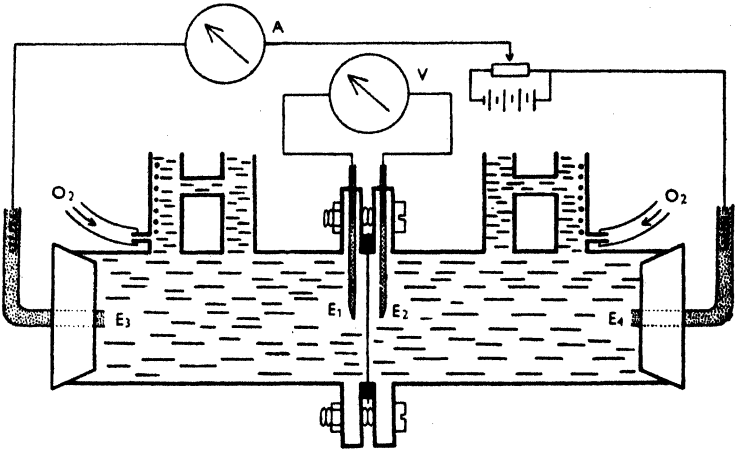


Fig. 67. Chamber for studying electroproduction in frog skin. For details see text.

In a similar way the significance of chlorides for the membrane potential may be tested in a new preparation. After first recording potentials in normal Ringer solution, this is replaced in both halves by Ringer without Cl and again measurements are continued for a further 20—30 minutes.

2) The short circuit current of frog skin and its dependence on composition of the external solution.

If the large Ag-AgCl electrodes at the extreme ends of the apparatus are connected to a galvanometer, the electroproduction of the frog skin can be registered in units of current. The short circuiting is not complete, however, since part of the electric energy results in polarisation of the skin, which can be registered with the capillary electrodes as a certain potential difference between the two skin surfaces. In order to exclude this component and thus to obtain the actual short circuit current (Ussing and Zerahn 1951), a low variable resistance and a dry cell are inserted into the circuit of the large Ag-AgCl electrodes. With these the value of the current is adjusted so as to reduce the potential gradient across the frog skin (recorded simultaneously on the capillary electrodes) to zero. When $I \cdot R_s + E_s = 0$ (R_s = skin resistance, E_s = skin e. m. f.), the short circuit current I reaches a maximum. If under these conditions there are identical solutions on both sides, then no

concentration or electrochemical gradient exists for any ion and transport can only be maintained by active metabolic processes. The left part of the apparatus is then filled with solutions of different sodium concentrations and the short circuit current is always measured for 20—30 minutes.

3) The dependence of electrical phenomena in frog skin on metabolic processes.

A mixture of 95% O_2 and 5% CO_2 is introduced into both parts of the apparatus instead of O_2 when the short circuit current is clearly in evidence. The resulting changes in the potential or current are observed for 60 minutes. Severe interference with skin electroproduction may be obtained with metabolic inhibitors, e. g. by adding KCN to the solution on both sides of the skin. The transport mechanism may further be influenced by some drugs. This is well demonstrated by adding adrenaline to a final concentration of 10^{-6} to the internal solution. In another experiment atropine (final concentration from 1 to 10 mM) is added to the external solution.

Results: 1) With Ringer solution on both sides, the inner surface of the skin is positive to the outer surface. With decreasing Na concentrations in the external fluid, the potential at first rises from 50—70 mV to 130—150 mV at 10 mM Na. If the frog skin behaved like a passive membrane selectively permeable to cations, then the corresponding Na potential would be of opposite polarity (the more dilute solution positive, cf. p. 22). Only when the solution in contact with the exterior contains no sodium, does the potential drop to zero and finally reaches negative values (—3 to —5 mV — Galeotti 1904, Kirschner 1955a). If chloride ion is replaced by sulphate ion in the bathing solution on both sides of the skin, the skin potential increases considerably. This is due to low skin permeability for SO_4^{--} and resulting elimination of the internal shunt caused by movement of chloride ions carrying charges across the skin in the opposite direction to sodium.

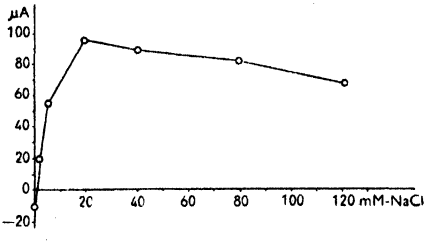


Fig. 68. Dependence of the short-circuit current of frog skin on the Na content of the external solution. Na^+ replaced by choline.

2) The short circuit current has similar characteristics. At a zero sodium concentration in the external solution it is negative (10—20 µA), then it rises rapidly and reaches a maximum of 200—400 µA at a concentration of 20—40 mM Na (Fig. 68).

As was shown in experiments with simultaneous measurements of movements of Na^{24} and Na^{22} (Ussing 1952, Kirschner 1955a) across the frog skin in both directions, the short circuit current corresponds to net sodium transport

$$Na_{in} - Na_{out} = Na_{net}$$

(Na_{in} = influx of sodium, Na_{out} = outflux of sodium, Na_{net} = net movement of sodium.) We can thus calculate the net transport of sodium directly in microequivalents from the average values of the short circuit current (I) during a certain period (t) using the equation

$$Na_{net} = \frac{I \cdot t}{F}$$

(F = Faraday constant 96 494 coulombs, I = current in microamperes, t = time in seconds). If, for instance, the average short circuit current during one hour was 200 μA, then during that time $\frac{200 \cdot 3600}{96\,494} = 7{\cdot}46$ μequ. Na were transported.

3) Aeration of Ringer solution with 95% O_2 + 5% CO_2 suppresses the potential and the short circuit current within several tens of minutes (Ussing and Zerahn 1951). A similar effect is obtained with NaCN (Lund 1926, 1928b, Ussing 1949) but initial values are only decreased by 70—80%. Addition of adrenaline (Ussing 1949) to the internal or atropine to the external solution (Kirschner 1955b) increases the short circuit current.

Conclusion: The source of the potential difference in frog skin is an active, metabolically maintained transport of sodium ions. A number of data indicate that similar transport mechanisms exist in cellular surface structures (sodium pump, Hodgkin 1951) and in other tissues (kidney, glands). Frog skin is a suitable and accessible model for the experimental approach to problems of active transport.

b) Positive demarcation potential of gastric mucosa

Problem: Determine the potential difference between a normal and an injured site of the gastric mucosa or serosa.

Principle: The orientation of the classical demarcation potential of nerves and muscles does not mean that an injured site must always be negative to an uninjured region. The inherent polarity of the structure at the site of a lesion is the determining factor. In muscle and nerve fibres the injury potential depends upon the polarity of the membrane (outside positive, inside negative). In structures with reversed orientation of the elementary dipoles, the demarcation potential is positive. This occurs in *Nitella* after reversing its membrane polarity with potassium (Osterhout 1936). Similar conditions also exist in tissues, e. g. in the gastric mucosa, which is negative to the serosa (Titaev 1938, Venchikov 1938, 1954, Rehm 1943).

Object: Dog weighing 5—10 kg.

Apparatus: A cathode follower with a galvanometer or a cathode ray oscilloscope with a D. C. amplification of at least 5 mV/cm. A calibrator, 3 compensators.

Other requirements: 4 calomel electrodes. Ringer-agar bridges in glass tubes (diameter 3—4 mm) in a special holder as shown in Fig. 69a. Surgical instruments for operation. 96% alcohol, ether. Electrode stands. 10% dial.

Procedure: The dog is fasted for 24 hours. The abdomen is opened under dial anaesthesia in the midline, the stomach is exposed and opened by an incision 6—8 cm long between the greater and lesser curvatures, avoiding injury to the blood supply as far as possible. The lower pair of agar bridges are inserted into the incision, thus making contact with the mucosa. The upper pair is

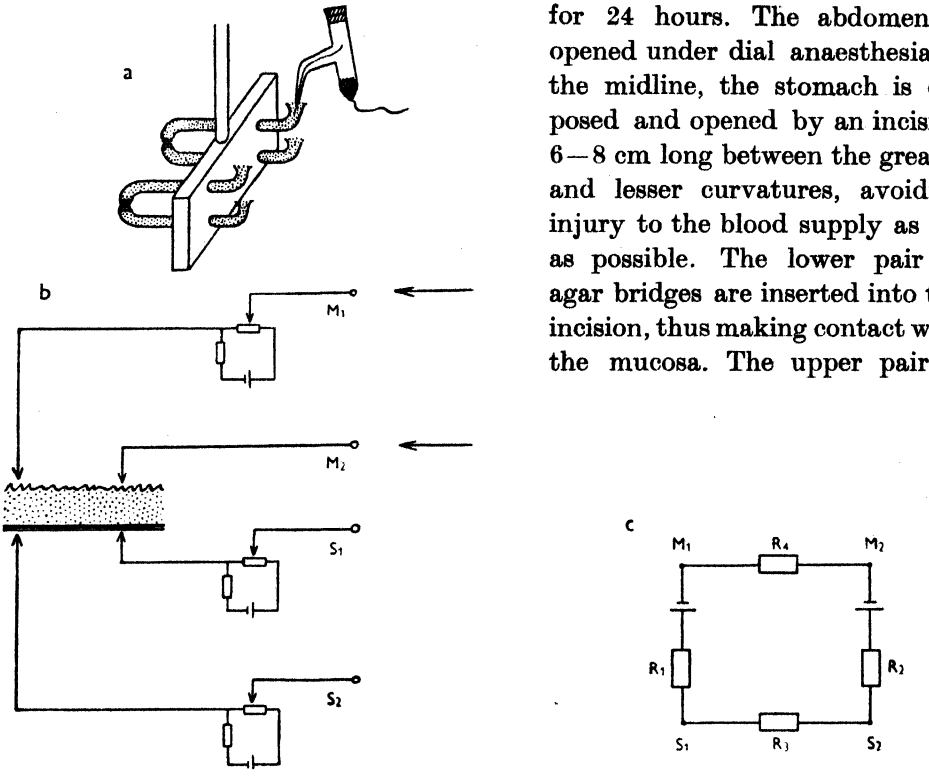


Fig. 69. Agar bridge for measuring the potential of the gastric wall (a) and diagram for connecting the electrodes with the balancing circuits (b). The input leads of the amplifier drawn opposite to M_1M_2 may be connected with any electrode pair. c) Diagrammatic representation of resistances and electromotive forces in the gastric wall.

applied to the serosa just opposite to the lower pair. Such an arrangement is suitable for experiments with freely accessible serosa. If, however, we intend to work with the mucosa, the lower edge of the incision is flapped back so that the mucosa lies uppermost. The gastric wall is fixed between the pairs of bridges in that position (mucosa upward) by two sutures fastened to the holder. The Ringer-agar bridges are then connected to the calomel electrodes and measurements may be started. There must initially, of course, be no

potential difference between the electrodes. This is attained by balancing three of the electrodes against the fourth (Fig. 69b). The potential differences across the stomach wall (M_1S_1, M_2S_2) are recorded, followed by measurement of surface potentials (S_1S_2, M_1M_2).

After these fundamental measurements have been made, the area M_1 is treated with 96% alcohol or ether and potential M_1M_2 is recorded. Then again S_1M_1, S_2M_2 and S_1S_2 are measured. In another experiment, 96% alcohol is applied to area S_1, and again similar recordings are made.

Results: Usually only small potential differences are found between two sites of the gastric serosa (S_1S_2) or mucosa (M_1M_2). The potential across the gastric wall, on the other hand, (M_1S_1, M_2S_2) usually attains several tens of mV, mucosa negative. Destruction of the mucosa below M_1 results in positivity of this area with respect to the normal mucosal surface (M_2). Thus a positive demarcation potential is formed.

On the serosa, on the other hand, the potential difference S_1S_2 remains nearly unchanged. Theoretically the following relation holds good for the above potential differences

$$M_1M_2 = M_1S_1 - M_2S_2 + S_1S_2 ,$$

the sign of the potential always referring to the first electrode of the pair. The fact that after destruction of the mucosal surface the potential difference on the mucosa is much higher than on the serosa ($M_1M_2 \gg S_1S_2$) probably follows from the resistance and e. m. f. distribution in the circuit (cf. diagram in Fig. 69c). This corresponds to $R_3 < R_4$ and a source of the potential near to the internal surface of the stomach. Rehm (1946) could actually demonstrate, using microelectrodes, that nearly the whole potential of the gastric wall originates in secretory elements of the mucosa. This conclusion is also supported by experiments in which 96% alcohol is applied to the serosa. Usually there is no change in the potential difference between the normal and injured serosal surface and the potential difference across the gastric wall is also unaltered.

Conclusion: The finding of a positive injury potential in tissues does not contradict the classical definition of a demarcation potential. It is the result of reversed tissue polarity.

c) Cell dipoles in series

Problem: Examine the electric gradient along an onion root.

Principle: The frog skin and gastric wall belong to tissues in which cell dipoles are directed radially towards the surface, i. e. they are connected in parallel from an electrical point of view. There are, however, tissues with

cellular dipoles arranged in series. A well known example is the electric organ of the electric eel, which can produce potentials of up to several hundred volts (Albe-Fessard et al. 1951, Grundfest 1957) because several thousand units with a voltage of approximately 0·1 V are connected in series. The principle of in series connection of cell potentials can, however, be demonstrated on a more accessible object — the roots of plants. The potential differences between individual sites along the root from the tip to the base are determined

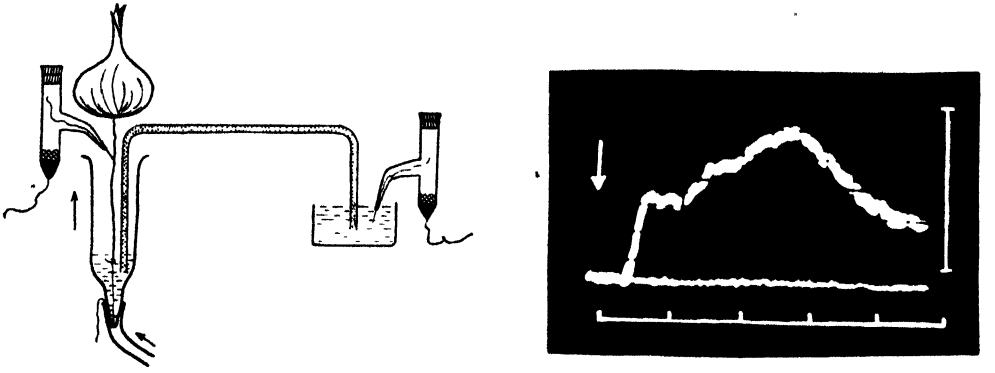


Fig. 70. a) Experimental layout for demonstrating the potential distribution along an onion root using a moving boundary. b) Oscillographic tracing. Reference electrode at the root base. Negativity upward. Calibration: 50 mV. Horizontal scale: 5 sec. = 5 mm.

by the size and orientation of the potential differences between the apical and basal ends of individual cells in different areas of the root (Lund and Kenyon 1927, Marsh 1928, Rosene 1935, Scott et al. 1955).

Object: Fine young root of the onion *(Alium cepa)*, water hyacinth *(Eichhornia crassipes)*, or bean *(Pisum sativum)*.

Apparatus: A cathode follower with a galvanometer or a cathode-ray oscilloscope with a D. C. amplification of at least 5 mV/cm. A calibrator and compensator.

Other requirements: A paraffin plate for fixation of the roots. A special burette (Fig. 70). A Mariotte's bottle. Physiological saline. 20% alcohol, vaseline. Calomel electrodes and agar bridges.

Procedure: A young growing root of an onion 3—5 cm in length and not separated from the plant is placed into the ridge of a paraffine plate and fixed at its ends with vaseline. One to two hours after fixation, the potential difference between different areas of the root is measured. The tip or base is used as reference point, the other electrode being slowly moved along the root. The potential difference can also be measured using a pair of electrodes with a fixed interelectrode distance of 2 mm, that are moved together along the root. The results of both types of measurement made rapidly one after the other on the same root are compared.

After determining the distribution of the potential, a part of the root 5 mm in length is sealed off with vaseline on both sides and 20% alcohol is dropped on it. The potential difference between the apex and the base of the root is measured and the effect of alcohol is observed, especially with respect to its localisation.

The potential distribution along the root can be rapidly and simply recorded with a cathode-ray oscilloscope and a D. C. amplifier. The root is fixed with threads in a vertical position, tip downwards, in a glass burette (Fig. 70a). The burette can be rapidly filled with saline from a Marriotte bottle. The wick of a calomel electrode is in contact with the base of the root, the other electrode is connected through an agar bridge with the solution in the vessel. Both electrodes (first adjusted to zero difference with the compensator) measure the potential difference between the base of the root and that area to which the level of the saline in the vessel reaches. By adjusting the rate of filling in such a way that the steadily rising level of the saline will cover the tip-base distance in 5 sec. and by adjusting the time base of the oscilloscope to this rate, we can then record the distribution curve of the potential along the root by simultaneously switching on the sweep and opening the flow of saline. Recordings are only made while the vessel is being filled. Between individual measurements, the root is washed with water and allowed to dry somewhat to prevent short circuiting of the measured potential.

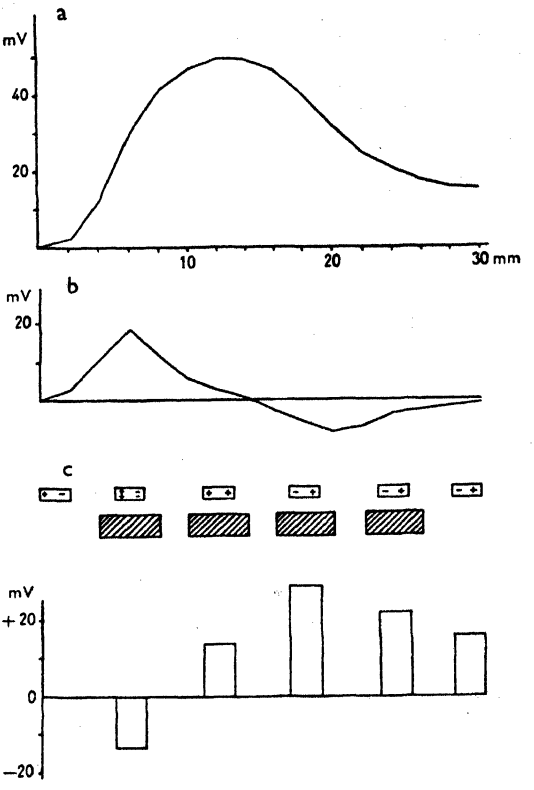


Fig. 71. Distribution of potential along an onion root a) in a monopolar recording (reference electrode at tip 0 mm). b) in a bipolar tracing (interelectrode distance 2 mm). c) Above: diagramatic representation of cell dipoles in different parts of the root. Below: change in potential difference between tip and base after excluding 4 mm section of the root (shaded rectangles) with alcohol (20%).

Results: The tip of the root is usually positive against the middle parts and these again are negative against the base (cf. Fig. 71a). The shape of the monopolar potential distribution along the root will be the same regardless

of whether the reference electrode is applied on the base or on the tip. Only the zero level will be shifted. When using a pair of electrodes with a constant interelectrode distance, the distribution of the bipolar potential differences plotted against the right border of the corresponding sectors is fundamentally the derivative of the monopolar distribution (Fig. 71b). This curve has its maximum where the first curve has the steepest slope and the minimum is found where the first curve shows constant values.

The minimum of the potential gradient at the 14th mm. and the change in polarity of the potential difference about this point is probably related to the fact that in this region of the root the cellular dipoles arranged in series change their orientation as is shown diagrammatically in Fig. 71c. This is also confirmed in experiments using alcohol. Depending on the position of the treated area and thus on the direction and size of the affected dipoles the potential difference measured between the base and the tip will be either increased or decreased (Fig. 71c). Simple short circuiting of a certain area by saline which practically abolishes the contribution of this area to the total potential difference has similar effect as alcohol (Rosene 1935).

Fig. 70b shows a typical oscillographic recording of the potential distribution along an onion root obtained by the method described. The length scale added permits direct transformation of the voltage-time function to a voltage-distance function.

Conclusion: The potential difference measured between two points of a tissue may be the result of vectorial addition of individual cellular dipoles in the interpolar area. Without detailed analysis of the electric field between the electrodes, it is not possible to draw any conclusion concerning the distribution of electromotive forces between them.

C. Electric phenomena in plants

Although plant material has already been used twice for demonstration of bioelectric phenomena (internodes of *Chara* or *Nitella*, plant roots), it is felt that a special chapter must be devoted to the electrophysiology of plants.

It was demonstrated as early as in the second half of the last century that plants may be a source of electric potentials (Buff 1854, Burdon-Sanderson 1882). Yet plant electrophysiology has remained far behind that of animals. The latter rapidly developed, particularly in connection with study of electrical changes accompanying the function of nerves and muscles. A knowledge of electrical phenomena in plants permits a demonstration of some bioelectrical processes under simple conditions, and also makes it possible to consider electroproduction as a universal property of living matter, both in cells and

tissues and in whole organisms. For the above reasons some fundamental experiments directly arising out of the last chapter will be described.

a) The biolectric potential of photosynthesis

Problem: Determine the potential difference arising between an illuminated and a darkened area of a green leaf or between two areas with different chlorophyl content.

Principle: The intensity of metabolic processes of photosynthesis differs considerably in illuminated and darkened parts of a plant. This is indicated not only by differences in O_2 production and CO_2 consumption, but also by a characteristic potential difference. Since photosynthesis depends upon the presence of chlorophyl, a similar potential difference depending upon the intensity of illumination can be observed also between the green and unpigmented sites of the variegated leaves.

Object: Elodea canadensis, Pelargonium zonale.

Apparatus: A cathode follower with a galvanometer and an optic kymograph, or a cathode-ray oscilloscope with a D. C. amplifier, total sensitivity of at least 5 mV/cm. A compensator, a calibrator.

Other requirements: Nonpolarisable wick calomel electrodes. A black dissecting plate of rubber or cork. Dissecting instruments. Pins. A light source (100 W bulb, microscope lamp). A water filter. A solution of black India-ink in water.

Procedure: An isolated *Elodea* leaf (length 10—12 mm, width 3—5 mm) is particularly suitable for demonstrating photosynthetic potentials because of its simple structure. It is composed of only two layers of cells without cuticle and has only one central rib.

1) In daylight such a leaf is fixed to the preparation plate and the wicks of calomel electrodes are placed onto two points of its surface. The electrode potential was previously balanced with a compensator. The potential difference between those two points is recorded. After some 10—20 minutes one half of the leaf is covered with a black cover through which the electrode passses, and the change in potential difference is followed for at least 20 minutes. The cover is then removed and the course of potential changes is again observed. A similar result is obtained if a drop of water is applied at one electrode and a drop of black India-ink covering an area of at least 5 mm in diameter at the other.

2) In a darkened room a leaf of *Elodea* is fixed onto a black plate with an opening 2 mm in diameter through which a beam of light is thrown from below across a water filter (a 5 cm layer of water for absorbing heat radiation, distance of a 100 W bulb 50 cm). The leaf is placed in such a way that light

falls either onto its apical or basal portion. One electrode is permanently placed onto the dark portion of the leaf; with the other, the potential of the illuminated part and the potential gradient along the whole leaf is examined (cf. Fig. 72a). For the experiment to be successful, the leaf must first be adapted to the dark (for, at least, half an hour). The bulb must be well screened in a black tube at the top of which the dissecting plate is mounted (Fig. 72a).

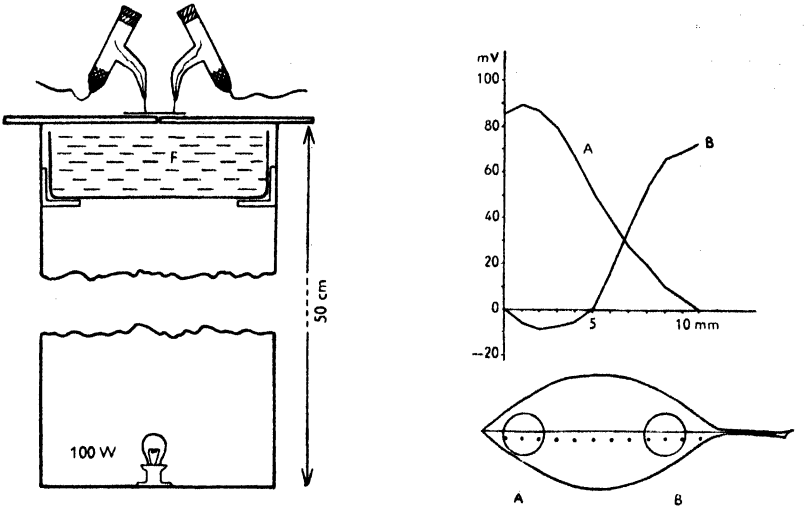


Fig. 72. a) Experimental layout for determining potential differences between illuminated and non-illuminated sites of an *Elodea* leaf. b) Distribution of potential along an *Elodea* leaf on illuminating the tip (A) or base (B). Reference electrode at base (A-11 mm.) or tip (B-0 mm). Positivity of the active electrode upwards.

3) The photosynthetic potential can simply be demostrated in so called variegated (mosaic) leaves of some plants, e. g. *Pelargonium zonale*. One electrode is placed on an unpigmented portion of the leaf, the other on a green portion. In the dark, there will be only a small potential difference between the two electrodes (previously balanced to zero). This difference will change characteristically on suddenly exposing the plant to light.

The photosynthetic potentials can be demonstrated in all green leaves of flowers, vegetables, trees, etc. Their polarity, size and time course differ considerably in various plants.

Results: 1) The darkened portion of an *Elodea* leaf becomes 50—100 mV negative to the illuminated portions within several minutes. Within certain limits, this potential difference is proportional to the difference in the intensity of illumination. After the dark cover is removed, the original potential reappears (Fig. 73). Light and dark periods can be alternated many times, always with the same effect (Glass 1933, Uspenskaya 1951).

2) Fig. 72b shows the potential distribution around an illuminated point of the leaf. It is evident that the electrical gradient is not so steep as would be expected from the distribution of light and shade, but is gradual, indicating some influence of the illuminated portion on the non-illuminated parts of the leaf. The metabolic gradient, which evidently forms the basis of these potential gradients, adds to the natural polarity of the leaf (Fig. 72b). The tip of the leaf remains positive to the more central parts, even if the maximum of positivity shifts to the illuminated basal portion (Glass 1933).

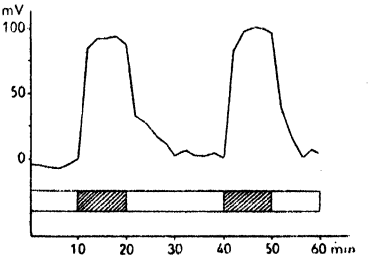


Fig. 73. Potential of photosynthesis in an *Elodea* leaf. Shaded rectangles-area of active electrode illuminated. White rectangles — the whole leaf in the dark. The illuminated site positive.

3) The green parts of a *Pelargonium* leaf are the more negative with respect to the non-pigmented portions the more intensive the illumination. This potential difference, however, begins to decrease within 10—15 minutes, even though the illumination remains unchanged. Different leaves show different direction of the potential difference between the illuminated and non-illuminated portions. The majority of leaves react like pelargonium (the illuminated part is negative) but the isolated leaves of cabbage, for example, give a positive potential at the site of illumination (Klein 1898, Waller A. D. 1900, Waller J. C. 1925, 1929).

Conclusion: Potential difference between the illuminated and darkened portions of a leaf are the result of metabolic processes of photosynthesis. They depend on the intensity of illumination and on the structure and metabolism of the leaf.

b) Resting electrical polarity of plants and the electrical response of plant tissue to stimulation

Problem: Determine the electrical gradient on the stem of a tree (or a stalk). Determine the spatial distribution and temporal course of electrical potentials evoked in plant tissue by different stimuli.

Principle: Irritability, a general property of living protoplasma, is present at all stages of development, and manifests itself as a characteristic change in cellular metabolism and function, produced by various stimuli. This is accompanied by a decrease in cellular membrane potential and changes in polarity of tissue. In contrast to action potentials in nerve or muscle, the electrical phenomena of irritability are gradual, depend upon the stimulus intensity

and are limited in space to the area of direct action of the stimulus (Wedensky's stationary excitation). Electrical potentials occur in plants in response to mechanical, chemical, thermal or electrical stimuli (Stern 1924).

In addition to these electrical responses, resting potential differences can also recorded in plants. These are mainly an expression of the metabolic

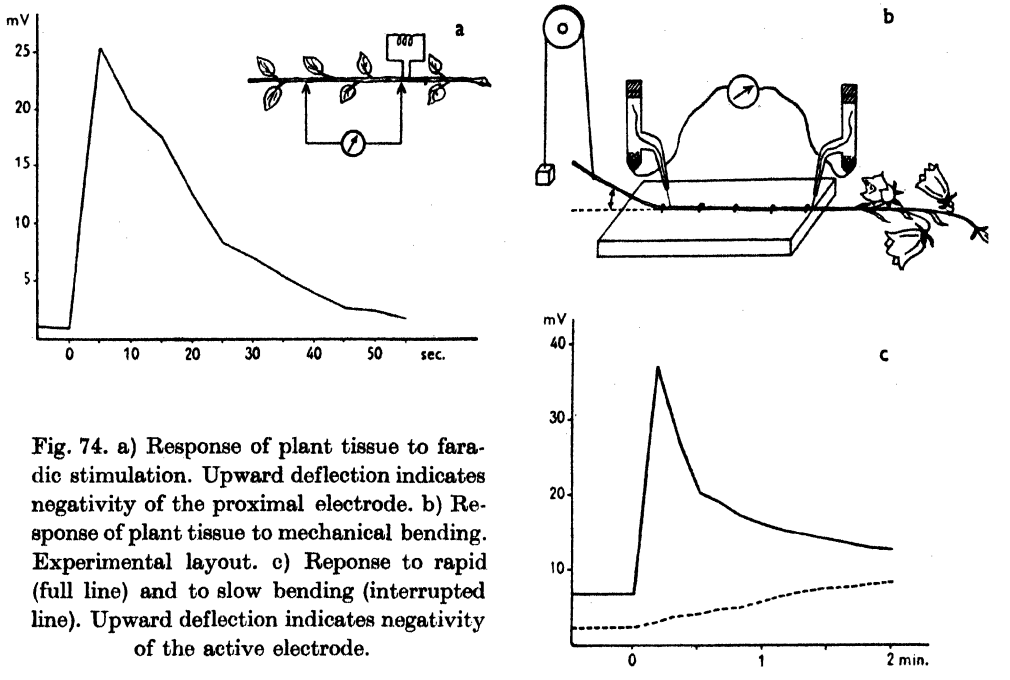


Fig. 74. a) Response of plant tissue to faradic stimulation. Upward deflection indicates negativity of the proximal electrode. b) Response of plant tissue to mechanical bending. Experimental layout. c) Reponse to rapid (full line) and to slow bending (interrupted line). Upward deflection indicates negativity of the active electrode.

gradient between the apical portions and the roots, corresponding to the transport of water, salts and nutrients (Opritov 1958).

Object: Branches, stalks and blades of different grasses, flowers and trees (e. g. *Taraxacum*, *Chenopodium album*, *Nicotiana rustica*, *Artemisium*) isolated from the plant or in situ.

Apparatus: A cathode follower with a galvanometer and an optic kymograph or a cathode-ray oscillograph with a D. C. amplifier, total sensitivity of at least 5 mV/cm and a slow time base. A simple stimulator and an anode battery with a key.

Other requirements: Nonpolarisable electrodes (calomel or Ag-AgCl), Ringer-agar bridges of suitable shape. Physiologial saline. A metal rod weighing 5—10 g moving in a tube of 3—4 mm diameter. A pulley, threads, clamps, stands. A drill for wood (diameter 5 mm), grafting wax.

Procedure: The recording calomel electrodes are placed onto the branch (stalk) moistened with 0·9% NaCl, in such a way that one is close to the stimulated point, the other further away (Fig. 74a). Wire Ag-AgCl electrodes

are used to stimulate the branch or stalk with a short train of stimuli (rectangular pulses or A. C. current), or with a pulse of D. C. current (about 1 sec.), and the response is recorded. This will differ in intensity according to the strength of the stimulus. In a similar way the effect of mechanical stimuli may be analysed either by applying constant pressure on the area of the electrode or by dropping the rod onto the area of recording from different heights. The stimulus can then be measured in units of energy (g.*mm.).

For determining electrical potentials on bending, the stalk is fixed at the middle (cf. Fig. 74b). One end of the stalk is firmly fastened to the dissecting plate while to the other a thread is tied leading over a pulley to a weight. By releasing the weight a sudden bending of the stalk is achieved. This can be measured exactly in degrees.

Very strong stimuli causing destruction of the plant tissue produce demarcation potentials between the injured and normal tissue. Their temporal and spatial course in a leaf is determined by placing one electrode onto the injured site (tissue crushed by pressure, thermocoagulated or treated with 96% alcohol), while the others are placed in a row towards the normal part of the leaf at 10 mm intervals (Fig. 75).

Resting polarity is demonstrated by placing one calomel electrode onto the part of the stalk close to earth. The other is moved upwards, and the potential differences are recorded. In trees the electrode must enter the trunk. The bark is removed from an area 2 × 2 cm, the cambium is exposed, and a hole is made to the centre with a drill. A glass tube of suitable diameter filled with 0·9% NaCl-agar is introduced, and fixed to the bark externally with grafting wax. Nonpolarisable calomel electrodes are connected to this agar bridge and the potential differences between electrodes situated at different heights are measured (Burr 1944, 1947).

Results: The electrical response to stimuli appears at the site of application as negativity, the amplitude of which depends upon the strength of the stimulus. The maximum negativity is attained within several seconds, but decrease lasts for much longer (Fig. 74a). Electrodes situated at greater distance from the site of application of the stimulus show a large spatial decrement of negativity. In general it may be stated that there is an exponential decrease with increasing distance from the site of the stimulus. If recordings are taken at various distances from the site of the stimulus, the rate of spreading of maximum negativity can also be determined, since despite a rapid decrease in amplitude, there still is a certain spreading of potential.

Bending is well suited to studying the effect of the rate of the stimulus onset on the character of the electrical response. Here the general law concerning the relationship between the rate of stimulus and rection formulated by du Bois-Reymond and elaborated by Hill (1936) holds good. A bend of a

certain angle realised suddenly (dropping the weight) gives a much larger electrical response (Fig. 74c) than one of the same angle occurring gradually over 20 seconds, as by slowly pulling the thread by hand (Kvasov 1949).

Fig. 75 shows the temporal course of the demarcation potentials and their distribution in space. These attain larger amplitudes than potentials

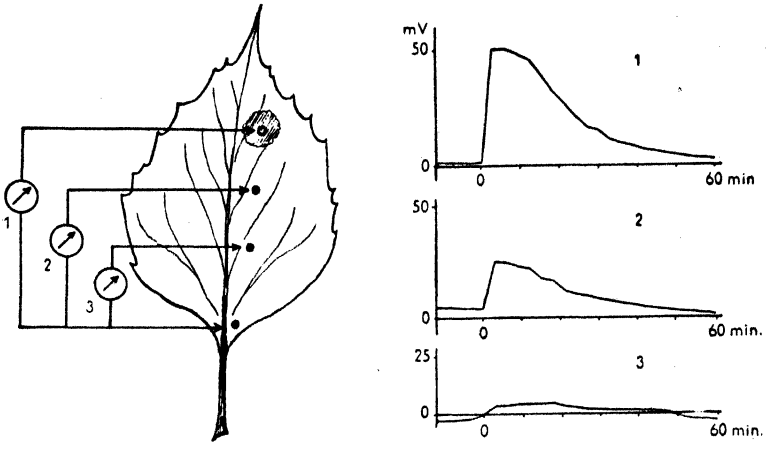


Fig. 75. Time course and spatial distribution of demarcation potential in plant tissue. Shaded region-tissue destroyed by thermocoagulation. Common reference electrode at the base of the leaf. 0 — moment of thermocoagulation.

produced by weaker stimuli. Even they, however, decrease in time at rates depending upon the type of tissue, area of injury, stimulus, etc. (Kümmel 1929). The decrease in demarcation potential is mainly due to reparatory processes in the cell layer separating the injured and normal tissue, where the membrane potential is distributed asymmetrically and in a polar fashion. The diffusion potential produced by different ion concentrations in the injured and normal regions also gradually disappears.

Measurements of the axial gradient show that the apical electrode is usually positive to the basal one. Sometimes potential differences of over 100 mV are recorded. These differences show diurnal changes (according to Burr, 1944 they are largest before dawn, lowest at noon) evidently related to cyclic changes in metabolic activity.

Conclusion: Electrical responses accompany all kinds of stimuli acting on plant tissue. They are characterised by a gradual change from threshold responses to phenomena related to irreversible tissue injury (demarcation potential), by a relatively long duration and a considerable spatial decrement.

c) Potential changes accompanying leaf movement in Mimosa pudica

Problem: Record the electrical phenomena due to the process mediating the spread of seismonastic movements in *Mimosa.*

Principle: In the majority of plants, reaction to external stimuli is limited to the stimulated area. There are some plants, however, which react with movements to stimulation of distant areas. This capacity may be of biological significance, e. g. in insectivorous plants (*Dionaea muscipula* — Burdon Sanderson 1882). The transmission of excitation for considerable distances can be demonstrated in *Mimosa.* The path of excitation appears mechanically as closing of leaves and electrically as a wave of negativity, which may be recorded in a monopolar or bipolar recording (Burr 1943). The spreading mechanism is probably a humoral one, some substance being released into the tracheal water stream.

Object: A young specimen of *Mimosa pudica,* at least 30 cm in height.

Apparatus: A cathode-ray oscilloscope with a slow time base (0·01—0·1 cycles/sec.) and a D. C. amplifier with an input resistance of at least 2 MΩ and total sensitivity at least 5 mV/cm. A two channel apparatus is best. A stimulator giving single electric pulses synchronised with the time base. A compensator for each channel. A calibrator.

Other requirements: An anode battery. Nonpolarisable electrodes. Physiological saline, a dropper, stands, electrocautery. A photographic camera.

Procedure: The condition of a mimosa plant is tested on the previous day by observing its reactibility to mechanical stimuli. The leaves of *Mimosa* consist of a primary petiole, some 5 cm in length, divided into two or four secondary pinnae, each of which is further subdivided into 10—20 oppositely arranged pinnules. All movements are performed by specialised organs, pulvini, localised at the articulations of two leaf subdivisions (pinnules with the secondary pinna, secondary pinna with the petiole) or of the petiole with the stem. Electrodes are placed according to the diagram in Fig. 76 on carefully cleaned parts of the stem and leaves, moistened with 0·9% NaCl. The referent electrode is placed low on the stem, the further electrode (electrodes) on the petiole or pinna of the leaf, the peripheral part of which will be stimulated electrically or by heat. Great care must be taken to avoid mechanical stimulation. First the potential difference between the reference point and the other electrodes is determined. The time base is then switched on synchronously with the stimulus, and the course of potential changes is recorded. Since after stimulation the leaf displays considerable movements, good contact between the electrodes and the site of recording must be ensured using long and freely movable wicks. An attempt may be made to obtain a more precise correlation between mechanical and electrical processes by photographing

the leaf at the moment when the wave of negativity commences below one of the electrodes or when it reaches its maximum.

Results: The peripheral electrode is usually 5—20 mV positive to the basal one at rest (this is similar to other plants, cf. p. 192). The response is somewhat variable. With weak stimuli, the spreading of the reaction may soon cease. Only a few of the nearest pinnules react by folding up, and the

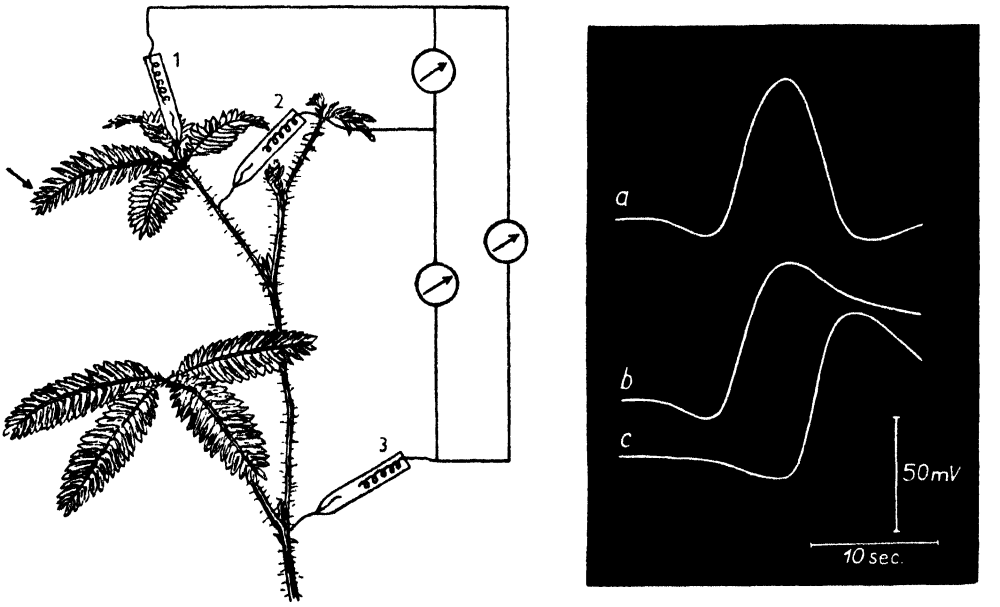


Fig. 76. a) Diagram of electrode distribution when recording action potentials in *Mimosa*. The arrow indicates the site of electrical or heat stimulation. b) Oscillographic tracing of the action potentials of *Mimosa*. Bipolar recording from electrodes 1—2; b, c — simultaneous monopolar recordings from electrodes 1—3, 2—3. Upward deflection — negativity of the first electrode.

negativity may reach only the nearest electrode or not even that. With stronger stimuli, however, the response spreads for a considerable distance and may affect the whole plant. It is then possible to follow not only basipetal but also acropetal transmission of excitation along the leaves from the junction of individual pinnae or petioles with the stem.

Electrically, such a response appears as a wave of negativity (Fig. 76b, c) attaining an amplitude of 50—100 mV and spreading from the site of the stimulus at a rate of 2—10 mm/sec. The wave attains its maximum within 1—2 seconds. Its ebb, on the other hand, is much slower and it takes sometimes tens of minutes before the original polarity is restored. This corresponds to the slow return of the mechanical reaction, for the leaves remain folded a relatively long time. This course of the electrical reaction is observed in monopolar

recordings with the reference electrode low on the stem. In bipolar recordings (cf. Fig. 76a), the shape of potential depends upon the electrode distance, and is somewhat different, since the potential wave passes below both electrodes.

As long as incomplete repolarisation persists, it is not possible to produce a new reaction. The prolonged negativity is thus an expression of the refractory period in *Mimosa*.

Conclusion: Transmission of motor reactions in *Mimosa* is somewhat analogous to impulses in nerve and muscle fibres of animals. Even though the mechanism of transmission is different, there is a striking similarity between the electrical phenomena in both cases.

D. Electrical polarity of the animal organism

The characteristic differences in organisation and functional significance between apical and basal parts of the body of lower organisms have led to the formulation of a theory of axial gradients (Child 1929). A number of data were obtained concerning metabolic gradients related to gradients in physiological functions. Electrical polarity is also an expression of this axial gradient. This was observed in a number of organisms (*Hydroids* — Mathews 1903, Lund 1926; *Amblystoma* — Burr and Hovland 1936a; chick embryo — Herman and Gendre 1885, Burr and Hovland 1936b). According to some authors, this polarity is no chance consequence of anatomical structure, but is of fundamental significance in formative processes of embryogenesis (Burr 1944). It was actually demonstrated that it is possible to suppress, direct or even completely reverse regeneration using an external electric field in *Obelia* (Lund 1923) and in *Dugesia tigrina* (Marsh and Beams 1952). The same was shown for the growth of nerve fibres in explanted nerve tissue from chick embryos (Marsh and Beams 1946). Electric polarity is the stronger the simpler the structure of the organism. Even then, however, it is usually not easy to determine which part of the body and which tissues are its source. All that has been said regarding polarity of whole tissues holds good in these cases, but conditions are much more complex. As an example, an experiment is described in which changes of electrical polarity are studied in the development of an organism under special conditions of registration.

Electrical phenomena of early embryogenesis in the chick

Problem: Observe the development of electrical potentials in the fertilised chicken egg during the first five days of incubation.

Principle: The chick embryo up to the 5th day of incubation is positive to the albumin or the yolk (Hermann and Gendre 1885). This potential dif-

ference can by measured even through the shell. Technical difficulties caused by the large resistance of the shell and by contact potentials arising at the site of application of the electrodes are eliminated by the method described by Vorontsov and Emchenko (1947). The method utilises the free mobility of the embryo in the shell. Because it is of lower specific weight than the yolk,

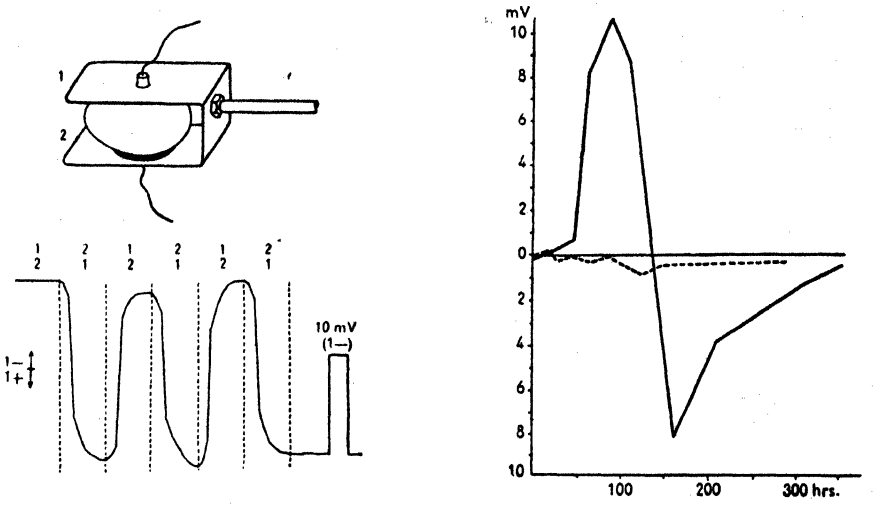


Fig. 77. Measuring the potential difference in a chick embryo. Above: fixation of the egg in the holder. Below: time course of potential difference between electrodes 1—2 when turning the holder 180°. Interval between rotations 1·5 min. Upward deflection due to negativisation of electrode 1. b) Development of potential difference between the upper and lower surface of a fertilised (full line) and unfertilised (interrupted line) hen egg during incubation. Upward deflection corresponds to negativity of the upper egg surface.

the embryo always floats within a few tens of seconds to the uppermost part of the egg. Electrodes situated at opposite sites of the egg (one on top, one at the bottom, cf. Fig. 77a) record the potential difference, which includes that of the emryo and the contact potentials.

If the egg, together with the electrodes, is turned 180° about its longitudinal axis, the contact potentials remain unchanged but the potential of the embryo changes its polarity with respect to the electrodes within a few tens of seconds. The embryo moves upwards and is again uppermost, but adjacent to the electrode which was underneath previously. The potential difference before and after turning the egg is double the potential difference produced by the embryo itself.

Object: Freshly-laid hen's eggs, preferably from a controlled breed.

Apparatus: A cathode follower, galvanometer with a sensitivity of 10^{-9} A/mm/m. A calibrator and compensator.

Other requirements: A turnable holder for the egg with special disc Ag-AgCl electrodes (Fig. 77a). Physiological saline, a laboratory incubator at 37°C.

Procedure: Ag-AgCl electrodes wrapped in cotton wool and stored in a dark bottle with saline are fitted to the holders and connected to the leads. Each egg is first cleaned with cotton wool soaked in warm water and then dried with a flannel cloth. It is placed into the holder in such a way that its longitudinal axis is parallel to the axis of rotation. During recording the electrodes are moistened occasionally. The deflection of the galvanometer is observed or the potential may be adjusted until it becomes stable, using a compensating circuit. The result is recorded. The egg, clamped between the electrodes, is then rotated through 180°. After a latency of several seconds, the potential begins to change, slowly at first and then more rapidly until finally (after 30—50 secs.), it comes to rest at a new value which is also recorded. Rotations are repeated several times at regular intervals (1·5 min.). Care is taken not to rotate the egg in the same direction every time. Finally, a calibration voltage of 10 mV is inserted into the electrode circuit with the egg in one of the two positions. This voltage is poled in such a way that it increases the negativity of one of the electrodes. A comparison of the direction and size of deflections when rotating the egg with deflections produced by the calibration signals makes it possible to determine the sign of the potential and its magnitude in mV. One and the same egg is first measured immediately after being laid and then at various intervals after the beginning of incubation (after 12—24 hours). If several measurements are made on one egg, the time the egg is outside the incubator must be kept to a minimum. Temporary short cooling and other procedures involved in recording potentials, however, do not impair the development of the embryo in any way.

Results: Fig. 77a shows the results of measurements made in an egg after 3 days of incubation. It also shows the way in which galvanometer deflections are changed to mV and how polarity is determined. In the example given, the average deflection, according to calibration, corresponds to 17 mV. The actual polarity of the embryo, however, as has been explained, is only half. Since a change over from position 2/1 to position 1/2 gives a deflection corresponding to negativisation of the upper electrode (deflection in the same direction as is produced by increasing negativity of electrode 1 with a calibration signal), the upper surface of the egg is negative to the lower one. In freshly-laid eggs, on the other hand, only irregular small deflections are recorded, not exceeding 1 mV in most cases. From the second day of incubation a characteristic potential difference begins to appear in fertilised eggs, while unfertilised ones continue to be electrically inert. The potential of fertilised eggs attains a maximum on the 4th day and then decreases again (Fig. 77b), evidently as a result of the increased volume of the embryo, its

decreased mobility and the altered distribution of the potential sources within the egg.

At the time when the polarity of the egg is maximal, this can be changed reversibly by warming the egg in warm water (50°C, 2 min.) or by cooling it. Such procedures decrease the polarity of the egg considerably but temporarily.

Conclusion: The potential differences between the chick embryo and the yolk or between different parts of the embryo produce an electrical field, which can be recorded even across the intact shell. According to changes in that field, it is possible to determine whether the egg is fertilised or not, to follow its development and reactions to changes in the environment.

IV

The electrophysiology of isolated excitable structures in vitro

The part of electrophysiology analysed in most detail to day is that of isolated excitable structures. Excitable structures are capable of sending signals — impulses — from the site of stimulation across considerable distances. Transmission occurs without decrement, since at each point energy for the impulse is obtained from local metabolic supplies.

In addition to their signaling function, excitable structures, including the whole nervous and muscular systems, also have a special function: analysing and synthesising signals received. This occurs at the synapses. Electrical activity in the latter case, in contrast to nervous or muscle impulses, is characterised by gradual electrical changes spreading for short distances only.

The anatomical accessibility of peripheral excitable structures and their relative resistance to changes in the external environment permit successful studies to be made under in vitro conditions.

Experiments in this chapter are arranged in such a way as to give, in addition to electrophysiological techniques, some orientation in the fundamentals of electrical activity in these structures and its relation to physiological functions. Both signalling functions and fundamental processes occurring at the synapses are described.

A. Electrical manifestations of a nerve impulse

The transmission of signals from the periphery to nerve centres, and hence to acting peripheral organs, is a specific function of nerve fibres. This signalling function is realised by nerve impulses. A nerve impulse is a complex biological process which spreads from the site of stimulation at a certain rate along the nerve fibre. It manifests itself by various electrical, chemical and volume changes, changes in heat production, etc.

Changes in electrical potential at the excited site are the most conspicuous. Consequently electrical signs of a nerve impulse, so called action potentials, have been studied most intensively and are the most usual indicator of the presence of a nerve impulse.

Hence it is necessary to master the technique of recording action potentials if any electrophysiological approach to the problems of nerve and muscle physiology is to be considered.

a) The action potential of peripheral nerves

Problem: Record the monophasic and biphasic action potential of the frog sciatic nerve.

Principle: The excited site is electrically negative with respect to a quiescent site. That portion of the nerve which is negative at any given moment is of different length in various nerves, ranging from several mm to several cm, and is called the wave length. The negativity spreads along the whole nerve, the wave length remaining constant. The electrical potential of a site through which the nerve impulse has passed returns to resting values.

When the nerve impulse is anywhere outside the electrodes connected to a cathode-ray oscilloscope (Fig. 78a), no deviation can be recorded since

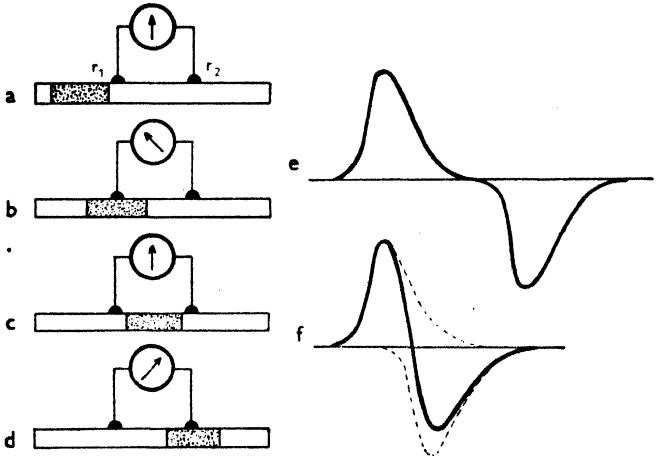


Fig. 78. Diagramatic representation of the origin of a biphasic action potential.

there is no potential difference between electrodes r_1 and r_2. When the nerve impulse reaches electrode r_1 (Fig. 78b) the part of the nerve below it becomes negative. A potential difference arises between electrodes r_1 and r_2 and a deflection is recorded. Fig. 78c shows the moment when the impulse is between

the electrodes. The tissue below both electrodes again has the same potential and consequently no deviations is recorded. When negativity reaches electrode r_2 (Fig. 78d), a situation similar to that illustrated in Fig. 78b arises. The only difference is that this time the deflection of the beam will be in the opposite direction, since the negativity is now below the second electrode.

If the potential is traced as a function of time, a curve as shown on the right is obtained (Fig. 78e). Since the onset and termination of negativity at a certain point are not instantaneous, but require a certain short time to reach a maximum, the ascending and descending parts of the curve have a certain slope. For the sake of clarity an example has been chosen which occurs only rarely in actual fact. Usually the wave length is longer than the distance between the electrodes. It is obvious that in such a case there will be no isopotential line between deflections corresponding to appearance of negativity under r_1 and r_2, as was the case in the previous example. The decay of negativity below one electrode will still continue while negativity already reaches the second one. Thus a curve as shown in Fig. 78f is obtained.

Similar considerations lead also to the conclusion that the amplitude of the deviation will be the smaller the smaller the distance between the two electrodes.

An action potential recorded in this way is termed biphasic. Negativity from the electrode closer to the point of stimulation is recorded in an upward direction and consequently negativity from the second electrode (more distant) in a downward direction.*)

This second phase of the biphasic action potential is often termed positive.**)

Recording of the so called monophasic action potential is more suitable when studying its exact shape. In such a recording the arrival of the impulse below the second recording electrode is prevented and only its course below the first electrode is traced. This can be done in various ways. The simplest is to clamp the nerve with forceps between the electrodes or to place electrode r_2 upon the killed end of a nerve. The most suitable and most effective method is depolarisation of the terminal part of the nerve using isotonic KCl solution.

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*) While in the technical literature upward deflection usually corresponds to positivity of the active electrode, in electrophysiology it denotes negativity in most cases. This convention is often not adhered to, however, especially in electrophysiology of cerebral cortex, in electroretinography and in intracelullar recordings. Also in this book upward deflection does not always indicate negativity of the active electrode and the reader is adviced to check always polarity of the individual records in the text or legends. Such a usage is of advantage in both studying and writing electrophysiological papers.

**) This is not correct since this might be mistaken for the actual positive phase of the action potential, which occurs in some non-myelinated nerves of invertebrates.

The recording of a monophasic action potential is diagramatically illustrated in Fig. 79.

Object: The frog sciatic nerve with the tibial and fibular branches.

Apparatus: A single or double beam cathode-ray oscilloscope and a preamplifier. Overall sensitivity of the apparatus 100 μV/cm. A voltage calibrator. Time marker 1000 c/sec. A stimulator giving square wave pulses (duration 0·2 msec.) synchronised with the time base. Screened chamber.

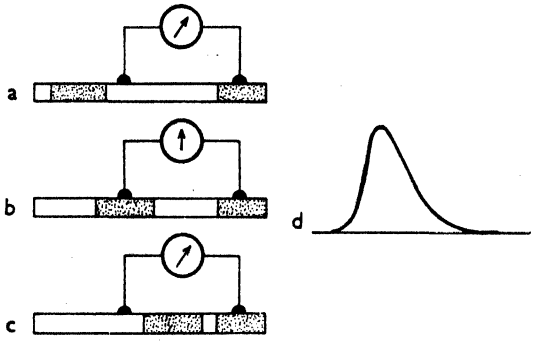


Fig. 79. Diagramatic representation of the origin of a monophasic action potential.

Other requirements: A moist chamber for the nerve preparation. Frog Ringer solution, gaseous mixture 5% CO_2 in O_2. A Petri dish with a layer of paraffin at the bottom. Dissecting instruments.

Procedure: Preparation of the sciatic and the tibial nerve. The nerves of poikilotherms are particularly suitable for learning the method of recording action potentials since they are, in contrast to those of homeotherms, much more easily maintained in good functional condition. For the same reasons the nerve-muscle preparation (sciatic nerve-gastrocnemius muscle) is a classical object for teaching physiology.

Fundamentally the same method is used to prepare the sciatic-tibial nerve preparation and the above mentioned nerve-muscle preparation.

The frog *(Rana esculenta* or *R. temporaria)* is decapitated with scissors. A probe is introduced into the spinal column and the spinal cord is destroyed. The frog is then fixed on its back to a board with a cork covering. The abdomen is opened and the gut is reflected so as to expose the roots of the sciatic nerve entering the spinal column. About 1 cm above that point of entry, the trunk of the frog is cut through, dividing the animal into two. The lower part is skinned, pulling as in taking off a glove. Care must be taken to prevent the skin from coming into contact with the solution in which the nerve is being prepared. The preparation thus obtained is placed into a Petri dish with about 0·5 cm of paraffin wax at the bottom. The preparation is fixed to the paraffin on its ventral side with pins, and frog Ringer is added until it is completely immersed.

The tibial nerve is cut above the talocrural joint and all side branches are cut cranially. The branches of the sciatic nerve are also removed. The fibular nerve is severed. After the tibial and sciatic nerves are thus freed of

all branches they can be easily freed by raising them carefully with a glass hook. It is not necessary to remove the connective tissue in the regions of the knee and hip joints. The same is done with the other hind limb. Then the preparation is turned and the sciatic and tibial nerves are pulled to the ventral side. In such a way the left and right tibial and sciatic nerves are freed up to

Fig. 80. Isolation of the sciatic and peroneal nerves in the frog.

their entry into the spinal column. It then suffices to cut the spinal column in two and the preparation is ready. The connection between the sciatic nerve and the spinal cord is not severed. A piece of spinal column to which the sciatic nerve is attached is used to hold the preparation with forceps when manipulating with it.

The main stages of dissection are shown in Fig. 80. The nerves are placed in small Petri dishes containing frog Ringer solution.

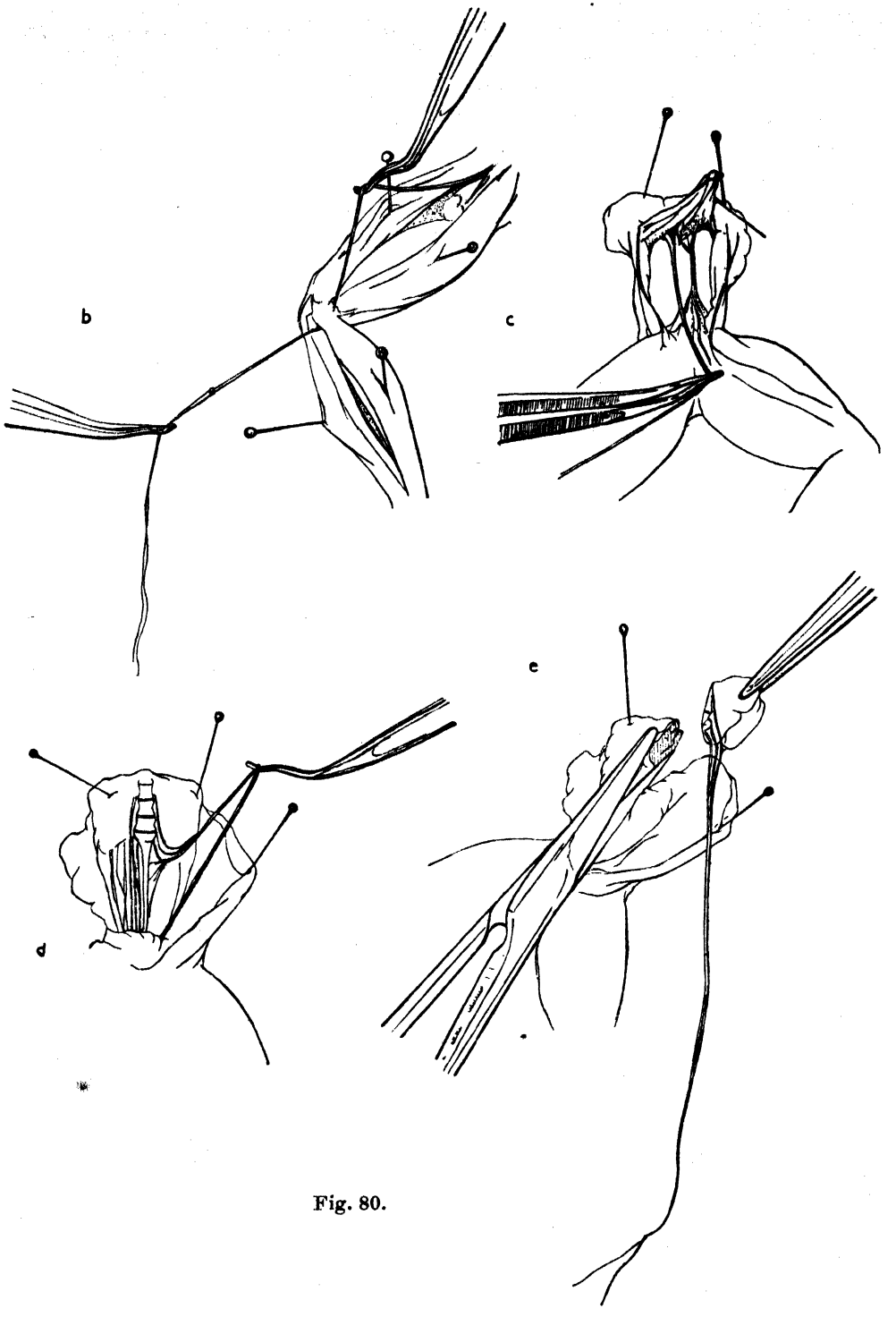


Fig. 80.

If the construction of the moist chamber does not permit supporting the nerve along its whole length, silk loops are fixed to each end of the nerve and are used to stretch it.

The moist chamber. This is intended to serve the following purposes:

1) to fix the nerve to the stimulating and recording electrodes so that stimuli may be applied and action potentials recorded,

2) to maintain the nerve in good state, i. e. produce in vitro conditions approximating those in vivo. It would, of course, be ideal if the nerve were placed in a solution of identical temperature and composition as the extracellular fluid of the animal from which it was taken. These conditions can be fulfilled ideally when using intracellular microelectrodes. Electric stimulation and recording of bioelectric potentials with external electrodes, however, require the absence of solution between the electrodes, it is a short-circuiting factor.

Moist chambers, of which there are as many modifications as there are laboratories using them, are always a certain compromise between those two conflicting requirements.

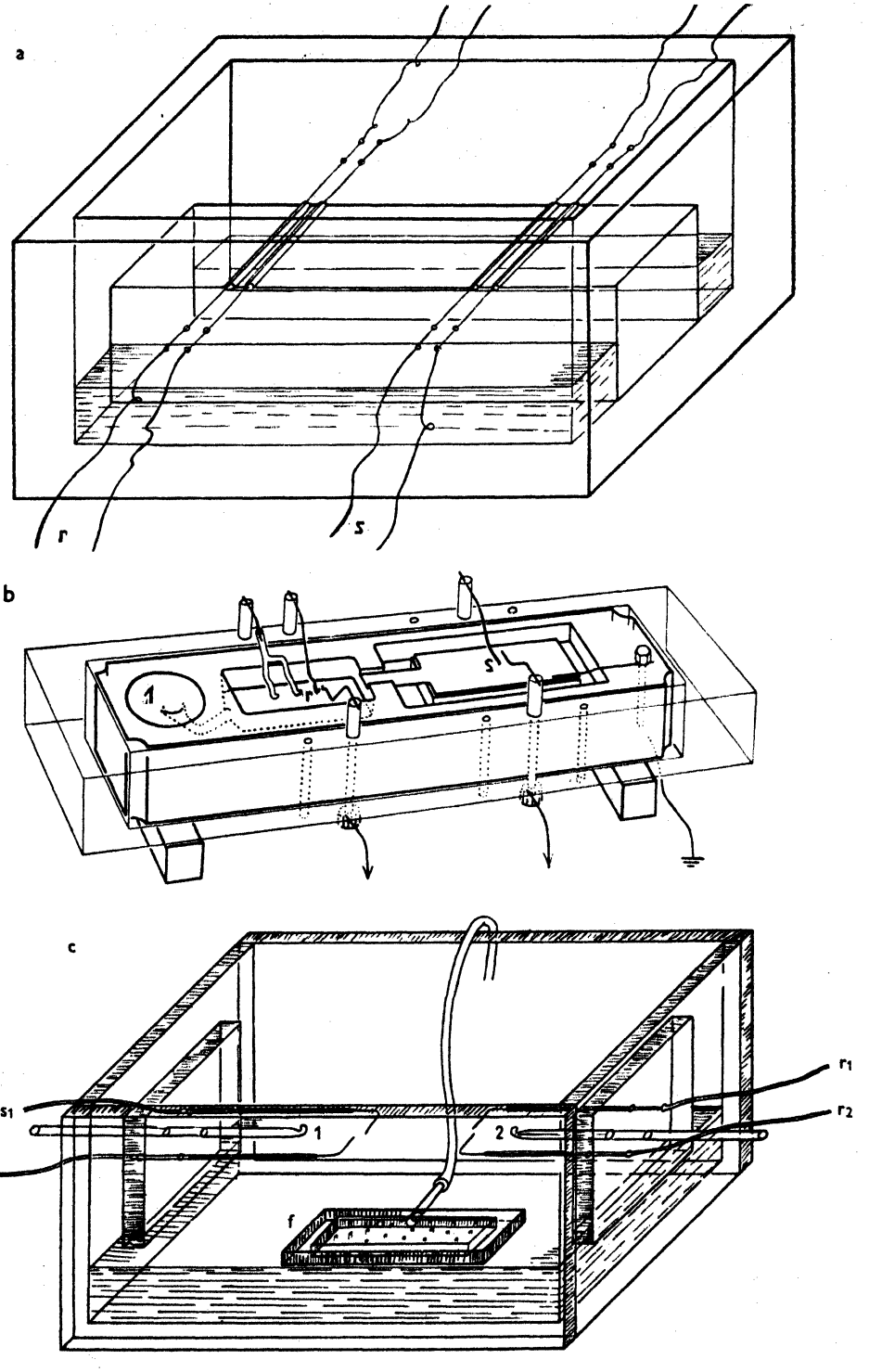
Fig. 81 shows several diagrammatic representations of moist chambers.

The simplest is a paraffin chamber with an elevation in the centre. Several pairs of platinum wire may be placed into small grooves in this elevation. The wires are connected to insulated leads leaving the chamber. The bottom of the chamber on both sides of the elevation contains Ringer solution. The nerve is placed on the electrodes and the chamber is covered with a glass plate. Evaporation of the solution in the closed chamber is sufficient to prevent the nerve from drying (Fig. 81a).

A similar chamber may be prepared from plexiglass. Stimulating and recording electrodes on movable riders are fixed to a bridge that can be inserted into two pairs of contacts on the lateral sides of the chamber. Contacts must be made from nonoxidisable material.

Fig. 81b shows a nerve chamber suitable for recording from isolated nerve fibres. The chamber is made of plexiglass and composed of a frame carrying the stimulating (s) and recording (r) electrodes and of an exchangeable bridge with a groove below the recording electrodes. The dissected nerve fibre is placed in the groove filled with Ringer solution so that its distal part lies on the recording electrodes. The Ringer solution in the groove is then replaced by paraffin oil from 1.

Another type of chamber is shown in Fig. 81c. The nerve has no firm support but is stretched between two glass hooks (1, 2). Platinum electrodes are sealed into the glass tubes so that they are connected to their leads outside the chamber (r_1, r_2, s_1, s_2,). A fine sintered glass filter through which a mixture of 5% CO_2 in O_2 or air is bubbled into the frog Ringer solution is placed at the bottom of the chamber (f). Bubbling results in fine dispersion of the solution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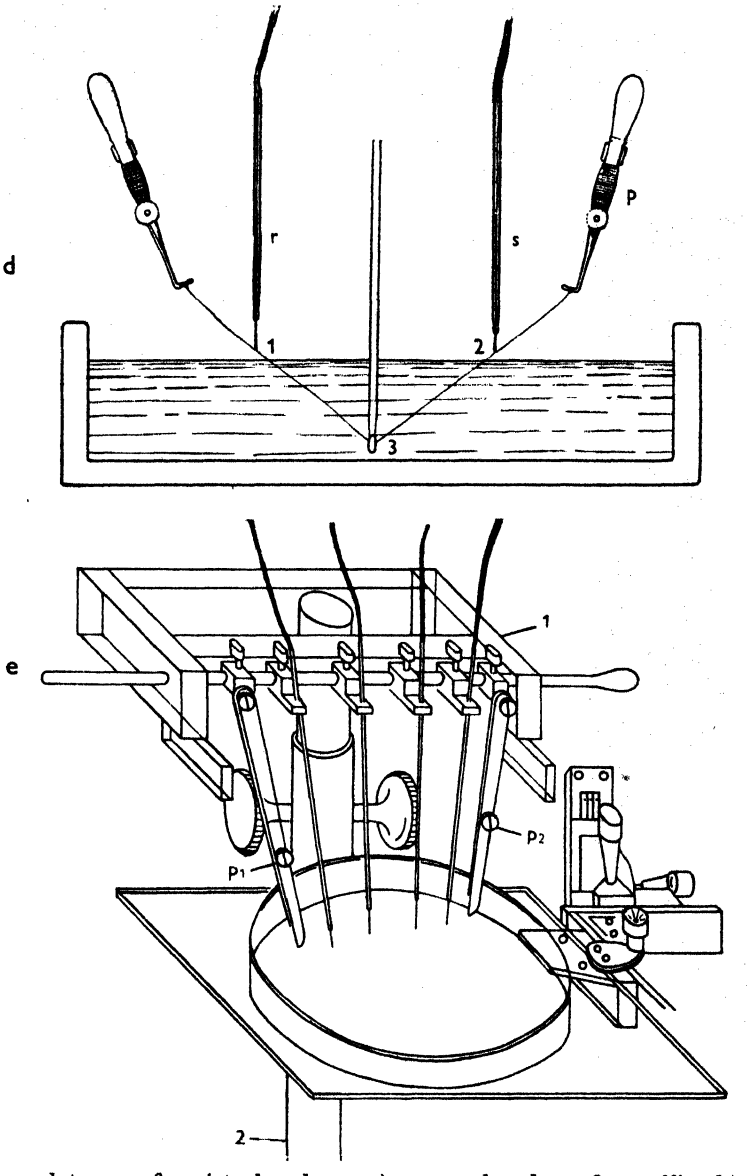


Fig. 81. Several types of moist chambers. a) nerve chamber of paraffin. b) plexiglass nerve chamber for isolated nerve fibres. c) moist chamber without a firm support for the nerve; 1,2 — glass hooks; r_1, r_2 — recording and s_1, s_2 — stimulating electrodes, f: fine filter for aeration of Ringer solution and introduction of gases into the chamber. d) arrangement for studying the effect of drugs on the nerve; p — screw controlled forceps, r, s — electrodes, 3 — point of contact of the glass hook with the nerve; 1, 2 — electrodes formed by the Ringer-air (or oil) interface, the common indifferent electrode is connected to the saline (e. g. silver plate). e) a commonly used arrangement for fixing the nerve and mounting the electrodes. p_1, p_2 — screw controlled forceps for holding the nerve, 1 — electrode support, 2 — stand for vertical movement of the electrode support.

within the chamber, thus causing the solution to settle on the nerve and maintaining the desired gas atmosphere around the nerve.

In some experiments the method illustrated in Fig. 81d is also suitable. The nerve is suspended in the solution at a certain angle. It may be kept in place at point 3 with a glass hook. The solution is earthed. The boundaries 1 and 2 between the solution and air act as the stimulating or recording electrode. The other stimulating or recording electrode is situated on that part of the nerve which is in air. By changing the level of the solution the distance between the recording and stimulating electrodes can be varied. This arrangement is used when studying the effect of different drugs on peripheral nerves.

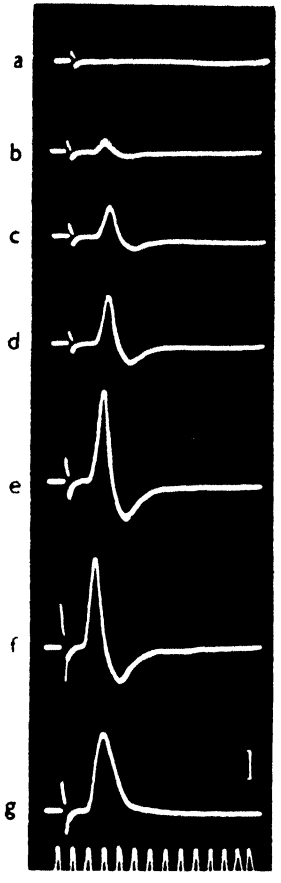


Fig. 82. Response of frog sciatic nerve to gradually increasing strength of the stimulating pulse. Time mark: 1000 c/sec. Voltage calibration: 1 mV.

Fig. 81e shows a diagram of a method used very frequently. Platinum hook electrodes are sealed into glass tubes fixed to a horizontal plate (1). The nerve is held between two forceps and the stimulating and recording electrodes carefully placed on it. The electrode holder can be moved along a vertical steel rod (2).

The electrodes together with the nerve can be immersed in Ringer solution when electric activity is not recorded, or raised to their original position when stimulating or recording.

Registration. The moist chamber (Fig. 81e) is placed inside a screened chamber. The stimulating and recording electrodes are connected to screened leads from the stimulator or amplifier. The nerve is fixed between forceps p_1 and p_2 and the stimulating and recording electrodes are carefully applied. For the time being the nerve is immersed in Ringer solution in a Petri dish. Stimulation frequency is adjusted to 20 c/sec. Each pulse of the stimulator is synchronised with the sweep of the oscilloscope. The nerve and electrodes are taken out of the solution and drops remaining between the electrodes are dried off. The voltage of the square wave pulse of the stimulator is gradually increased and the shape of the tracing is observed on the oscilloscope. First the stimulus artifact appears on the screen, i. e. the stimulating pulse which reaches the recording electrode by physical spread of the current. This indicates the moment of stimulation (Fig. 82a). By further increasing the voltage, the artifact

grows until a slight undulation appears on the tracing. This increases on further increasing the stimulus voltage (Fig. 82b, c, d) until a full sized action potential is obtained (Fig. 82e, f). In this case a biphasic action potential is recorded. Using a sharp forceps the nerve is now clamped between the electrodes r_1 and r_2. The second phase of the potential disappears, since the nerve impulse does not reach the second electrode. A monophasic action potential is then registered (Fig. 82g).

A voltage calibrator is connected to the amplifier input and the size of the calibration pulse is photographed from the screen of the oscilloscope. Amplification must remain the same as when photographing the action potential. The time marker is then connected to the oscilloscope input (a sine wave from the audio-oscillator) and is also photographed (Fig. 82). Here again the time base must remain the same as before.

Conclusions: The action potential of a preparation: sciatic nerve-tibial nerve has an average amplitude of 2 mV. The duration of the action potential is 2·5 msec. The ascending phase lasts for 0·5 — 1·0 msec, the descending phase 1·0 or 1·5 msec. Apparently the action potential does not obey the "all or nothing" law, since its amplitude increases from threshold to maximum values. This, however, is due to the fact that the sciatic nerve is composed of a large number of nerve fibres. By gradually increasing the voltage of the stimulus, more and more nerve fibres are stimulated. Each fibre responds according to the "all or nothing" law but the recorded compound action potential is a summation curve of a large number of spikes with different thresholds, rates of conduction and amplitudes. Since the rate of conduction is not known exactly, the wave length cannot be determined.

b) The action potential of isolated nerve fibres. The "all or nothing" law

Problem: Record the action potential in non-myelinated nerve fibres of the crayfish *(Astacus fluviatilis)*. Demonstrate the validity of the "all or nothing" law.

Principle: A nerve such as the sciatic nerve is composed of a large number of nerve fibres with different diameters, stimulation thresholds, conduction velocities and spike amplitudes. When such a nerve is stimulated, fibres with the lowest threshold are excited first.

The action potential of each individual fibre is subject to the "all or nothing" law. It is produced when a certain stimulus value is reached and does not increase on increasing the intensity of the stimulus. Other fibres, however, are activated. The action potential recorded from a nerve is the sum of action potentials of its individual fibres.

The result of a gradually increasing stimulus is a gradually increasing amplitude of the action potential. When all fibres are excited, the action potential amplitude does not increase further, even on further increasing the intensity of stimulation.

Non-myelinated invertebrate nerves are suitable for demonstrating these facts experimentally, since nerve bundles composed of a small number of nerve fibres, sometimes of only two or three or even one, can be found in them. It can be demostrated that in one fibre the maximal action potential is produced by a threshold stimulus. With invertebrate nerves containing one or only a few nerve fibres it is not necessary to remove the fibres one by one in order to obtain a one fibre preparation as is the case for vertebrate nerves.

Object: Three fibre preparation from the claw of the crayfish *(Astacus fluviatilis)*.

Apparatus: A single beam cathode-ray oscilloscope with a preamplifier, overall minimum sensitivity of 100 μV/cm. A calibrator. Time marker 1000 c/sec. A stimulator. A screened chamber.

Other requirements: A recording bridge for fixing the preparation. Dental drill, paraffin dish, crayfish solution (van Harreveld 1936; 12·6 g NaCl, 1·5 g $CaCl_2$, 0·4 g KCl, 0·25 g $MgCl_2$, 0·2 g $NaHCO_3$ per litre distilled water), paraffin oil, oxygen, solid paraffin. Dissecting instruments.

Procedure: Three-fibre preparation. The large and small nerves of the proximal joint of a crayfish (ischio- and mero-podite) are isolated and a fine nerve bundle having only three motor fibres is prepared. This separates in the carpopodite from the remaining fibres and branches multifidously at the propodite attachment.

The medial side of the large claw is used. First the hard shell is removed. This is facilitated by drilling holes into it with a dental drill. Lateral incisions are made together with incisions in the sulci of the ischio, mero- and carpopodite. A semicircular incision is drilled in the propodite at the border of its lower third. It leads from the edge of the m. abductor to the opposite edge of the claw (Fig. 83a).

The limb prepared in this fashion is cut off from the body in the coxopodite. The wound can be treated with heated paraffin to prevent bleeding.

The claw is now placed into the paraffin dissecting dish filled with crayfish saline.

It is best to start removing the shell from the ischiopodite. If the shell is not completely cut, scissors may be used to complete the incisions. The muscles attached to it are removed with a scalpel close below the inner face of the shell. The freed shell is removed up to the next incision. The same method is used for the other joints. Special attention is paid to the lateral sulcus of the meropodite and medial sulcus of the carpopodite. Here the nerve

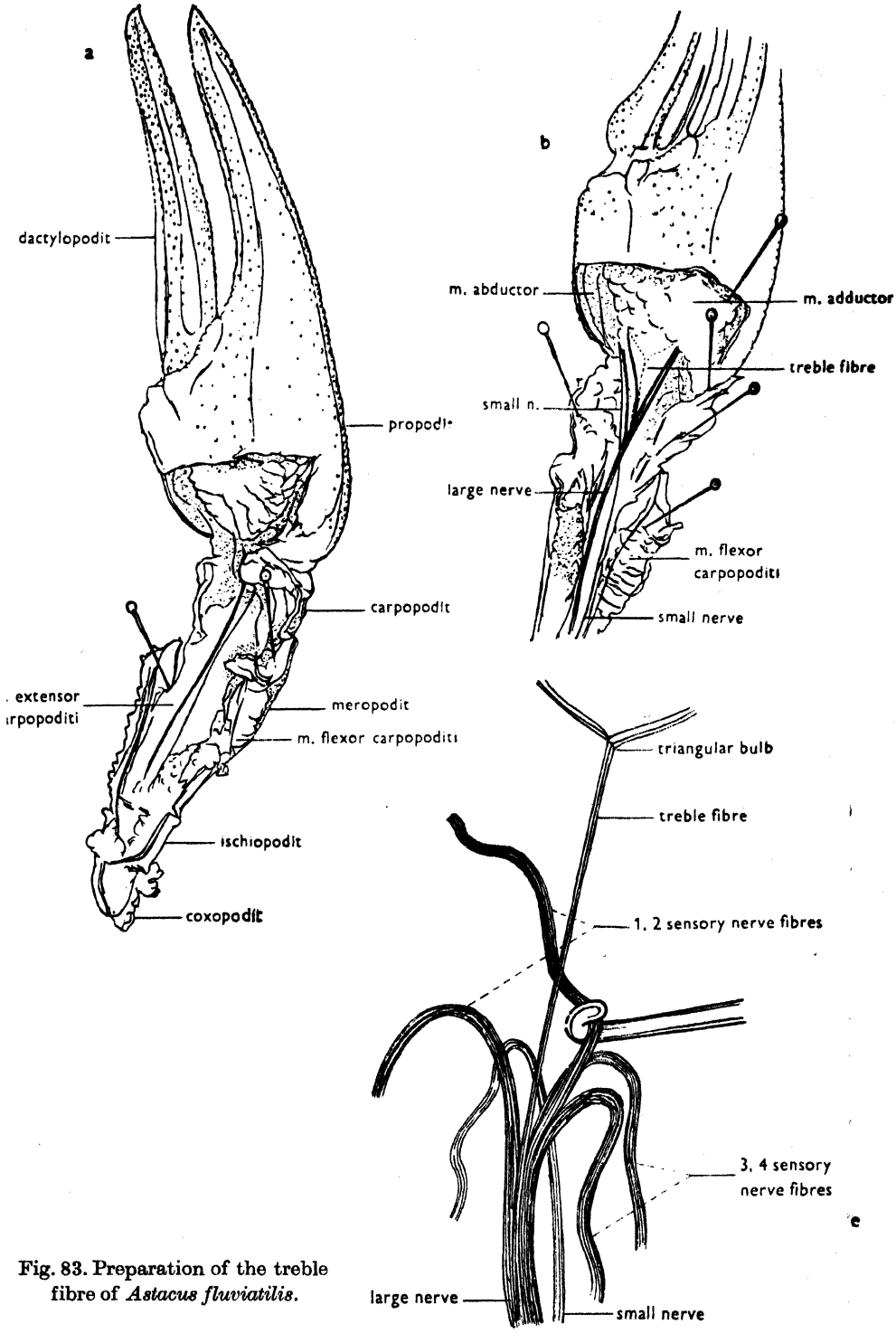


Fig. 83. Preparation of the treble fibre of *Astacus fluviatilis*.

trunk is superficially placed. The fine skin covering these areas is cut and pulled off with forceps.

The nerve trunk is then exposed. The preparation is fixed in the paraffin dish with pins and covered with saline. The site of dissection is illuminated with a focused light. Dissection is best started in the meropodite (Lewin 1927, Furusawa 1929, Monnier and Dubuisson 1931, Bayliss et al. 1935, Bogue and Rosenberg 1936).

A needle is used to fix the meropodite as laterally from the sulcus as possible, to prevent injury to nerves running beneath it.

After fixation of the meropodite, the central tendon of the m. flexor carpopoditi is held with forceps. The tendon is attached to the carpopodite and can be seen in the merocarpal fold as a white rectangle. The muscle is pulled away. This is easily done, since removal of the shell has freed its attachment.

When removing the muscle care must be taken not to injure the small nerve that is more deeply situated in the muscle. After removal of the flexor carpopoditi, the small and large nerves can readily be seen along the m. extensor carpopoditi. Both nerves can be followed in the ischiopodite, and here too they can be isolated from surrounding muscle bundles.

In the carpopodite the nerves run between two muscular bundles. They are situated superficially and their course can be traced immediately upon removal of the shell. Both muscle bundles are pulled laterally (with needles) in order to expose the nerves.

1) It can be seen that in about the middle of the carpopodite the small nerve passes below the large one, reaching the opposite side of the large nerve (it approaches the edge of the abductor muscle of the claw). (Fig. 83b).

2) The large nerve divides into several finer bundles. Two of those are directed towards the adductor of the claw and the other two run to the abductor muscle, together with a vessel, in the ridge between the abductor and adductor. The fifth and last bundle runs a nearly medial course. It is possible to see three reflecting axons (three fibres) under the dissecting microscope if good illumination is used. Each of the three axons divides near the attachment of the muscle. A triangular bulb can be seen at each bifurcation (Fig. 83b, c).

A 2—3 cm length of the fibres must be isolated. This is obtained by cutting the four sensory branches somewhat above the division of the three fibre bundle and by gradually pulling them one by one with a forceps towards the meropodite. Thus the motor fibre of the adductor muscle (three fibre bundle) is freed from the other sensory nerve fibres.

When the bundle is exposed for a length of 2—3 cm, the other nerve bundles which have been pulled away are severed and all tissue connections and side branches are removed in the exposed part. The large nerve is then

isolated from the muscles in the mero- and ischiopodite. In such a way a preparation is obtained composed of the large nerve ending with a 2—3 cm long portion of the small nerve bundle.

It is advisable to leave some muscle tissue intact at both ends of the preparation, i. e. both at the end of the large nerve and the end of the three fibre bundle. This makes fixation of the preparation in the recording bridge easier.

Recording and fixing arrangement. The three fibre preparation must not remain in contact with air, since it dries rapidly. Recording can therefore only be made under paraffin oil. The arrangement shown in Fig. 81e is therefore useful. The treble fibre together with crayfish saline is sucked into a wide tube and transfered into the crayfish saline in a Petri dish. Using forceps p_1 and p_2 the ends of the treble fibre and the nerve are held. Using microscrews the preparation is slightly stretched. The recording electrodes are carefully placed on the treble fibre and the stimulating electrodes on the nerve. A layer of paraffin oil is carefully placed on the crayfish saline. Using microscrew the whole electrode holder is now carefully raised into the paraffin oil. The solution forms a very fine film surrounding the preparation. This ensures a normal ionic environment around the nerve and also makes it possible to stimulate and record. The paraffin must be pure and it is advisable to bubble oxygen through it before the experiment is started.

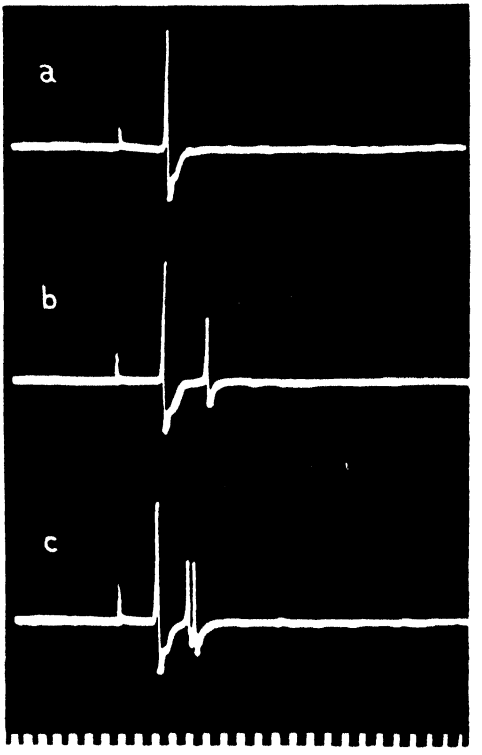


Fig. 84. Response of the treble fibre of *Astacus fluviatilis* to gradually increasing stimulus strength. Time mark: 1 msec.

Registration. The procedure is similar to that in the previous experiment. The voltage of the stimulating square wave pulse is gradually increased. First the stimulus artifact appears on the screen. This gradually increases with increasing stimulus. At a certain value of the stimulating voltage, the action potential of one fibre suddenly appears. On further increasing the stimulus intensity its amplitude does not change but a new action potential appears, when the threshold of another fibre has been attained. Then the stimulating

voltage is further increased until the action potential of the third fibre appears. It can be seen that the amplitude of the previous action potentials has not increased. After the threshold of the third fibre has been reached, further increase of stimulus voltage will not result in the appearance of further action potentials (three fibres!), nor will the amplitude of the action potentials change (Fig. 84).

When decreasing the intensity of stimulation, the action potentials of the individual fibres disappear in the reverse order. The time mark (sine-wave 1000 c/sec. from the audio oscillator) and the amplitude calibration pulse are photographed without changing the time base and sensitivity of the apparatus.

After registration is over, the electrodes and the preparation are immersed in crayfish saline. The experiment may be repeated several times. The fibre is capable of functioning for several hours.

Conclusion: The action potential of an isolated fibre arises at a certain threshold value of the stimulus. This occurs suddenly and the amplitude does not change on further increasing the intensity of the stimulus. The "all or none" law holds good for one fibre. The distance of the action potential from the stimulus artifact indicates a conduction velocity that is much smaller than in vertebrate nerves.

B. Propagation of nervous impulses

It is generally agreed that the cause of spreading of nerve impulses along a nerve fibre is the ability of the excited zone to stimulate the adjoining regions. The active point is depolarised and negative. Thus a potential difference arises between this point and the neighbouring region. This potential difference produces local currents (hence the term: local circuit theory), which flow from the intact surface to the depolarised point and thus progressively depolarise the adjacent inactive region.

The potential difference caused by an action potential is several times larger than that necessary for the production of a nervous impulse in a neighbouring point. There is thus a large safety factor, permitting conduction to occurr even under adverse conditions, e. g. during the relative refractory period after a nervous impulse.

Examples given in this section are chosen to show the technique of measuring the rate of conduction as well as some classical experiments which led to the formulation of the local circuit theory.

a) Measurement of conduction velocity of a nervous impulse

Problem: Determine the conduction rate of a nervous impulse in A-fibres of the frog sciatic nerve.

Principle: The sciatic nerve of the frog is a mixed nerve. It contains fibres having diameters from 3—29 μ. The corresponding differences in conduction velocities are not evident at small distances from the point of stimulation. The recorded action potential is smooth, although it represents the sum of potentials from hundreds of fibres with different conduction velocities. At greater distances from the point of stimulation, the different conduction velocities manifest themselves as small humps on the descending limb of the action potential. At very great distances the action potential of the most rapidly conducting fibres is separated from that of more slowly conducting ones.

Hence recordings must be made at sufficient distances from the stimulating electrode in order to differentiate between groups of fibres.

The conduction velocity can be calculated in two ways. Both require a determination of the path travelled by the nerve impulse and time necessary for it.

The first method records the action potential at a certain distance from the stimulating electrode. Time is measured from the stimulus artifact to the onset of the ascending phase of the action potential. This method is less exact, since the point of stimulation cannot be determined accurately enough because of the possibility of stimulating with virtual cathodes (p. 236).

The second method uses registration of action potentials from two points that are separated by a given distance. This avoids the difficulty of determining accurately the point of origin of the action potential, but is more elaborate. A dual-beam oscilloscope with two differential amplifiers is needed. A single beam oscilloscope may also be used by alternating the connection of the electrodes to the amplifier input.

Object: A sciatic nerve — peroneal nerve preparation from the largest frogs available. Length more than 13 cm.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier. Overall sensitivity of the apparatus 100 μV/cm. A time marker 1 msec. A square wave stimulator.

Other requirements: A moist chamber for the nerve with 5 platinum electrodes. Otherwise as in experiment IVAa.

Procedure: The nerve is fixed in the moist chamber using silk loops tied to glass rods and slightly stretched (Fig. 81c). Glass hooks are placed under the nerve at several points. The stimulating electrodes are placed close to

the origin of the sciatic nerve from the spinal cord. The first recording electrode (r_1) is situated at the beginning of the peroneal nerve, the second (r_2) is close to the end of the preparation. The reference electrode r_i is at the end of the nerve (monophasic recording).

First r_1 against r_i is connected to the amplifier input. The intensity of the stimulus is increased and the amplitude of the action potential is observed. An action potential first corresponding to A fibres, group α is formed. Gradually group β appears, and finally γ fibres are also visible. After photographing the recording from the first electrode, electrode r_2 is connected to the amplifier input and, using the same intensity of stimulation, the action potential from a more distant part of the nerve is recorded (Fig. 85).

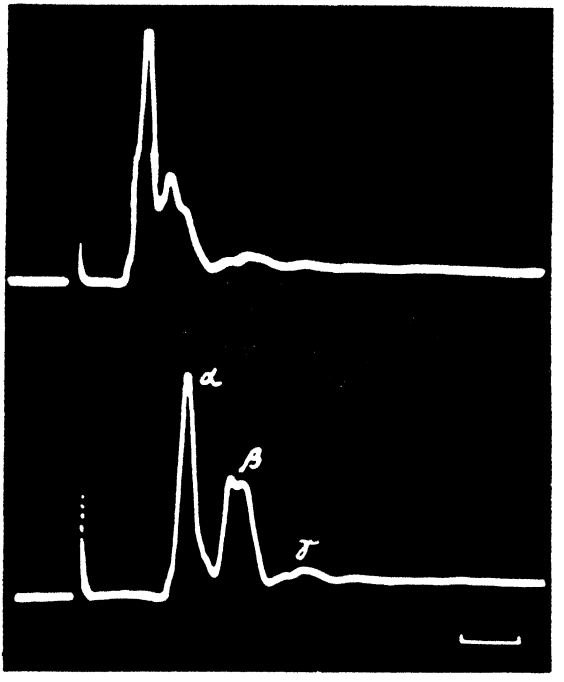


Fig. 85. Conduction velocity in frog sciatic and peroneal nerve A fibres. Time mark: 2 msec.

The time signal and amplitude calibration are photograhped and the distance between electrodes r_1 and r_2 is measured with calipers. This gives all the data necessary for calculating the rate of conduction of different groups of A-fibres.

The time from the onset of the action potential at the first electrode r_1 to the onset of the action potential at electrode r_2 is either measured in enlarged positive pictures, care being taken to have the same enlargement, or from negative films projected onto paper on which the time scale has been marked previously. The same sweep, starting from the same point of the screen must be used when recording the time mark and the action potentials in order to avoid error due to a non-linear course of the time base.

When enlarging, systematic errors must also be avoided. These may be due to thickness of the enlarged tracing when measurements are not made from a standard point, e. g. always from the upper contour of the trace.

With these precautions, the time differences between action potentials

of groups α, β and γ are determined. The conduction velocity is calculated by the equation:

$$\bar{v} = \frac{s}{t}$$

where $\bar{v}$ = the mean conduction velocity in m/sec., s = the length of the path in m (the distance from the first recording electrode to the second one), t = = time between the appearance of the action potential at r_1 and r_2 in seconds.

Results from an experiment similar to that shown in Fig. 85 are given in Table 3. The distance $r_1 - r_2 = 0{\cdot}055$ m.

TABLE 3

	t	$\bar{v}$
α-fibres	0·0013 sec	42 m/sec
β-fibres	0·0022 sec	25 m/sec
γ-fibres	0·0034 sec	16 m/sec

Conclusion: The conduction velocity of the most rapidly conducting group of fibres (A) was determined in the frog's peroneal nerve. It has 4 components α, β, γ, δ. The conduction velocity was determined for the first three. With greater amplification, the conduction velocity in the B and C fibre groups can also be measured. These have a smaller spike-amplitude and a lower conduction velocity (B-4·2 m/sec; C-0·4 m/sec). From the aspect of central coordination, the hundredfold differences in conduction velocity in fibres of the same nerve are undoubtedly important.

b) Relation between fibre size and conduction velocity

Problem: Compare the conduction velocities of two nerve fibres of different diameters.

Principle: Within the same phylogenetic species it is possible to demonstrate a relationship between fibre diameter and conduction velocity of a nervous impulse. The original concept, that conduction velocity is proportional to the square of fibre diameter (Blair, Erlanger 1933) has been modified. Today it is believed that the relationship is linear (Gasser and Grundfest 1939) in mammalian nerves. Multiplying the diameter of the myelinated nerve fibre (in μ) by 6 gives the approximate conduction velocity in m/sec.

This cannot be applied to fibres from different species. During phylogeny the rate of conduction increased by increasing fibre diameter (e. g. giant fibres) in invertebrates, whereas in vertebrates, this come about rather by

myelinisation and development of the mechanism of saltatory conduction. In different species, the product of fibre diameter times birefringence of the fibre sheath is approximately constant (Taylor 1942).

It is difficult to determine the relationship between the conduction velocity and the fibre diameter in mixed nerves. One of the methods used is to correlate the maximum conduction velocity in different mixed nerves with the corresponding maximum fibre diameter as determined histologically (Hursh, 1939).

A preparation with few fibres is better for demonstrating these relations. The simplest way is to compare the rate of conduction in two fibres with differing diameters. The rate of conduction of each fibre is determined in the usual way and its diameter is then measured under the microscope.

Object: A motor double fibre, an inhibitory and excitatory nerve, innervating the abductor of the claw of the crayfish *(Astacus fluviatilis)*.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier, total sensitivity 100 μV/cm. A voltage calibrator. A time marker 1000 c/sec.

Other requirements: Two pairs of recording electrodes. Otherwise as in experiment IV. Ab.

Procedure:

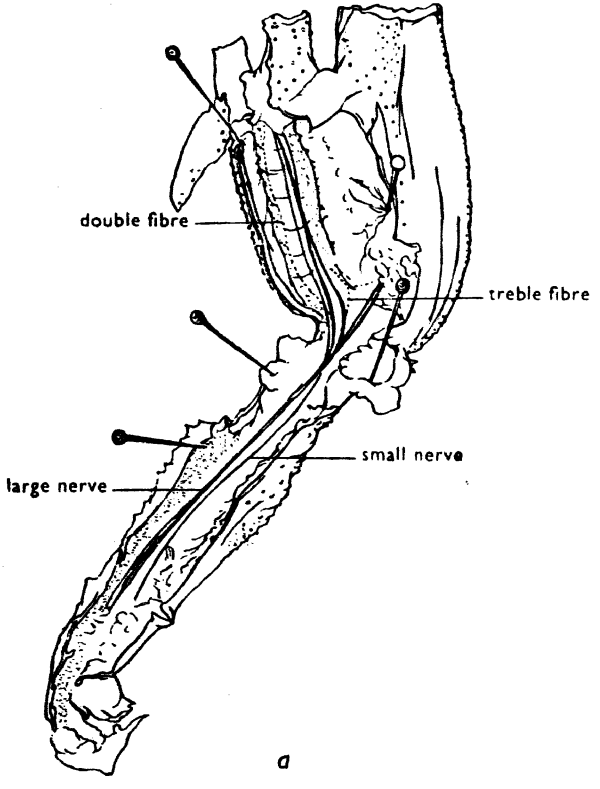
a) Preparation of the double fibre.

The fibre consists of a small and large nerve trunk and a 1—3 cm portion of the inhibitory and excitatory fibre innervating the abductor muscle of the crayfish claw. In the proximal portion the inhibitory fibre is contained in the large and the excitatory fibre in the small trunk. Both fibres separate from the other fibres in the distal part of the limb. Enveloped in the same sheath they form in the propodite the so called double fibre (Fig. 86).

Opening of the shell and isolation of the nerve trunks is basically performed in the same way as described for the treble fibre (page 210). Differences in the preparation are desribed below.

When incising the shell in the propodite four longitudinal incisions are made in addition to the semicircular one at the boundary of the lower third of the claw. These are joined in the upper parts in such a way that three rectangular fields are formed. Then the shell is easily removed. It is important to make a special hole on the dorsal side of the propodite in the ridge between the abductor and adductor muscles. The incision does not reach the lower edge of the propodite so that on deflecting the shell it remains connected to the rest of the shell.

When preparing the trunks it is necessary to avoid injuring the small nerve trunk in the lower parts of the mero-, ischio- and carpopodite. Special care must be taken in the carpopodite in which the small nerve passes below the large one and comes to lie on the opposite side. This point must remain free from injury.



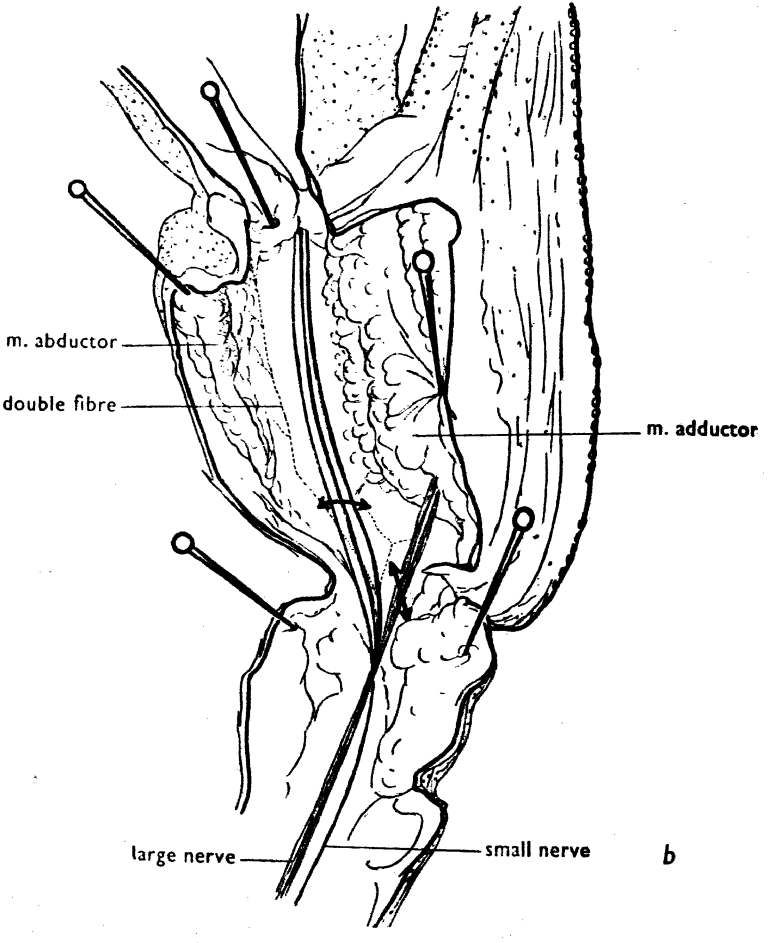


Fig. 86. Preparation of the double fibre in *Astacus fluviatilis*.

The double fibre is prepared under the microscope. The propodite must be fixed. The adductor muscles is pulled away with a firm needle which is also used to fix the propodite to the board. The other side (edge) of the adductor muscle is also pushed onto the board and a second needle is passed through the internal side of the cut stump of the daktylopodite and fixed as far laterally as possible to the bottom of the preparation dish.

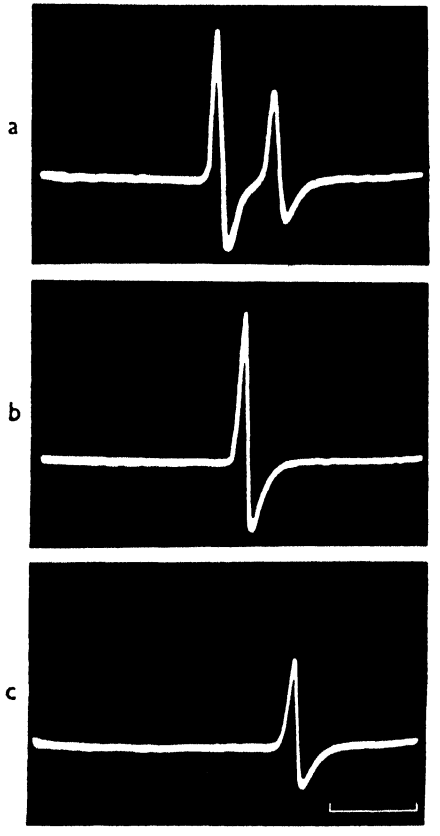


Fig. 87. Action potential of the excitatory and inhibitory nerve fibre of *Astacus fluviatilis*. a — double fibre, b — inhibitory fibre, c — excitatory fibre. Time mark: 10 msec.

After cutting the connective tissue between the two musles these are separated from each other. It is best to leave the small nerve and the two sensory branches of the large nerve together with the vessel on the side of the adductor muscle and to separate the abductor. Then it is possible to pull away carefully the freed edge of the abductor muscle and to look for the double fibre. Using good concentric illumination and correctly turning the dish the axons reflect the light and are seen as two bright fibres running parallel to each other and enveloped in a connective tissue sheat. The double fibre sends off branches while passing along the abductor muscle. These are cut. In the lower third the double fibre changes its direction and runs obliquely downward towards the sensory fibres. Here it passes below a vessel. After separating the double fibre from the muscle along its whole length the sensory fibre is severed just where it passes below the vessel. Usually the double fibre is only prepared up to this point but sometimes, especially if a longer sector is required, it may further be isolated and separated from the vessel (arrow 1 — Fig. 86).

Finally the nerve trunks are separated from the muscle in the ischio-, mero- and carpopodite up to the free part of the double fibre. Here special care must be taken in the carpopodite. The double fibre runs deeply submerged in the muscles and separates. The inhibitory fibre joins the large nerve, the excitatory one the small nerve. A binocular lens is always used for dissecting. The sensory fibres are severed and separated from the muscle (arrow 2 — Fig. 86).

Then the most distal portion of the fibre is exposed and separated where it finally branches. A piece of muscle in which the fibre terminates is also taken so that the preparation may be held in forceps by this muscle.

b) The preparation is fixed as in experiment IV. Ab. First the large nerve which contains the inhibitory nerve is placed on the stimulating electrodes. On stimulation the action potential of the inhibitory fibre is recorded at the recording electrodes (Fig. 87b). Stimulation of the small nerve, on the other hand, evokes an action potential in the excitatory fibre (Fig. 87c). When both are stimulated at the same time the action potential of both fibres is obtained (Fig. 87a). The conduction velocity in both fibres can be estimated from the distance between the stimulus artifact and the action potential. More accurate results are obtained when two pairs of recording electrodes, which can be connected alternately to the amplifier imput, are placed on the double fibre. First the action potential from electrodes r_1, and then that from the more distant electrodes r_2, is recorded. Finally the distance between the first electrodes of both pairs of recording electrodes is measured.

Conclusion: The inhibitory fibre has a diameter of 18 μ and the excitatory fibre one of 14 μ (Eckert and Zacharová 1957). It is therefore to be expected that the rate of conduction in the inhibitory fibre is greater. The experiment recorded in Fig. 87 confirms this assumption. Fig. 87b shows a recording from the double fibre when stimulating the large nerve, which contains the inhibitory axon, and the action potential recorded, consequently, belongs to that of the inhibitory fibre. Fig. 87c shows the action potential of the excitatory axon evoked by stimulation of the small nerve, which contains this fibre. It can be seen that the inhibitory axon, having a larger diameter, conducts impulses at a greater rate than the excitatory fibre. This is shown in Fig. 87a where both nerve trunks were stimulated simultaneously and action potentials from both fibres were evoked. The action potential can be identified not only from the conduction velocity, but also by its amplitude. The spike-amplitude of the inhibitory axon is greater than that of the excitatory one. This is in full agreement with the rule that the conduction velocity and spike amplitude are directly and the threshold to electric stimulation indirectly proportional to the fibre diameter.

c) Extrinsic potentials

Problem: Record the extrinsic potentials and changes in excitability of a peripheral nerve beyond a cold block.

Principle: This experiment tests the hypothesis that local currents are important for the propagation of nerve impulses. The excited point causes increased excitability in adjacent regions of the nerve. The same is true for

the nervous impulse arriving at a nerve block. Changes in excitability are produced beyond the latter even if the impulse itself cannot pass it. This effect has been long known in the literature as Wedensky facilitation across a block (1903). The method for ascertaining such changes in excitability is shown in Fig. 88a. A block is induced at site B, e. g. by clamping the nerve. One pair of stimulating electrodes is above the block (s_1), the other beyond the block (s_2). At a certain distance from them the recording electrodes are placed at C. When a block is formed at B no potential is recorded from C on stimulating at s_1. At s_2 subthreshold stimulation is applied and again no action potential is recorded at C. If, however, s_2 is stimulated at the moment when the nerve impulse elicited at s_1 reaches block B, then it may be expected, according to the local circuit theory, that part of the local currents will flow from a site beyond the block towards the stretch of the nerve in front of the block depolarised by the arriving impulse (Fig. 88b). In such a way local currents produce increased excitability beyond the block. A subthreshold stimulus at s_2 will become effective now, and an action potential is recorded at C.

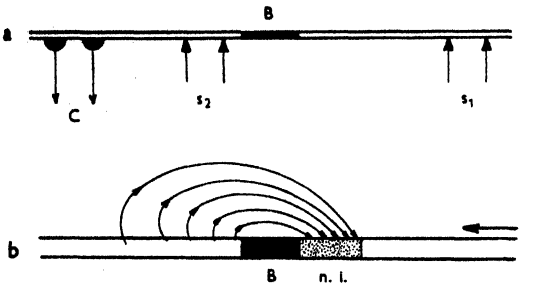


Fig. 88. Determination of excitability changes beyond a block. a) — electrode arrangement. s_1, s_2 — stimulating electrodes, C — recording electrodes, B — blocked nerve segment, n. i. — nerve impulse. b) flow of local currents beyond the block after the arrival of the nerve impulse in the blocked segment.

This experiment was already performed by Wedensky, who concluded that the nervous impulse increases the stationary excitation in the blocked site for a certain short period, and produces corresponding periparabiotic excitability changes (Wedensky 1903).

Hodgkin in 1937 recorded electrical changes occurring beyond the block after arrival of the nervous impulse and corresponding to the excitability increase predicted by the local circuit theory. This potential was termed the "extrinsic" potential by him. It differs from the action potential in having a lower amplitude and a spatial decrement (a decrease to 1/e of its value in two mm).

Apparatus: Cathode-ray oscilloscope with a preamplifier, total amplification at least 100 μV/cm. Two pairs of stimulating and one pair of recording platinum electrodes. A stimulator with two outputs. The time interval between the first and second output pulse can be changed continuously. Time marker 1000 c/sec. A voltage calibrator.

Other requirements: A moist chamber (e. g. Fig. 81c). An arrangement for producing cold block. This is an L shaped glass tube (diameter 5 cm) closed

at both ends with rubber stoppers. The tube is filled to ¼ its volume with ether through an opening in the upper stopper. Ether vapours are sucked off through another opening in the same stopper using a water vacuum pump (exhauster). A temperature of −10 to −15°C can easily be attained. A silver wire (diameter 0·5 cm.) passes through the lower stopper. This serves to lead off heat from the nerve to the tube. The area of the wire touching the nerve can be changed at will by soldering silver plates of different dimensions to the end of the wire. The wire is introduced into the chamber through a longitudinal fissure in its side, thus permitting movement of the cooling apparatus and placing of the cooled wire below any part of the nerve. The temperature act-

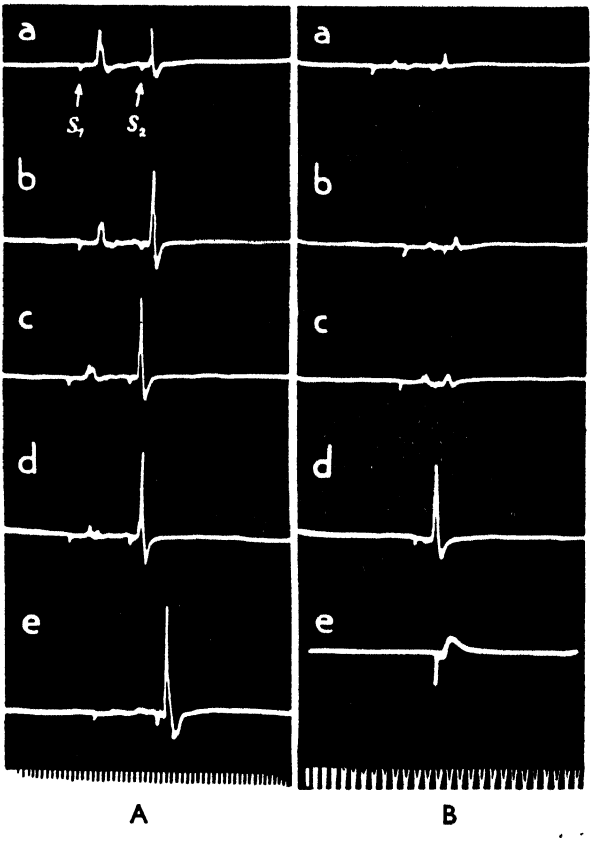


Fig. 89. Changes of excitability in the vicinity of a cold block. A) increase of excitability in the vicinity of a developing cold block. Time mark: 0,5 msec. B) changes in excitability produced by a nerve impulse beyond the cold block. Time mark: 1 msec.

ing on the nerve can be changed by changing the rate of exhaustion or by switching off the pump. Nerve blocks below −6°C, i. e. supercooled (Boyd and Ets 1934) are usually irreversible. It is advisable to use reversible blocks of about −1°C. This may be simply achieved (Hodgkin 1937) by encasing the whole wire in paraffin and leaving a slit along its whole length, thus maintaining contact with the air. A layer of ice begins to form at the cooler end of such a slit. This gradually progresses along the wire and reaches the nerve, and thus supercooling does not occur.

Object: Sciatic nerve — peroneal nerve of the frog *(Rana esculenta* or *Rana temporaria)*.

Procedure: The nerve is stretched as described previously (p. 208) in a moist chamber. Two pairs of stimulating electrodes, recording electrodes and the silver cooling wire are placed on the nerve as shown in Fig. 88a.

The nerve is stimulated with a maximal stimulus at s_1. A subthreshold stimulus is applied to point s_2 after a certain time interval greater than the duration of the refractory period. A maximum action potential of A fibres is obtained in response to stimulus s_1. No action potential appears after stimulation of s_2. Then cooling with the silver wire is started. Gradually a cold block develops in more and more nervous fibres. This is evident from the fact that the action potential produced at s_1 and recorded beyond the block becomes smaller and smaller. Since, on the other hand, the excitability near the block increases (in Wedensky's terminology: near the parabiotic focus is a peripara-biotic increase in excitability), the subthreshold stimulus at s_2 becomes a suprathreshold one, and consequently a submaximal action potential is evoked also in response to stimulus s_2. On further cooling, the action potential, evoked at s_1 continues to decrease until it finally desappears altogether,while that elicited from s_2 continues to rise. Fig. 89A shows oscillograms illustrating this part of the experiment.

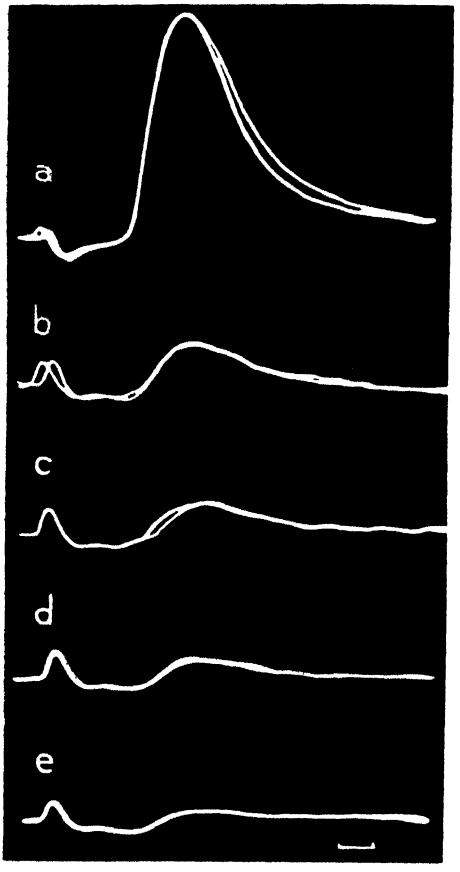


Fig. 90. Spatial decrement of extrinsic potentials. Time mark: 1 msec.

After warming the nerve, the experiment is repeated as follows. When the block at B is nearly complete, the stimulus intensity at s_2 is lowered so as to make the stimulus subthreshold or just threshold once again (cf. osc. 89Ba). When now the interval between stimuli is reduced so that the impulse from s_1 reaches block B just at the instant when the stimulus from s_2 begins to act, an action potential appears on the recording electrodes in response to stimulus s_2. This indicates that the impulse from s_1, although already blocked, produces an increase in excitability beyond the block (osc. d, Fig. 89B).

If the stimulating electrodes s_2 are disconnected from the stimulator

and connected to the amplifier input instead of electrodes C, the extrinsic potential is recorded beyond the block.

The next experiment is arranged in that way. One recording electrode is proximal to the block and four beyond the block. One after the other, these five electrodes are connected, against the distal recording electrode, to the amplifier input so that the spatial decrement of extrinsic potentials can be observed.

Fig. 90 shows a recording from such an experiment.

Conclusion: The first experiment showed that a periparabiotic excitability increase occurs around the parabiotic focus in the sense of the terms as used by Wedensky. The mechanism of this excitability change can be explained in a similar way as the excitability increase ahead of a nerve impulse, i. e. by assuming a flow of current from the periparabiotic areas to the parabiotic region. The only difference is due to the fact that in this case stationary depolarisation (Wedensky's stationary excitation) is developing much more slowly.

Further experiments confirmed the assumption deduced from the local circuit theory. After arrival of the impulse at the block, an excitability increase is found beyond the block accompanied by an electric potential having a large potential decrement along the nerve. The extrinsic potential is an electrotonic effect of the action potential. A nearly identical spatial distribution was obtained by Hodgkin (1937) when he applied a short catelectrotonic pulse to the blocked site. The extrinsic potential precedes the impulse wave when the latter is normally propagated along a nerve and represents the foot of the ascending phase of the spike potential. This phase is not active. The active phase follows it only after a certain period, as is also shown by simultaneous recording of the action potential and of impedance changes during a nervous impulse (Cole and Curtis 1939).

d) The law of independent conduction

Problem: Demonstrate that α and β spikes of the A group of nerve fibres in the frog sciatic nerve are conducted by separate fibres.

Principle: This demonstration is based on differences in threshold, and also utilises the refractory period (p. 247). If α and β spikes are conducted by separate nerve fibres, the test stimulus maximal for α and β fibres following a stimulus maximal only for α fibres, should produce an action potential only in β fibres, since it falls into the refractory period of the previously stimulated α fibres.

Object: Frog sciatic nerve — peroneal nerve preparation, at least 12 cm in length *(Rana esculenta* or *Rana temporaria)*. Large frogs must be chosen so that separation of the α and β spikes caused by different conduction velocities in α and β fibres will be apparent.

Apparatus: A cathode-ray oscilloscope with a preamplifier. Total sensitivity 100 μ/cm. A time calibrator. A stimulator giving paired square wave pulses with independently adjustable delay and amplitudes. A radiofrequency output unit. A moist chamber and other requirements as in experiment 1VAa.

Procedure: The nerve is fixed in the moist chamber in the usual way. The stimulating electrodes are placed close to the exit of the sciatic nerve from the spinal column, and the recording electrodes on the end of the peroneal nerve. Since the distal recording electrode is situated at the point where the silk loop is attached, a monophasic recording is made. A sufficiently large interval between stimulating pulses is chosen; the second stimulus is applied only after the end of the refractory period following the first stimulus. The voltage of the first stimulating pulse (s_1) is adjusted so that only α fibres are stimulated, that of the second (s_2) is maximal also for β fibres. A recording as shown in Fig. 91a and b is obtained.

With the voltage of both pulses remaining unchanged the delay between them is shortened. The action potential of the α fibres in response to stimulus s_2 gradually decreases (Fig. 91 d) until it disappears completely (Fig. 91e). Only the response of β fibres to the second stimulus s_2 remains. The stimuli may be permitted to merge, but the β spike evoked by s_2 does not change.

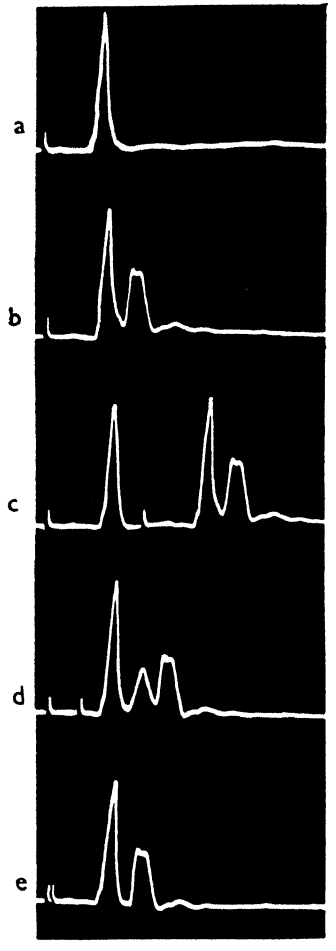


Fig. 91. Demonstration of independent conduction in α and β frog nerve fibres. The time interval between the shocks is 5·5 msec. in c.

Conclusion: This experiment, first performed by Erlanger and Gasser (1937) demonstrated that the β spike is conducted by other fibres than the α spike. According to the conduction velocity it may be judged that the β fibres have a smaller diameter (cf. p. 217).

e) Interaction between nerve fibres

Problem: Determine excitability changes in one fibre when an impulse passes in another closely adjacent fibre.

Principle: Under physiological condition a nervous impulse cannot pass from one nerve fibre to another. This, however, does not mean that the impulse does not affect the excitability of adjacent fibres (Katz and Schmitt, 1940). This effect can also be deduced from the local circuit theory. Imagine two fibres lying next to each other as in Fig. 92. The shaded area indicates the nervous impulse moving from left to right (fibre I). Arrows indicate the direction of current flowing ahead of and behind the impulse. Any point of the active fibre will be successively a source, sink and again source of current, a small fraction of which will flow through the inactive fibre. With respect to the inactive fibre, the current flow is successively anodal, cathodal and anodal. Accordingly, thresholds are at first increased, then decreased and finally again increased. The course of excitability changes in the inactive fibre is thus opposite to that in the active fibre.

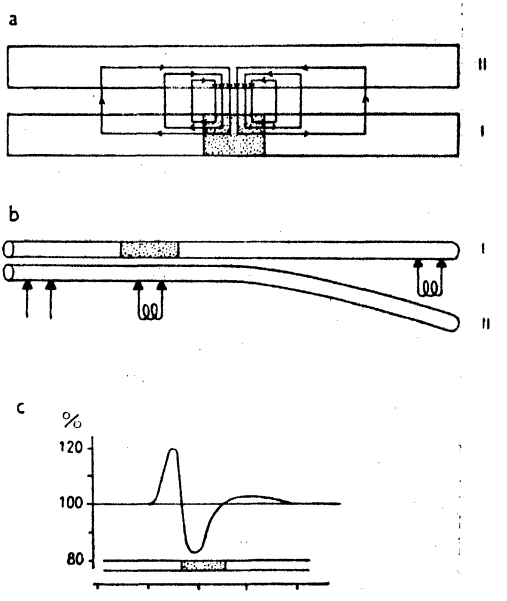


Fig. 92. Interaction between two nerve fibres. a) course of lines of current flow in fibre II when the impulse is in fibre I (dotted area). b) diagramatic representation of experiment designed to determine the interaction between two nerve fibres. c) threshold representation of changes in threshold in fibre II when the nerve impulse is in fibre I.

To demonstrate this process, two fibres lying next to each other are necessary. It must be possible to stimulate one fibre separately as is shown diagramatically in Fig. 92. An impulse is evoked in fibre I with stimulating electrodes s_1. Excitability changes in fibre II are determined with stimulating electrodes s_2 during the passage of the impulse through fibre I. Action potentials from both fibres are recorded with the same recording electrodes.

Apparatus: A cathode-ray oscilloscope with a preamplifier. Total sensitivity about 100 μV/cm. A stimulator with two independent outputs, continuous adjustment of pulse delay, independent regulation of the amplitude of both pulses.

Other requirements: as in experiment IVAb.

Object: An isolated double fibre - inhibitory and excitatory axons of the claw abductor muscle of the crayfish *(Astacus fluviatilis)* — with the large and small nerve trunks (cf. p. 218).

Procedure: The object is prepared as described previously (p. 218). The nerve trunk containing the excitatory axon (I) is placed on electrodes s_1. Electrod-

es s_2 and r are placed on the isolated double fibre. It was shown previously that the spike amplitude of the excitatory axon is lower than that of the inhibitory one. This helps to identify the fibres. In addition the threshold is lower in the inhibitory axon. The fibre I is given a suprathreshold stimulus with electrodes s_1. The action potential of this fibre appears on the recording electrodes r. If a suprathreshold stimulus is applied through electrodes s_2 the action potential of both fibres is obtained on electrodes r (Fig. 93a). The larger spike is from fibre II, the smaller one from fibre I. The intensity of stimulation at s_2 is then gradually decreased. The spike of fibre I disappears first (fig. 93b), since it has a higher threshold. The spike of fibre II then also falls out (Fig. 93c). The intensity of the stimulus at s_2 is kept just below threshold while a suprathreshold stimulus is applied at s_1. Now the interval between the stimulating pulses s_1 and s_2 is changed. If it is such that the cathode s_2 begins to stimulate with subthreshold intensity at the moment when negativity of the nervous impulse occurs in the adjacent region of the I fibre, the spike of the inhibitory fibre also appears at the recording electrodes (Fig. 93d,e). For other delays between the pulses s_1 and s_2 the spike of the inhibitory fibre disappears again.

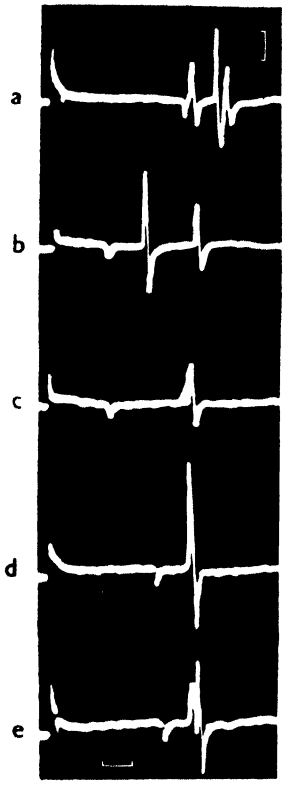


Fig. 93. Demonstration of interaction between inhibitory and excitatory axons in the double fibre of the crayfish. Voltage calibration: 2 mV. Time mark: 5 msec.

The second experiment is performed as follows: The intensity of the stimulus s_2 is kept just at threshold value for fibre II. At s_1 the intensity of the stimulus is suprathreshold for fibre I as in the preceding experiment. If now the delay between the two pulses is changed the following results are obtained. If cathode s_2 stimulates much earlier than the I impulse wave arrives, both action potentials are registered. If the s_2 stimulus immediately precedes the arrival of the I impulse into the stimulated area the action potential from fibre II disappears. This lasts until the beginning of stimulation at s_2 coincides with the moment the I impulse arrives at the point, next to which fibre II is stimulated. At that moment the action potential of fibre II again appears. It is sufficient, however, to increase the interval between the impulses only slightly for the action potential of the inhibitory fibre to disappear again. If

the interval is further increased a point is reached after which the action potential again appears.

Conclusion: The first experiment shows that the excitability of a point in fibre II increases during the passage of a spike through a corresponding point of fibre I (decreased excitability). The second experiment indicates that for a certain period before and after the passage of a nerve impulse in fibre I, a decrease in excitability occurs in fibre II. These experiments thus confirm the deduction made from the local circuit theory. In such a way the exact time course of excitability changes in fibre II could be mapped out when a nerve impulse passes in the adjacent fibre I. Fig. 92c shows such a map using a similar preparation. It is evident that the course of changes is the same as was demonstrated qualitatively in the experiment presented here.

The threshold changes shown in this experiment are small and so do not contradict the principle of isolated conduction. Impulses connot jump from one fibre to another under natural conditions. This may be achieved artificially by increasing the excitability of the fibre e. g. by decalcification. Local currents entering the adjacent inactive fibre become suprathreshold. This was shown by Kwasow and Naumenko (1936) in the nerve-muscle preparation and Arvanitaki (1942) in the nerves of *Lolligo*. She called this artificial synapse ephapsis.

C. The initiation of nerve impulses

Nerve impulses may be elicited by different chemical, mechanical and electrical stimuli. In the organism either stimulation of a receptor organ or excitatory activity of preceding neurones constitutes physiological stimuli. During propagation of the nerve impulse along a nerve fibre the impulse itself is the stimulus as is postulated by the local circuit theory. Fundamentally this is an electrical stimulus formed successively along the nerve fibre by the nervous tissue itself.

Practically, electric stimuli are the most suitable artificial stimuli. They can be characterised well and produced simply and repeatedly even in a short period of time, since their action is completely reversible. For those reasons they are very frequently, or even exclusively, used when analysing nerve impulses.

Natural or artificial stimuli result in changes in the nerve membrane that make the latter more permeable to Na ions (cf. chapter I).

Experiments to be described here are intended to give practical examples of conditions under which an electric stimulus evokes excitation of a nerve. Changes occurring in nervous tissue under the influence of an electric current and the effect of Na ions on the action potential of a nerve will be demonstrated.

a) Electrotonus and nerve excitability

Problem: Demonstrate changes in excitability occurring in the frog nerve as the result of cat- and anelectrotonus. Record electrotonic potentials.

Principle: It is instructive to consider a nerve fibre as a core-conductor (Herman 1879) when studying the effect of an electric current on a nerve. Such a conductor consists of a core (the interior of the axon) that has a small resistance or high conductivity and is separated from the extracellular space (also having a high conductivity) by a membrane composed of high resistance and capacitance units (Fig. 94a).

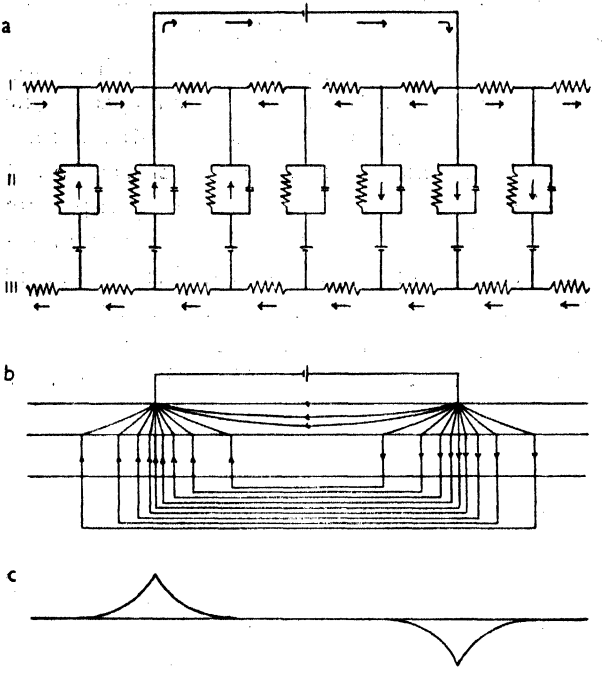


Fig. 94. a) diagram of the electrical equivalent of the nerve membrane. Arrows indicate current flow after switching on the current in the model. On the right — anode, on the left — cathode. I. — equivalent of extracellular space, III. — intracellular space, II. — membrane. b) current distribution in the membrane. c) current density below the cathode and anode.

If a current is applied to a nerve through extracellular electrodes, it will flow as shown by arrows in Fig. 94b. Part of the current flows through the extracellular space and part across the membrane into the axon, through the conductive interior and again across the membrane to the cathode.

The density of the current flowing across the membrane is greatest at the site of application of the current, i. e. at the point of electrode contact. It decreases in an approximately exponential manner on both sides (Fig. 94c). When the current flowing from the axoplasma across the membrane to the outside attains a critical level, a propagated response is produced. Changes occuring in the nerve at the cathode when applying subthreshold currents result only in an increase in excitability. The spatial distribution of increased excitability corresponds to that of the current density, i. e. it decreases exponentially with increasing distance. At the anode opposite changes occur — excitability decreases.

In general these changes produced by a flow of current in the nerve are termed electrotonus. At the point where the current enters the axon anelectrotonic changes occur, whereas at the point where it leaves the axon catelectrotonic changes are encountered.

The above changes in excitability form the basis of Pflüger's rule (1859). Werigo (1883, 1901) found that the cathodic increase in excitability represents only a temporally limited part of the effect of a cathodic current on the nerve. If the cathode is permitted to act on the nerve for a longer period, the increase in excitability is not so evident as in the beginning. Finally, immediately below the cathode, a decrease in excitability, so-called cathodic depression, is produced. The zone of increased excitability shifts towards the sides as is shown in Fig. 95.

If during this cathodic depression the anode acts on the nerve, it has the opposite effect from that predicted by Pflüger's rule. It restores the decreased excitability — or in other words, raises it to normal. According to Werigo (1901), the anode effect depends upon the state of the nerve's excitability. When excitability is decreased by strong cathodic current below r (Fig. 95d) the anode returns it to normal value, i. e. it increases excitability. If excitability is increased by weaker cathodic action, the anode again returns it to value r — i. e. in this case it decreases excitability.

Woronzov (1924, 1925) discovered that the anode acts in this way (increases excitability) also if excitability is decreased by the action of a KCl solution (Woronzov's phenomenon).

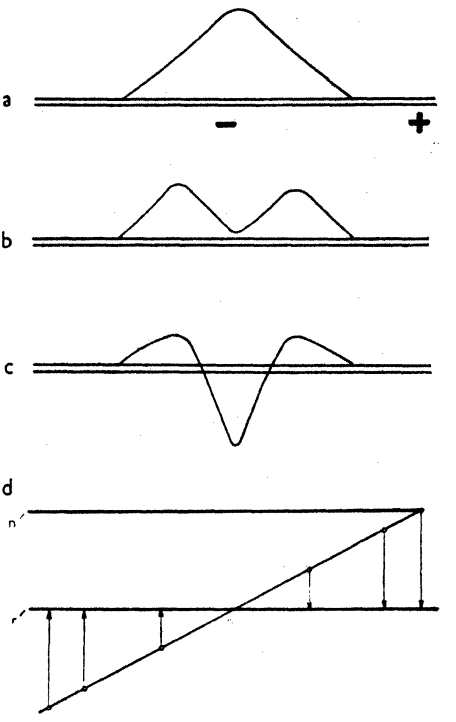


Fig. 95. Diagram of the development of Werigo's cathodal depression. a) excitability changes immediately on switching on the cathode. b, c) after prolonged cathode action on the nerve. d) diagramatic representation of the anode effect on the nerve in different states of excitability according to Werigo. n — normal excitability, r — line of reversal of anodal effect. Arrows indicate the direction of excitability changes due to the anode acting at different initial states of the nerve.

Changes in membrane potential produced by an external current are termed electrotonic potentials.

In order to determine qualitative cat- and anelectrotonic excitability changes in a mixed nerve it is sufficient to observe the effect of electrotonus on

the height of the spike amplitude after submaximal stimulation with electrodes near the cathode or anode of the polarising external current.

Recording of electrotonic potentials is performed as illustrated in Fig. 99. The letters p_1 and p_2 are electrodes through which the current is applied, r_1 and r_2 are the recording electrodes. If electrode r_2 is at a sufficient distance from p_1, no measurable current flows below it. Its potential may be regarded as constant and is conventionally marked as zero. In such a way the electrotonic potential is only determined by the position of electrode r_1 and the current below it.

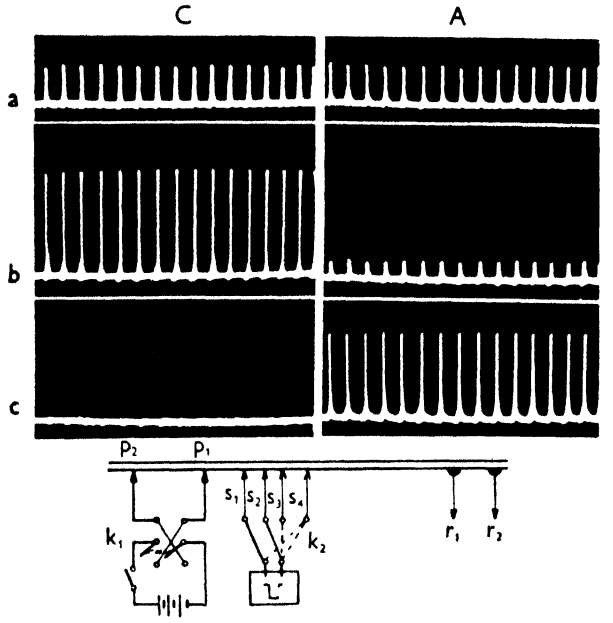


Fig. 96. Effect of cathode (column C) and anode (column A) on the excitability threshold of the nerve. a) before the make, b) 2 sec. after the make, c) 3 sec. after break of the D. C. current. Below is the arrangement of electrodes for qualitative determination of excitability changes in the vicinity of the cathode and anode of the applied current. p_1, p_2 — polarising electrodes, s_1, s_2, s_3, s_4 — stimulating test electrodes, r_1, r_2 — recording electrodes, k_1 — commutator, k_2 — selector switch.

The time course of the electrotonic potential obviously depends upon the time course of the applied current. According to Lorente de Nó (1947), it is of advantage to use rectangular current pulses since this considerably simplifies experimental conditions. The electrotonic potential is closely related to the mechanism maintaining the resting membrane potential. According to Lorente de Nó, the membrane potential consists of two parts: Q (quick) and L (labile). Two components of electrotonus (so-called slow and quick electrotonus) correspond to these two fractions of the membrane potential. The slow one is an expression of fraction L, the rapid one of fraction Q.

Both components of the membrane potential depend on the state of the nerve, which can be changed by changing the gaseous or fluid medium of the nerve. In such a way it is possible to emphasise or supress one or the other component.

Object: Sciatic nerve — peroneal nerve preparation of the frog *(Rana esculenta* or *Rana temporaria).*

Apparatus: A D. C. cathode-ray oscilloscope with a D. C. preamplifier,

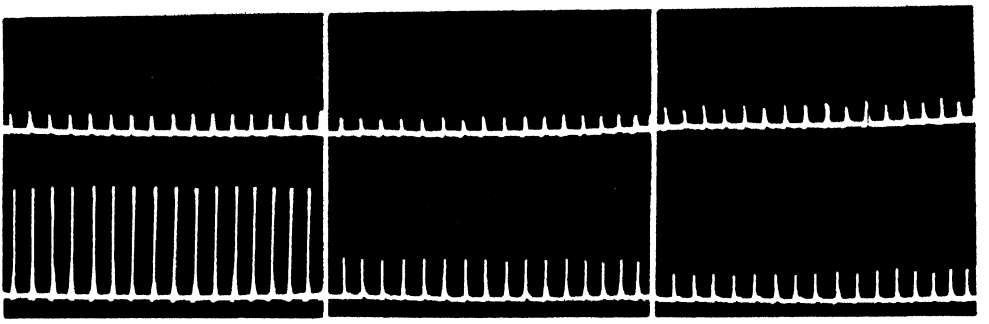


Fig. 97. Excitability changes produced by the cathode at various distances from the point of its application. Upper oscillograms: activity before the cathode is applied. Lower oscillograms: at distances 3, 6 and 9 mm from the point at which the cathode is applied.

total sensitivity of at least 100 μV/cm. Time marker 0·1 sec. A stimulator giving direct current pulses from 0—50 μA. A nerve chamber. Nonpolarisable electrodes (Fig. 55).

Other requirements: As for experiment IVAa. 95% O_2 and 5% CO_2.

Procedure:

1) Changes in nerve excitability near the cathode and anode.

The nerve is fixed in the nerve chamber by silk loops on both ends of the preparation. Electrodes are distributed as shown in Fig. 96. Electrodes p_1 and p_2 are 30 mm apart. The nerve is severed in the middle of the interpolar distance. The first stimulating electrode s_1 is situated 3 mm from electrode p_1. The remaining electrodes are also 3 mm apart. An A. C. amplifier suffices for this experiment.

Using the pole-changing switch ((k_1), electrodes p_1 and p_2 are connected so that p_1 is the cathode of the applied current. Using the second switch (k_2), stimulating electrodes s_1 and s_2 are connected to the stimulator. The nerve is stimulated with a train of submaximal stimuli and the result is observed (Fig. 96). The current is allowed to flow in the circuit $p_1—p_2$, the electrode p_1 being the cathode. The amplitude of the spikes has increased considerably while the intensity of stimulation in s_1 s_2 remained the same. After switching off the polarising current, the spike amplitude decreases until it nearly disappears. The experiment is repeated with p_1 as the anode. Spike amplitude decreases greatly (Fig. 96), and it increases again when the current is switched off. Using the same current intensity the experiment is repeated for different distances between the stimulating electrodes and electrode p_1 (Fig. 97).

2) Werigo's cathodic depression and the phenomenon of Woronzov.

A freshly prepared nerve is fixed as in the previous experiment. It is

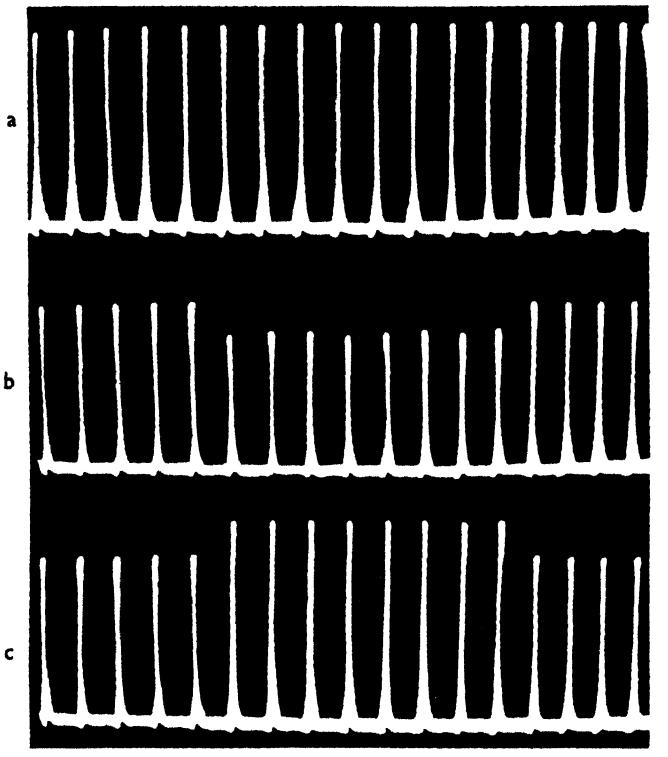


Fig. 98. Woronzov's effect. a) normal responses to maximal stimuli for A-fibres. b) 2 min. 8 sec. after application of 0·15M-KCl; decrease due to the cathode. c) increase due to the anode. Stimulation frequency 100 c/sec.

stimulated with a frequency of 100 c/sec. through electrodes s_1 — s_2 using a stimulus maximal for A fibres. The intensity of stimulation is then halved (submaximal stimulus), and a polarising current of 7 μA is allowed to pass. The amplitude of the spikes increases at first. As the polarising current is permitted to continue, this increase gradually becomes less and less evident until spikes are as low as at the beginning — Werigo's cathodic depression sets in. Finally the action potentials disappear.

The polarising current is switched off. The amplitude of the spikes begins to increase only very slowly and when the anodic current is switched on action potentials increase.

Woronzov's experiment is performed on another nerve. This is placed on the electrodes in a nerve chamber similar to that shown diagramatically in Fig. 99. A stimulus maximal for A fibres is applied to the nerve through electrodes s_1—s_2 situated in the middle between p_1 and p_2 electrodes. A vessel containing 0·15 M-KCl solution is placed between electrodes p_1 and r_1. At

first neither anodal (7 μA) nor cathodal polarisation causes any changes. After some minutes, however, the cathode already produces a block in a large number of nerve fibres (Fig. 98b) and the anode restores spike amplitude to normal (Fig. 98c). Later, until complete block occurs, these changes are even more pronounced.

3) Electrotonic potentials.

Electrodes are arranged on the nerve as shown in the diagram (Fig. 99). The distance between p_1 and p_2 is 3 cm. Electrode r_1 is situated successively

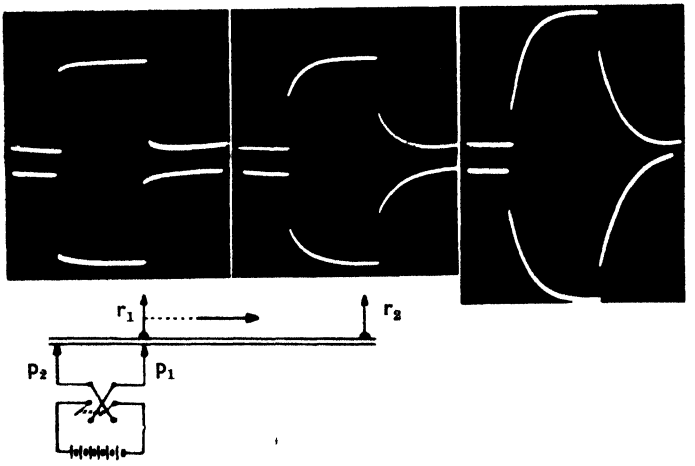


Fig. 99. Electrotonic potentials 1, 2 and 5 mm from the polarising electrode. Upper row: catelectrotonic potentials. Lower row: anelectrotonic potentials. Nerves in 95% O_2 + + 5% CO_2. Below: diagramatic representation of the experiment. Electrotonic potentials 2 mm and 5 mm from the p_1-electrode are recorded at 2·5 and 5 times higher amplification respectively in comparison with 1 mm distance recordings.

1, 3 and 5 mm from p_1. Electrode r_2 is 30 mm from r_1. A rectangular current pulse of 1500 msec. duration is applied through electrodes p_1, p_2. The intensity of the polarising current is gradually increased and changes are observed on the screen of the oscilloscope. The nerve is in 95% O_2 + 5% CO_2 atmosphere.

The first column of Fig. 99a show tracings recorded with electrode r_1 at a distance of 1 mm. The second column the same for a distance of 3 mm and the third for a distance of 5 mm from electrode p_1.

Both fractions of the electronic potential can be demonstrated in Fig. 99. A discontinuity can be seen at the beginning and end of the electrotonic potential due to a rapid vertical movement of the spot. This corresponds to fast electrotonus. Only its amplitude can be evaluated from the tracing. The discontinuity is followed by a more slowly developing potential increase. This part of the electrotonic potential is the slow electrotonus.

It can be seen from the tracings that, taking the amplification of the apparatus into consideration, both components of the electrotonic potential show a considerable spatial decrement. The rate of decrease is less steep for the slow than for the fast electrotonus. At a distance of 5 mm the slow electrotonus forms a more conspicuous part of the tracing then when recording 1 mm from the polarising electrode.

Now the same experiment is repeated with a fresh nerve in an O_2 atmosphere with electrode r_1 at a distance of 5 mm from p_1. It can be seen that slow electrotonus has disappeared. Small rapid undulations can be seen on the sweep, indicating that the nerve is in an exalted state (asynchronous automatic activity). Introducing 95% O_2 and 5% CO_2 considerably changes the electrotonic potential. A pronounced increase (on the cathode), or decrease (on the anode), of the slow electrotonus occurs. This is an expression of changes in the L-fraction of the membrane potential which increases in a gaseous medium of this composition.

Conclusion: The above experiments demonstrate two of three fundamental theses of Pflüger's rule (1859):

1) the cathode of an external current decreases and the anode increases the stimulation threshold in a nerve.

2) after the end of polarisation, excitability is decreased at the cathode and increased at the anode.

3) both effects — the excitability increase at the cathode and decrease at the anode, are maximal during the action of the current. If the excitability of the nerve is tested at intervals during the action of a polarising current the curve of excitability changes during a long lasting polarisation may be drawn. Smoothing of the initial excitability changes during the prolonged action of the same current is an expression of accomodation (Nernst, 1908), i. e. the ability of the nerve to act against the tendency of an external current to excite or to inhibit the nerve (p. 240).

b) Virtual cathodes

Problem: Demonstrate the existence of a virtual cathode in the interpolar segment of a nerve.

Principle: A nervous impulse arises at the cathode of the stimulating electrode. This can be demostrated by compression of the nerve between the stimulating electrodes. If the stimulating electrode proximal to the recording ones is the anode, no response is obtained. It happens frequently, however, that the action potential does not disappear after this procedure. Consequently, especially in the older literature, statements are found that an impulse may arise also at the anode after the closure of the current and at the cat-

hode after the opening of current. This phenomenon was explained by Bernstein (cf. Biedermann 1895) by the formation of a so called virtual cathode. This is especially easily formed during transcutaneous stimulation of nerves in situ, i. e. lying in a volume conductor.

A virtual cathode, however, may also arise when working with isolated or exposed nerves, though much less often. The formation of such virtual cathodes is explained by irregularities in the cross-section of the interpolar segment of the nerve (Lorente de Nó 1947). If this segment has a uniform diameter, the applied current will only flow across the membrane near the cathode and anode. If, however, irregularities are present the current may also leave the axon at the narrowest stretch of the nerve between the electrodes as if a cathode were present at that point (hence the term virtual cathode). When switching on the current an impulse may arise even if the point below the cathode is anaesthetised. With currents of sufficient strength such virtual cathodes may occur close to the anode if a branch is cut or a small drop of Ringer solution left nearby.

The method of crushing the nerve in the interpolar zone, however, is not adequate for determining which electrode is stimulating since it produces irregularity of the interpolar segment. The existence of a virtual electrode can be demonstrated in an experiment in which the anode of the polarising current is placed in the vicinity of the cut branch of the sciatic nerve. The segment of the nerve at the cathode is treated with cocaine.

Object: The sciatic-peroneal nerve preparation of *Rana esculenta* or *R. temporaria.*

Apparatus: A single beam cathode-ray oscilloscope with an A. C. preamplifier, total sensitivity 100 μV/cm. A stimulator giving D. C. pulses of variable amplitude and duration.

Other requirements: A nerve chamber (Fig. 81c), a pair of platinum electrodes and a pair of nonpolarisable electrodes. A 1% solution of cocaine. Ringer solution and Ringer solution saturated with ether.

Procedure: The sciatic-peroneal nerve preparation is placed into the nerve chamber so that the stump of the fibular nerve is 5 mm from electrode p_1, to which the positive pole of the polarising current source is connected. The site where electrode p_2 connected to the cathode is situated is painted with the cocaine solution. The pulse length is 100 msec. The current intensity of the polarising pulse is gradually increased until an action potential appears on the recording electrodes although the nerve below the cathode is anaesthetised.

If now the branch is treated with Ringer saturated with ether the action potential rapidly disappears. After washing this point with pure Ringer solution the action potential reappears.

If now the anode is moved further away from the cut nerve branch, so that the latter is nearer to the cathode, no action potential is obtained, since the virtual cathode at the branch has now become a virtual anode.

Conclusion: The majority of cases described in the literature of impulses appearing after the closure of current at the anode, are due to the existence of virtual cathodes. It is, however, possible to initiate the impulse also by the action of the anode when according to Lorente de Nó (1947), the following conditions are fulfilled

1) the current is very weak,
2) the nerve is in a nearly complete rhytmical state so that its threshold is very low.

c) Strength-duration curve. Chronaxie

Problem: Determine the relation between the strength of the stimulating current and its minimum duration necessary to excite. Measure the chronaxie of the frog's sciatic nerve.

Principle: The threshold intensity of a stimulating current depends on its duration. This relation is characteristic for various excitable tissues and is expressed by the Hoorweg-Weiss' curve which is essentially a hyperbola (Fig. 100). The intensity of the current and the duration of its action are indirectly proportional: $i \,.\, t = k$, or $i = \frac{k_1}{t} + k_2$; k, k_1 and k_2 being constants. The shorter the stimulating pulse the greater is the current intensity necessary to produce an impulse. The t — axis asymptote of the hyperbola signifies the existence of a threshold intensity below which the impulse cannot be evoked regardless of the stimulus duration. This value was termed rheobase by Lapicque (1926). The shortest time during which a rheobasic current must flow in order to produce excitation is termed utilisation time. The asymptote to the ordinate signifies that also a certain minimal time is necessary to produce an impulse.

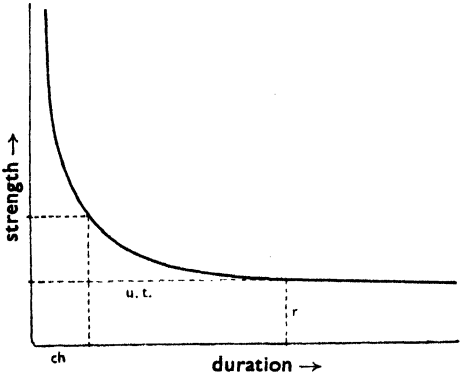


Fig. 100. Strength-duration curve. r — rheobase, ch — chronaxie, u. t. — utilisation time.

The strength-duration curve can be constructed in such a way that -t-coordinates for different -i-coordinates are determined or vice versa. Measurements are most exact in the middle part of the curve.

Lapicque (1926) introduced the so called *chronaxie*: the time during which a current of twice the rheobasic strength must flow in order to excite. Excitability of different tissues may be compared using chronaxie. Chronaxie is measured by determining the minimal stimulus duration for twice the rheobase. Rheobase is a threshold intensity of sufficiently long lasting stimuli.

Object: The sciatic-peroneal nerve preparation of *Rana temporaria* or *R. esculenta.*

Apparatus: A double beam cathode-ray oscilloscope with a preamplifier, total sensitivity 100 μV/cm. A stimulator giving rectangular pulses of variable duration and amplitude and capable of doubling the amplitude. A time and amplitude marker.

Other requirements: A nerve chamber with one pair of platinum (recording) and one pair of Ag-AgCl (stimulating) electrodes.

Procedure: The preparation is placed into the chamber on the recording and stimulating electrodes in the usual fashion. The threshold for a pulse of 100 msec duration is determined. The pulses are then progressively shortened and for each time interval the threshold intensity is determined. The appearance of slight activity on the oscilloscope screen indicates the threshold. The threshold is that stimulus intensity which just fails to evoke activity. The intensity and duration of the pulse are found by photographing it from the oscilloscope together with amplitude and time markings for each point of the curve. If the duration of the pulse is graduated on the stimulator, photographing is not necessary, and strength values may be expressed in relative figures. The stimulator, however, must be calibrated in volts, ampères or arbitrary units.

Chronaxie is determined on the same nerve. A pulse of 100 msec duration is applied and the threshold determined. Using the voltage or current doubler the pulse amplitude is increased to twice the threshold value. A large action potential appears on the screen. The duration of the pulse is then shortened and the value at which the action potential just disappears is found. This time value is the chronaxie.

Conclusions: The paired values obtained and plotted onto paper give the strength-duration curve. The chronaxie may be determined graphically from it. On comparing it with the value determined directly good agreement is found.

The chronaxie determined in the above experiment is that of the most excitable nerve fibres.

Before the development of electronic stimulators, chronaxie was determined with simple chronaximeters in which the duration of the stimulating pulse was determined by the duration of a condenser discharge. Chronaxie was then calculated from the equation:

$ch = 0{\cdot}37 \, . \, R \, . \, C$

where ch = chronaxie (msec),
C = capacity (μF),
R = resistance of the discharge circuit (kΩ).

If the tissue and electrode resistances are to be neglected, a constant current stimulator (p. 33) must be used. A high internal resistance is chosen, so that to obtain chronaxie, capacity C must be multiplied by a whole number, thus simplifying calculation.

d) Accommodation

Problem: Determine the accommodation constant λ in frog nerve.

Principle: In addition to a certain strength and duration, an electrical stimulus must also have a minimum rate of rise in order to produce an impulse. This feature is an expression of the ability of excitable tissues to adapt to external stimuli.

There are a number of theories attempting to explain this phenomenon (Monnier 1930, Rashevsky 1933, Hill 1936). Hill's theory is the most widely accepted.

When a current pulse is applied to a nerve two processes occur. These take place simultaneously, but with different time constants. An exponentially increasing state of excitation begins to build up when a rectangular current pulse is applied to the nerve. After termination of the pulse there is again an exponential decrease to the original value, which has a time constant k.

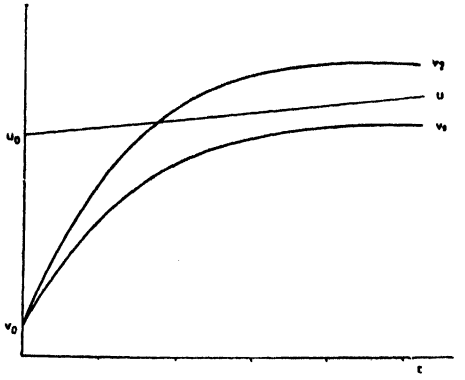


Fig. 101. Origin of excitation in a nerve according to Hill's theory. u — rise in excitability threshold. v_1 — development of a state of excitation for subthreshold stimuli, v_2 — for supraliminal stimuli.

Simultaneously a second process occurs: an exponential increase of the absolute threshold, with a time constant λ.

If the increase in the former (V) is slower than that in the latter (U) excitation does not occur. It does occur if the increase in threshold U is slower (Fig. 101).

The constant λ is an expression of the accomodation capacity of excitable tissue.

In order to determine this constant, a curve of the threshold increase U must be plotted by determining the threshold values for stimuli with various rates of increase. (Solandt, 1936.)

In practice measurements are made according to the principle shown in Fig. 102. Resistance R_1 and R_2, a capacitance C and a switch are connected to the square wave stimulator output. The slowly increasing voltage across the condenser C is used for stimulation (for discussion of similar RC circuits see p. 40). The capacitance C is variable and with its aid the rate of increase of the stimulus can be regulated. At a certain value of R_1, the threshold is

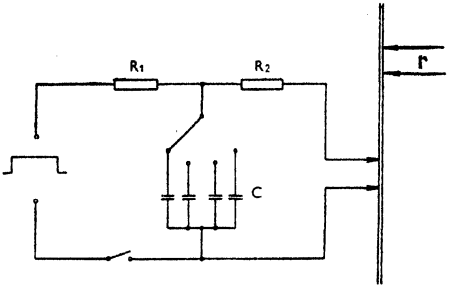


Fig. 102. Electrical circuit in the output of a rectangular pulse stimulator for determining accommodation. R_1, R_2 — resistances, C — variable capacitors, r-recording electrodes.

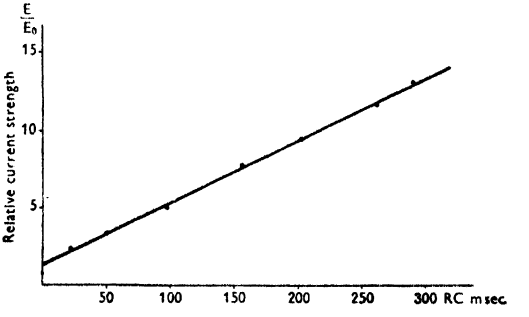


Fig. 103. Accommodation curve. E_0 — threshold voltage of rectangular pulse, E — threshold voltage of a pulse with RC circuit inserted. RC — time constant of pulse rise.

determined without the capacitance C (Eo) and then with the capacitance C, i. e. with a definite time constant (E).

The values obtained are plotted with the ordinate $-\frac{E}{E_0}$ and the abscissa — the time constant $R \cdot C$. The slope of the curve is determined and its reciprocal gives the value of λ.

Apparatus: A cathode-ray oscilloscope with a preamplifier, total sensitivity 100 μV/cm. An amplitude and time marker. A stimulator giving rectangular pulses. A circuit for obtaining exponentially rising pulses which can be connected to the output.

Other requirements: as in exp. IV. Aa.

Object: The sciatic-peroneal nerve preparation.

Procedure: The nerve preparation is fixed in the nerve chamber in the usual way (p. 208). A pair of platinum recording electrodes and a pair of non-polarisable Ag-AgCl stimulating electrodes are placed on the nerve. The threshold is determined with a given value of the variable capacitance C starting with small values of C. The threshold current or voltage E is read from the amplitude regulator at the stimulator output. The threshold E_0 is then determined without the condenser. The time constant of the rate of rise may be obtained by recording and photographing the pulse on the screen of the

oscilloscope and the constant $R \cdot C$ is then calculated from the pulse shape. The time constant can also be computed directly from the R and C values of the circuit. The whole procedure is repeated several times with different C values. The values obtained are plotted onto a graph with $\frac{E}{E_0}$ in arbitrary units of the output amplitude control on the ordinate and the time constant $R \cdot C$ in msec. on the abscissa (Fig. 103). A curve is drawn through those points. The reciprocal value of the slope of the curve is

$$\lambda = \frac{b}{a}.$$

If the value a (ordinate) is set equal to 1, λ is directly read on the abscissa in msec. as the value of b.

Conclusion: The determined value of λ for the frog nerve in this experiment is 21 msec. The time constant k (Hill) can be calculated from the chronaxie determined in the preceding experiment, since a simple relation exists between k and the chronaxie: $k = 1{\cdot}44\, ch$. Here $k = 0{\cdot}25$ msec, 84 times smaller than λ.

Accommodation can also be measured with other methods (cf. Kries 1884, Schriever 1931, Lapicque 1926). Values thus obtained can be converted to λ by simple calculation (Schaefer 1940).

e) *The local response of a nerve*

Problem: Record the local response in the non-myelinated nerve of the crayfish *(Astacus fluviatilis)*.

Principle: Catelectrotonus applied to a nerve evokes a propagated response only if the intensity of the stimulus produces a certain critical level of depolarisation. If the response is studied at some distance from the cathode subthreshold changes at the site of stimulus action are overlooked. If such changes are to be recorded they must be registered directly at the stimulated point. The stimulating electrode is also one of the recording electrodes. The most difficult problem when recording local responses is suppression of the stimulus artifact.

Object: The isolated excitatory or inhibitory nerve fibre of the claw of the crayfish exposed where both axons run separately (for preparation cf. p. 220).

Apparatus: A cathode-ray oscilloscope with an A. C. amplifier having a long time constant (3 sec.). A stimulator giving rectangular pulses. A radio-frequency isolation unit.

Other requirements: as in experiment IVAb.

Procedure: Three electrodes are placed on an isolated fibre suspended in paraffin oil (Fig. 104). The middle electrode is simultaneously a stimulating and a recording electrode, and those at the ends one a stimulating and the other a recording electrode. The stimulus strength is slowly increased and the response evoked is recorded. If the middle electrode is the cathode, a small negativity is produced by a short pulse. This has a steep initial slope and decreases exponentially. After reversing the stimulus polarity using the same strength, a mirror response is obtained at the anode. When increasing the stimulus strength a small negative hump is produced at the cathode (Fig. 104a). This is not evident in the anodic response. It is a local potential. If the stimulus intensity is further increased, a spike potential is produced on this negative hump (Fig. 104b) while at the anode only a passive electrotonic response is found.

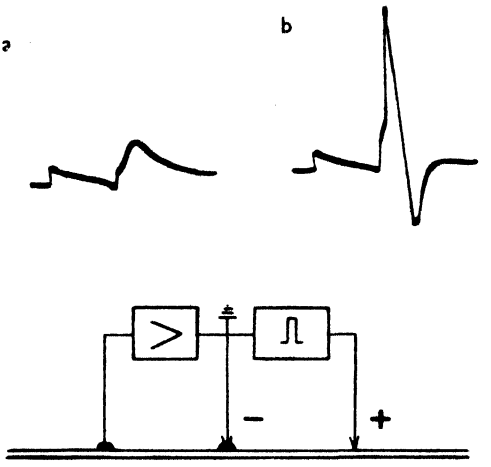


Fig. 104. Local potentials. a) passive electrical response and local potential of inhibitory nerve fibre. b) passive electrical response and spike potential of inhibitory fibre. Interval between stimuli — 10 msec. Below: arrangement of electrodes for determining local potentials.

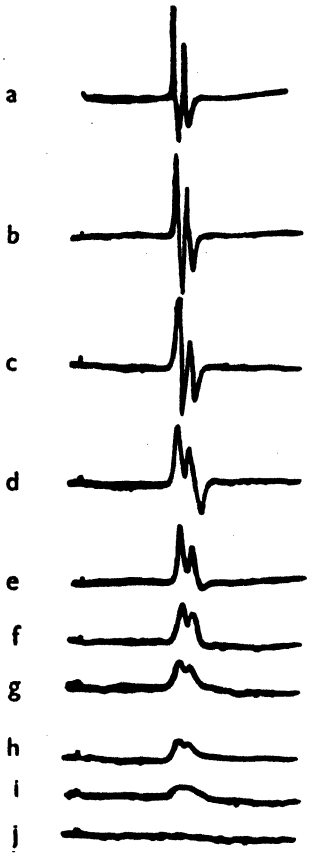
Conclusion: The local response evoked at the cathode by a stimulating pulse differs in several ways from a propagated response (Hodgkin 1938, Katz 1939, Rushton 1937). It is gradual and does not comply with the "all or nothing" law. It spreads with a decrement along the fibre. Because of these characteristics it is similar to postsynaptic potentials (page 314). Changes in excitability corresponding to the local potentials have been described (Katz 1939).

f) The effect of sodium ions on the production of an action potentials

Problem: Demonstrate the effect of a lack of sodium ions on the production of an action potential in the isolated nerve fibre of the crayfish *(Astacus fluviatilis)*.

Principle: Overton (1902) first demonstrated the significance of Na ions for the maintenance of muscle excitability. Recently this dependence upon sodium ions was confirmed for the majority of excitable tissues (cf.

Hodgkin 1951, 1958). An action potential does not arise in a sodium-free medium. In order to demonstrate this an isolated nerve fibre must be used since it is very difficult to wash out extracellular Na^+ from a whole nerve trunk.



Object: The excitatory and inhibitory axon of *Astacus fluviatilis*. For preparation see p. 218.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier. Time marker 1000 c/sec. An amplitude marker.

Other requirements: As for experiment IVAb. Crayfish saline with NaCl replaced by choline chloride.

Procedure: The preparation is fixed to the bridge as in experiment IVAb. A small vessel containing crayfish (van Harreveld solution) saline in which choline replaces Na^+ is placed below the double fibre at the site of recording. The action potential is recorded at the recording electrodes (Fig. 105a). This segment of the double fibre is then immersed in the vessel containing the above mentioned solution. From time to time the double fibre is pulled up into the paraffin oil and changes in action potentials are observed (Fig. 105b—j.).

Fig. 105. Effect of Na^+ ions on the production of an action potential. a) in normal solution. b–j) at successive intervals following exposure to a sodium-free environment: b — 11 sec., c — 23 sec., d — 33 sec., e — 45 sec., f — 52 sec., g — 60 sec., h — 72 sec., i — 83 sec., j — 96 sec. (Zacharová 1957).

The double fibre is then immersed in normal crayfish saline and after a short period, recordings are made in paraffin oil. It is found that conductivity is renewed after addition of Na^+.

Conclusion: Na^+ is indispensible for the initiation of an action potential in both nerve fibres. This can well be explained on the basis of the ionic hypothesis of nerve impulses, according to which action potentials are produced by increased flow of Na ions from the extracellular space (cf. p. 23) into the axon.

D. Recovery processes following a nerve impulse

During the passage of a nerve impulse through a certain stretch of the nerve fibre, the interior of the axon gains a certain amount of sodium and loses a corresponding amount of potassium. The quantities concerned are so small that the distribution of ions on both sides of the membrane is changed only insignificantly and further nerve impulses may pass through the axon. After prolonged activity it is nevertheless necessary to restore the original ionic composition. This is achieved by active transport of ions against concentration gradients, which occurs in the resting axon and requires metabolic energy. This is the recovery cycle of the nerve fibre in general. In the narrower sense the recovery cycle of the nerve impulse signifies changes occurring in the membrane immediately after the termination of the action potential and lasting tens of msec. During this period membrane permeability becomes normalised and the membrane becomes again capable of generating a new impulse. Electrophysiologically this period is characterised by after-potentials and by changes in excitability as absolute refractory period, relative refractory period, supernormal period, subnormal period and return to normal excitability.

From the physiological point of view, excitable tissues can be characterised by their ability to give a maximum number of responses per unit time. This maximal frequency is given by excitability changes following a nerve impulse.

Experiments described in the following section are concerned with this recovery cycle in the narrower sense of the word.

a) After-potentials

Problem: Record after-potentials in the sciatic nerve of the frog following a single stimulus and a train of stimuli; demonstrate the effect of veratrine on the negative after-potential.

Principle: In addition to the spike potential, a sequence of small changes in membrane potential may be recorded during the passage of a nerve impulse, if high amplification and a slow sweep are used. They can be recorded only in monophasic leads. The negative after-potential is represented by a slow decrease of the spike negativity towards the original level. With a slow sweep its beginning on the descending limb of the spike potential is well in evidence. This potential is followed by a positive after-potential.

After-potentials are a very sensitive component of a nervous response. They change during repetitive stimulation and when the external medium of the nerve is altered. The negative after-potential changes vary considerably under the influence of veratrine.

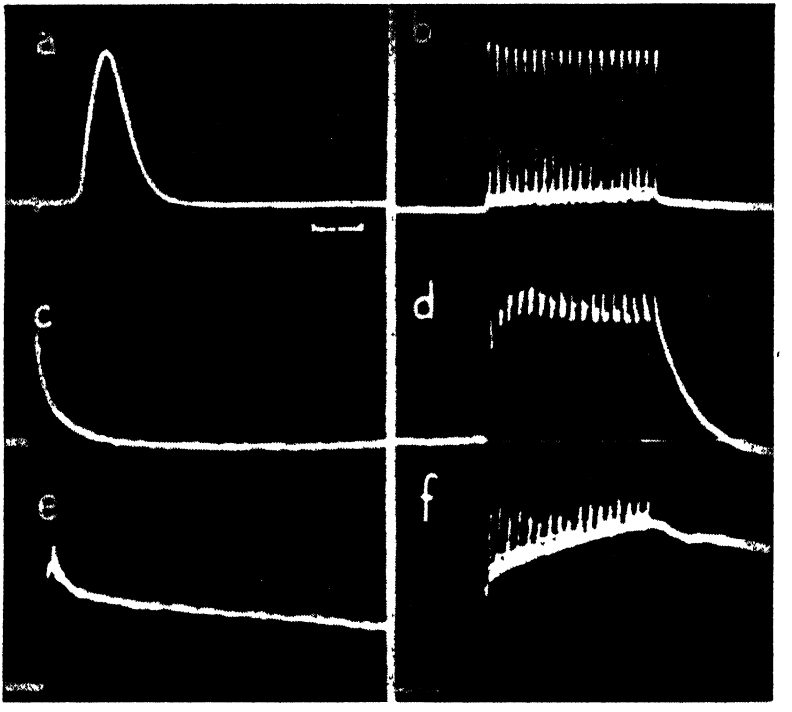


Fig. 106. After-potentials in the frog sciatic nerve. a) monophasic action potential. c) the same on fivefold amplification and slower time base. b–d) after-potentials on repetitive stimulation. e) after-potential in veratrine-treated nerve to one stimulus. f) same as e), with repetitive stimulation. Time scale: for a) 1 msec, for b–f) 40 msec.

After-potentials are accompanied by corresponding changes in excitability. During the negative after-potential an increase, and during the positive one a decrease in excitability can be observed.

Object: The sciatic-tibial or sciatic-fibular nerve preparation of *Rana esculenta* or *Rana temporaria*.

Apparatus: A cathode-ray oscilloscope with a preamplifier (D. C. or with a long time constant) total sensitivity 100 μV/cm. A stimulator giving at its output either a single stimulus or a train of electric shocks (10–20) with a variable frequency. Time marker; voltage calibrator.

Other requirements: A vessel for veratrine application to the nerve (as in experiment IVCf.). Veratrine (1 : 50 000).

Procedure: The preparation (sciatic-tibial nerve) is fixed in a nerve chamber (cf. experiment IVAa.) and placed on one pair of stimulating and one of recording electrodes. The nerve is crushed between the recording electrodes. It is then stimulated with a single electric pulse and the monophasic

maximal action potential is recorded (Fig. 106a). The sweep is slowed down so that the ascending and descending limbs of the spike potential fuse together and the amplification is increased fivefold (Fig. 106c). The crest of the spike is now beyond the screen of the oscilloscope. A slowly falling negative after-potential changing to prolonged positivity can be seen on the descending part of the spike potential.

The nerve is then stimulated with a volley of 20 pulses with a frequency of 140/sec. and the responses are recorded (Fig. 106b, d).

Subsequently the nerve is immersed in veratrine (1 : 50 000) and the response of the nerve to a single stimulus (106e) and a train of stimuli is observed at 15 min intervals (Fig. 106f).

Conclusion: During tetanic stimulation negative after potentials of the individual responses at first summate, but the effect of the positive afterpotentials predominates. After interruption of stimulation, a large positive afterpotential is observed following the last response.

In 1938 Gasser showed that during long lasting and frequent stimulation, two components can be seen on the positive after potential. Sometimes these are separated by a negative hump. In Gasser's terminology the first component P_1 is connected with the spike process, the second component P_2 with the negative afterpotential.

As follows from the recordings 106e, f, veratrine enormously enhances and greatly prolongs the negative afterpotential. Since veratrine increases oxygen consumption and heat production in the nerve, afterpotentials are considered to be connected to metabolic recovery processes in the nerve after the passage of a nerve impulse. This is indicated by their sensitivity to changes in the composition of the external medium of the nerve and also by changes in excitability after a nerve impulse.

b) The absolute and relative refractory period. The supernormal and subnormal period

Problem: Determine the absolute and relative refractory period, and the supernormal and subnormal period of A-fibres in the sciatic nerve of the frog.

Principle: The nerve is stimulated with a stimulus maximal for A fibres. This stimulus is termed conditioning stimulus. After a variable time interval, the nerve is excited with a test stimulus of variable intensity. If the time interval between those stimuli is sufficiently long, the same amplitude of action potentials is obtained, provided the intensity of both stimuli is the

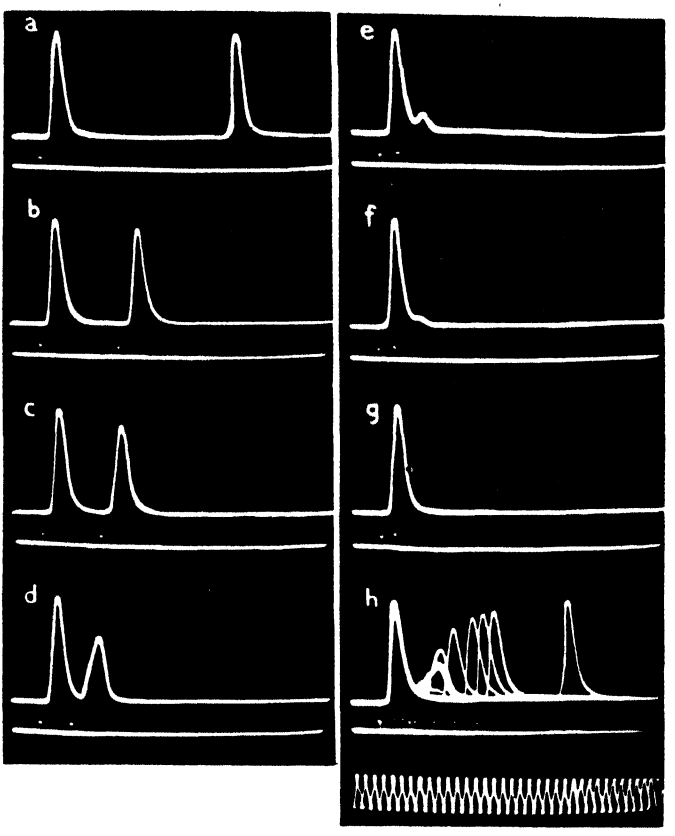


Fig. 107. Determination of the refractory period from the spike amplitude (a–g). The lower beam shows the intervals between stimulating pulses. Time mark: 1000 c/sec. Oscillogram h — the spike amplitudes at different intervals following the conditioning stimulus correspond to the course of the decrease in excitability during the relative refractory period.

same. The amplitude of the action potential produced by the test stimulus is decreased when the interval between stimuli is shorter than the relative refractory period. If the test stimulus falls into the absolute refractory period, no impulse is produced by its action.

The relative refractory period is due to a decrease in excitability of the nerve fibres. If the curve of changes in excitability during the relative refractory period is to be plotted quantitatively, the nerve must be stimulated at different intervals after the conditioning stimulus with a test stimulus, the intensity of which must be adjusted so that the same action potential is obtained. The relative refractory period is followed by further, less pronounced excitability changes. Excitability is increased at first, so that a less intense stimulus, as compared with the conditioning stimulus, is required to produce

the same spike amplitude. This supernormal period is followed by a period of subnormal excitability.

Apparatus: A double beam oscilloscope with one preamplifier, total amplification 100 μV/cm. A pair of recording and a pair of stimulating platinum electrodes. A stimulator giving paired rectangular pulses (variable delay between pulses and independently variable amplitude of both pulses). Time marker 1000 c/sec. A voltage calibrator.

Other requirements: As in experiment IVAa.

Procedure: The nerve is fixed in the moist chamber using silk loops tied to both ends. The latter are fixed to glass hooks and the nerve is slightly stretched. Stimulating electrodes (s) are placed at one end of the nerve. At the other end recording electrodes (r) are applied. The stimulator output is connected simultaneously to the (s) electrodes and to the input of the second channel of the oscilloscope so that the amplitude of the pulses can be observed.

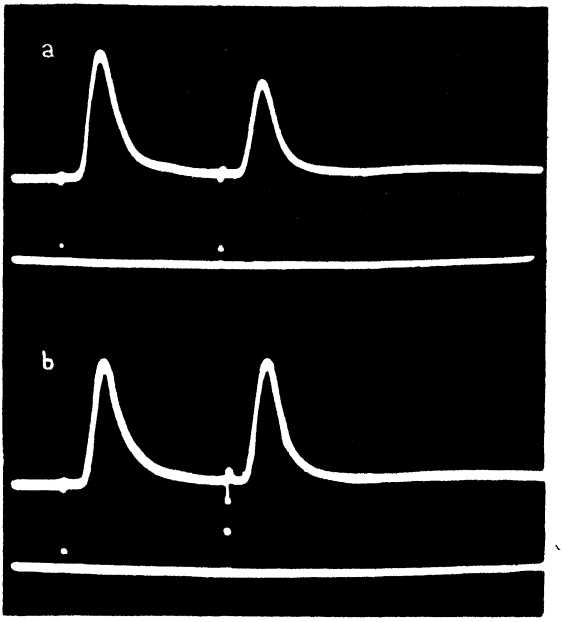


Fig. 108. A study of the refractory period according to changes in excitability. a) test stimulus of equal intensity as the conditioning stimulus in the relative refractory period. b) necessary increase in test stimulus for overcoming the decrease in excitability during the same time interval.

First the nerve is stimulated with the conditioning stimulus (c) alone, so that a maximal action potential for A fibres is just obtained. The sensitivity of the calibrating beam of the oscilloscope is increased so that the amplitude of the stimulus can be easily observed. The time interval between the pulses is then adjusted to about 20 msec. and a test stimulus of the same intensity is applied (Fig. 107a). It can be seen that the amplitude of both action potentials is the same. Maintaining the same pulse strength, the delay of the test pulse is gradually decreased. Starting from a certain delay, the amplitude of the action potential produced by the test stimulus begins to decrease. This is one limit of the relative refractory period. Finally the action potential disappears. This is the end of the absolute and the beginning of the relative refractory period (Fig. 107b, c, d, e, f, g).

The experiment is repeated, but this time the strength of the test stimulus is increased while shortening the time delay until the original amplitude of the action potential is obtained (Fig. 108).

The increase in amplitude of the test stimulus is calculated from the tracings for each delay used as percentage of the amplitude of the conditioning stimulus. The existence of the supernormal period is demonstrated as follows: With a 200 msec. interval between the pulses a threshold intensity of the test pulse is chosen. With unchanged pulse strength the delay is decreased. At a certain delay the threshold stimulus becomes a suprathreshold one.

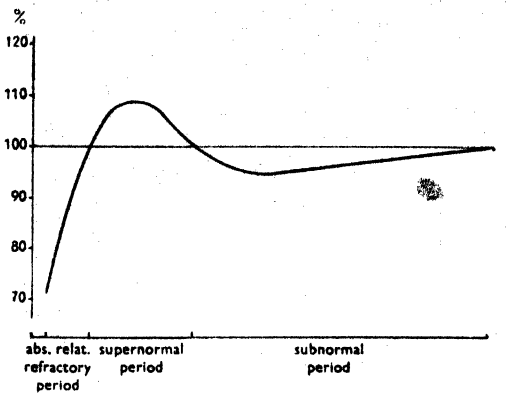


Fig. 109. The course of excitability changes after a nerve impulse. Ordinate: excitability in terms of resting threshold set at 100%. Abscissa: intervals between conditioning and test stimuli on a relative time scale.

The percentage decrease in the excitation threshold is determined for the supernormal period as in the case of the relative refractory period.

The subnormal period can be demonstrated in a similar way.

If the obtained values are plotted on a graph a curve as shown in Fig. 109 is obtained.

Conclusion: The curve shows that for 1·5 msec. after the stimulus the frog nerve is absolutely refractory since no impulse can be produced in it even when using very strong test stimuli. After 1·5 msec. the nerve is relatively refractory since an impulse can be produced only by applying more intense test stimuli. The relative refractory period ends after 4 msec. when the threshold has returned to normal. The supernormal period, with increased excitability, then begins. This phase lasts 15—40 msec. the excitability threshold then returns to normal a second time, and is followed by a period of decreased excitability, the subnormal period. Such a course of changes can be observed in the majority of excitable tissues. The supernormal period is very dependent on the composition of the external medium (Adrian 1920, Lorente de Nó 1947).

c) The maximal rhythm of a nerve fibre. The lability of Wedensky

Problem: Determine the maximum stimulus frequency to which the isolated inhibitory and excitatory nerve fibres of *Astacus fluviatilis* can respond.

Principle: The maximum number of impulses which a nerve is capable of transmitting in unit time in agreement with the rhythm of stimulation is

termed the maximal rhythm of the nerve. The maximum rhythm is a measure of the "lability" of excitable tissue (Wedensky 1884). It is mainly, but not solely, determined by the absolute refractory period, since it also includes cummulative changes occuring during longer lasting processes of the recovery cycle of the nerve impulse.

Maximal rhythm differ for different tissues.

If the stimulus frequency is greater than the maximal rhythm, the nerve does not respond to every stimulus, but transforms the rhythm of responses to a new one. At very high frequencies it only reacts with one response at the beginning. An activity similar to that due to a direct current or spontaneous activity may be started.

In general, determination of the maximal rhythm of a nerve requires application of maximal stimuli with variable frequencies to the nerve.

Transformation phenomena are very clearly demostrated when using a constant frequency of maximal stimuli close to the maximal rhythm while simultaneously cooling, thus decreasing the "lability" of the nerve (in the sense used by Wedensky).

Object: The double fibre with the small and large nerve of *Astacus fluviatilis* as in experiment IVBb.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier, total sensitivity 100 μV/cm. A stimulator giving rectangular pulses with a variable frequency and amplitude (an audio oscillator is also sufficient). Time marker.

Other requirements: A fixing bridge (Fig. 81). Cooling arrangement (page 222).

Procedure: The preparation is placed in the fixing arrangement. The recording electrodes are placed on the double fibre, the stimulating electrodes once on the large and the second time on the small nerve.

The stimulator is connected to the stimulating electrodes. The threshold is determined and then the inhibitory fibre (the small nerve) is stimulated with a short train of stimuli of twice the threshold strength. The frequency is gradually increased (Fig. 110). It can be seen that the inhibitory axon is capable of transmitting 100, 150, 200, 300 and 350 c/sec. without transformation. On applying 400 c/sec. transformation commences and is very regular, since every third action potential is dropped. There is always a longer time interval of about 4·3 msec. between the second and third action potentials, while the first two action potentials are separated by about 2·8 msec. The resultant frequency is about 290 c/sec. When applying 350 c/sec. it can also be seen that the one but last action potential dropped out of the series of responses traced in Fig. 110e. This indicates that the inhibitory fibre is not capable of responding with the maximal rhythm for prolonged periods. The maximal rhythm of the excitatory fibre is found in a similar way. It is 400 c/sec.

Conclusion: The maximal rhythm of the inhibitory fibre is 350 c/sec., and that of the excitatory fibre 400 c/sec. The transformation of the response to the 400 c/sec. stimulus may be explained on the basis of excitability changes during the recovery cycle following the nerve impulse. The second impulse of the train falls into the relative refractory period of the preceding impulse. This results in a prolongation of the latent period of the second response. Hence the time interval between the first two spikes is longer than in the preceding oscillogram, when a frequency of 350 c/sec. was applied. The third stimulus falls into the absolute refractory period of the second impulse and consequently no action potential is produced. Changes in the spike amplitude corresponding to changes in rhythm of responses may also be observed. At higher frequencies of 300 to 350 c/sec., the action potential is about $\frac{1}{7}$ smaller than at a frequency of 100 c/sec. The dependence of the amplitude on the time interval between individual spikes is evident also when stimulating with a frequency of 400/sec. The amplitude of a spike potential produced after a longer interval is greater than that of one occuring after a shorter time interval.

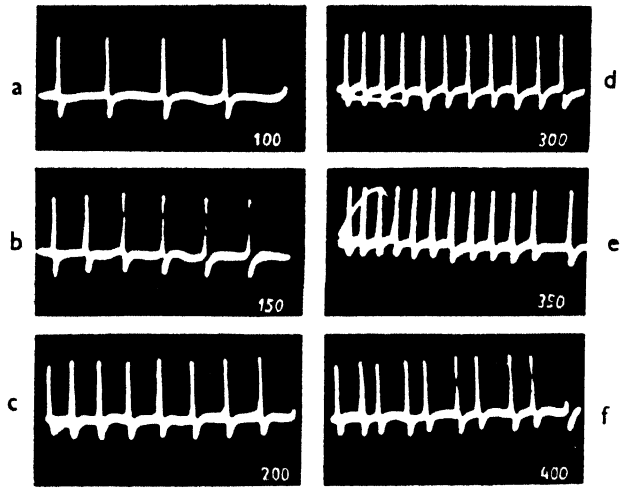


Fig. 110. The maximal rhythm of the inhibitory fibre of *Astacus fluviatilis*.

The same experiment can be performed in a frog sciatic nerve, but a large number of nerve fibres of different lability makes impossible the exact determination of the maximal rhythm.

E. Electrophysiology of the isolated skeletal muscle

In comparison to the isolated nerve, the isolated muscle is relatively much less used for studying fundamental questions of excitability with the help of electrophysiological methods.

For this there are several reasons. In the first place, muscle contractions may produce artifacts during recording. In addition, the diameter of the

muscle is irregular and large, and last, but not least, nervous structures and nerve endings are intimately associated with muscle elements.

A muscle may be activated either *directly*, i. e. by directly stimulating muscle fibres with an artificial stimulus, or *indirectly* by a physiological stimulus, a nerve impulse.

In this section only direct stimulation of a muscle will be considered. Indirect stimulation is described in connection with problems of neuromuscular transmission (p. 255). Chapter V deals with problems of the recording of action potentials from muscle fibres in situ.

All the characteristics of excitable tissues as described for nerve fibres can be demonstrated in muscle. In addition, it is possible to study the mechanism of activation of the contractile process by the muscle action potential.

The action potential of a skeletal muscle

Problem: Record the biphasic and monophasic action potential of the frog skeletal muscle evoked by direct electrical stimulation, and determine the latent period of the mechanical response.

Principle: The method of stimulation and recording is the same as for nerves. In order to eliminate indirect stimulation of the muscle through nerve endings, the site of stimulation on the muscle must not contain nerve endings and plates. The muscle is stretched so that only isometric contractions with minimum movement occur.

Both the muscle action potential at the point of stimulation and the muscle contraction must be recorded simultaneously in order to determine the latent period of the mechanical response.

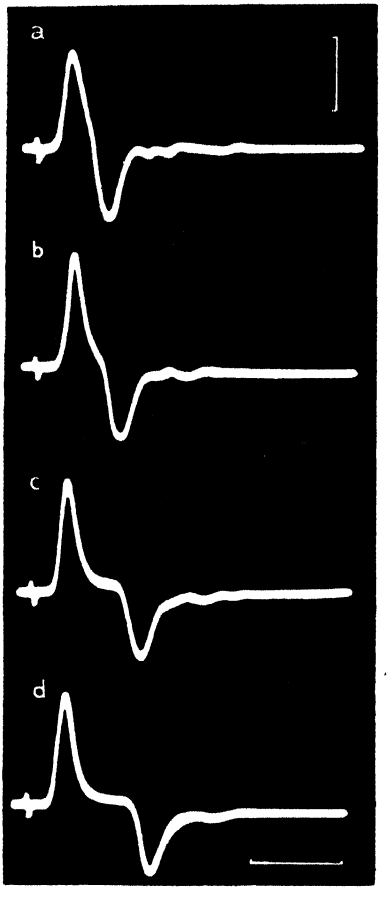
Object: The sartorius muscle of *Rana esculenta* or *R. temporaria*. This muscle is particularly suitable since its end-plates are concentrated at one or only a few points, and certain parts of the muscle are free of nervous elements. It is best to determine the area containing the end plates first (see. p. 256). Usually the pelvic part has no nervous elements.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier, total sensitivity 100 μV/cm. A double beam oscilloscope with one D. C. channel or at least a long time constant (4 sec.) is required for recording the "mechanogram" (record of muscle contraction). A stimulator giving rectangular pulses. A radiofrequency output unit for reducing the stimulus artifact. A time marker. A calibrator. A liquid rheostat (see. Fig. 128).

Other requirements: Ag-AgCl electrodes with cotton wool wicks for stimulation and recording. Dissecting instruments.

Procedure: Biphasic and monophasic action potentials: The muscle is stretched in the recording arrangement as described on p. 256. The stimulating

electrodes are placed 3 mm apart on the pelvic end of the muscle. The first recording electrode is situated about 5 mm peripherally from the stimulating electrodes, the other is placed successively 3, 5, 7 and 9 mm from the first. The action potential is recorded for each position of the recording electrodes (Fig. 111a, b, c, d). The further removed the second recording electrode the more distant are both phases of the biphasic action potential. For the last position they are separated by a clear-cut isopotential line.



The monophasic recording is simply made by placing the second recording electrode onto the knee tendon of the sartorius muscle.

The amplitude and duration of the individual parts of the muscle spike are measured on the oscillogram.

The latent period of the mechanical response. The muscle is fixed in a recording arrangement. The knee tendon of the muscle is connected to a liquid rheostat which transforms the mechanical contraction into an electric potential. The latter is recorded on the second beam of the oscilloscope.

Fig. 111. Action potential of the frog sartorius muscle produced by direct stimulation. The interelectrode distance of recording electrodes (a) 3, (b) 5, (c) 7 and (d) 9 mm. Time mark: 10 msec. Calibration: 10 mV.

The stimulating electrodes are placed on the muscle as in the preceding experiment. The first recording electrode is connected to the stimulating cathode, the second is on the knee tendon of the muscle.

In such a way both the action potential and the mechanical response are recorded for different stimulus intensities.

Conclusion: The shape and duration of a muscle action potential are similar to that of a nerve. The ascending phase lasts 1 msec., the total duration of the monophasic spike is 2 msec. The rate of conduction calculated indirectly (p. 215) is very slow: 2 m/sec. The short spike duration and the small conduction velocity indicate a small wave length of the impulse. This also

explains the fact that both phases of a biphasic action potential can be separated by increasing the interelectrode distance to only several mm.

The mechanical latent period is about 4 msec., so that the whole action potential is recorded before the contraction commences. Technically this is important for excluding movement artefacts, especially when using microelectrodes. Theoretically this demonstrates that the action potential is not an expression of muscle contraction but a part of the mechanism preparing it.

Transmission at peripheral synaptic junctions

Introduction

Peripheral synaptic junctions are those sites where the nerve impulse is transferred from the nerve endings to the effector organ (muscle, gland) or to another neurone situated outside the central nervous system (autonomic ganglia).

The fundamental characteristics of synaptic transmission have been studied chiefly on peripheral synapses, due to their easy accessibility. Excepting the mechanism of direct inhibition, these synapses have the same fundamental properties as synapses in the central nervous system.

Anatomically they contain the basis of a divergent and a convergent mechanism. The divergent mechanism ensures only diffusion of excitation into a larger area of the effector. Convergence, on the other hand, is the basis of elementary integrating activity.

Transmission in peripheral synapses is achieved chemically by means of mediators.

Electrophysiologically transmission across synapses is characterised by synaptic potentials (Eccles 1943).

In this chapter experiments are described which demonstrate the fundamental characteristics of peripheral synaptic transmission in the most commonly studied peripheral synapses, in the end-plate and in the superior cervical ganglia.

F. Neuromuscular transmission in skeletal muscle

The anatomical basis of neuromuscular transmission is the end-plate (Couteaux 1944). Before its termination the nerve fibre is no longer myelinised. There is no continuity between the nerve ending and the muscle fibre but only

very close contact. The muscle tissue immediately below the nerve endings has special structural features. It appears as a palisade of small rods. Developmentally it is of mixed origin, the rods originating from Schwann's sheath. This specialised junction is called the motor end-plate. It is at this junction that the nerve impulse is converted into a muscle action potential.

a) Electrophysiological localisation of the end-plates in skeletal muscle

Problem: Localise the end-plate region of the sartorius muscle of the frog using the latent period.

Principle: In addition to microscopic examination, two electrophysiological methods may be used to localise the end-plate region (Eccles, Katz and Kuffler, 1941). In normal muscle the minimal latent period is determined.

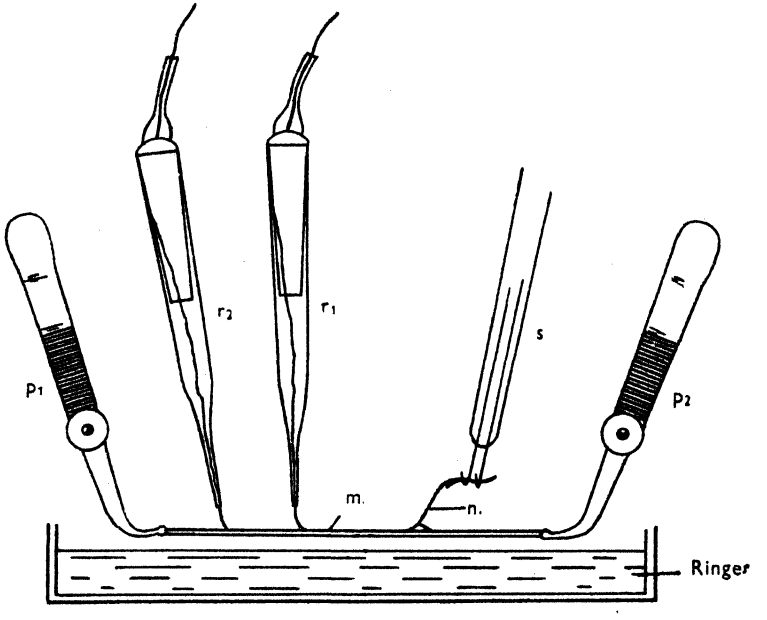


Fig. 112. Diagram of the arrangement for recording action potentials from indirectly-stimulated frog sartorius muscle. p_1, p_2 — screw-controlled forceps, s — stimulating electrodes, r_1, r_2 — recording electrodes, n — nerve, m — muscle.

If a nerve leading to a muscle is stimulated, the distance between the stimulus artefact and the muscle action potential will be the shorter the closer the recording electrode to the point at which the impulse in the muscle fibre originates, i. e. the nearer to the end-plate region. If the recording electrode

is above the end-plate region a certain minimal latent period is obtained. This includes the time required for conduction of the impulse along the nerve from the point of stimulation and the synaptic delay at the site of transmission. The time necessary for conducting the impulse along the muscle fibre in this case is not added. In the majority of muscles the end-plate region is not localised at the same point in all muscle fibres. For that reason when recording from the whole muscle, muscles like the frog sartorius, are suitable in which the majority of fibres are innervated in two or several discrete end-plate zones.

The number of such zones can be determined by this method as those points having a minimal latent period of muscular action potentials.

The second method which can be used for the curarised muscle is based on evaluation of the amplitude of the end-plate potential and will be described in the chapter on end-plate potentials.

Object: The frog *(Rana temporaria* or *R. esculenta)* sartorius muscle with the nerve leading to it.

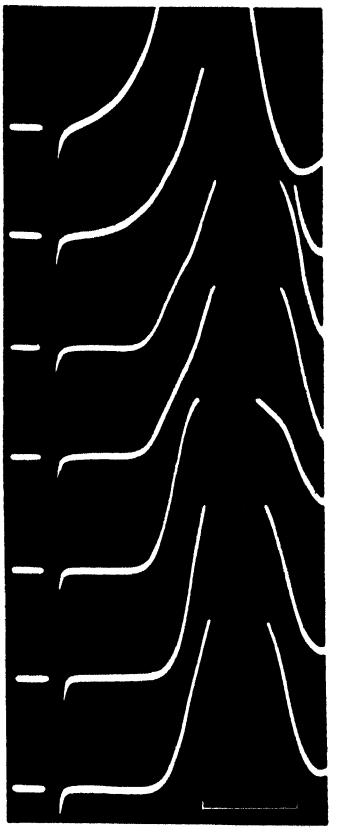


Fig. 113. Localisation of end-plates according to the latent period of the muscle action potential. Indifferent electrode on knee tendon. a) action potential above synaptic junction, b–g) in 2 mm distances each from the other. Time mark: 3 msec.

Apparatus: A cathode-ray oscilloscope and a A. C. preamplifier. The over-all sensitivity of the recording system about 100 μV/cm. A square wave stimulator and a time marker.

Other requirements: Fixing arrangement for the nerve-muscle preparation. A microdrive for one recording electrode.

Dissecting instruments, frog's Ringer solution, a pair of stimulating platinum electrodes and a pair of Ag-AgCl nonpolarizable electrodes.

Procedure: The preparation is fixed as shown in Fig. 112. Both the pelvic and the knee ends of the sartorius muscle are held by a screw-controlled forceps. The nerve is placed on the stimulating electrodes so that it is freely pendant. This prevents it from shifting during muscular contractions and thus

producing artifacts in the latent period. The cotton wick of one recording electrode is placed on the tendon, the other is placed on the muscle. The electrode is held in a holder that has a microdrive permitting movement along the muscle fibre.

The stimulating electrodes are then connected to the stimulator giving rectangular pulses. The time base of the oscilloscope is synchronised with this pulse. The nerve is stimulated with a suprathreshold stimulus for the muscle. Then the "active" recording electrode is moved by 1 mm and again the muscle action potential is recorded. In such a way the recording electrode traces the whole muscle (Fig. 113).

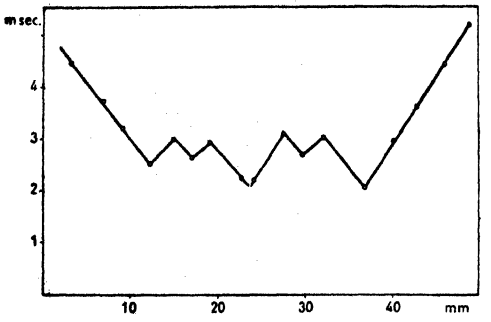


Fig. 114. Graphical evaluation of the end-plate localisation in frog sartorius muscle. Abscissa: distances at which action potentials were recorded. Ordinate: latent period of muscle response in msec.

Conclusion: If the distances from which the action potentials were recorded are plotted along the abscissa and the corresponding latent periods on the ordinate a curve with three minima is obtained (Fig. 114). Minimal latent periods occurred at distances 12, 23 and 37 mm. from the point between the knee tendon and the muscle. At those points the end-plate zones of the muscle are situated. The curve rises equally on both sides of these minima. This means that no end-plates are present in those muscular areas and the increase in latent period is due to conduction of the impulse along the mucsle fibre.

The rate of conduction of the muscle impulse can be calculated from the slope of the curve.

b) *End-plate potentials* (e. p. p.)

Problem: Record the end-plate potentials in the neuromuscular junction of the sartorius muscle of the frog under normal conditions and after treatment with curare. Determine the spatial distribution and Q_{10} of the individual components of the e. p. p.

Principle: The synaptic potential in the neuromuscular junction is termed end-plate potential. This differs from a nerve impulse in several ways. It does not comply with the "all or none" law, but depends upon stimulus strength, it arises at the point of transmission and does not spread into the surroundings and its amplitude decreases exponentially with increasing distance from the neuromuscular synapse. It is capable of summation and is

of longer duration than a nervous or muscular impulse. When its amplitude attains a certain level a muscular impulse is produced and this spreads along the whole muscle fibre (in vertebrates).

Consequently the end-plate potential must be recorded directly from the synaptic junction and, in order to determine its shape, stimuli which are subthreshold for the production of a muscular impulse must be used.

The first condition is fulfilled by locating end-plates as described in the previous experiment. The second condition can be complied with in several ways. Three methods for the normal and curarised muscle are given below.

When recording from the whole normal muscle it is not possible to obtain an e. p. p. uncomplicated by muscle spike, e. g. by making the nerve volley very small. Two stimuli are necessary. The second nerve volley is applied when the end-plate is in the refractory period after the first stimulus. During the refractory period the muscle spike either does not occur at all (absolute refractory period) or occurs with some delay on the e. p. p., and the latter thus becomes evident. The potential added in this way by the second nerve impulse is then obtained by subtraction and is plotted onto the same time-potential coordinates, the artefact of the second stimulus being zero time.

An easier method is to separate the action potential from the e. p. p. using graded curarisation. Subparalytic curarisation delays and eventually decreases the muscle spike, while the e. p. p. becomes progressively smaller without altering its latency or time course.

The third method consists of recording changes in action potential due to small shifts of the recording electrode from the focus of the end-plate zone. Here separation of the two potentials is obtained, since the muscle action potential arives later owing to the time necessary for conduction. The e. p. p., however, decreases exponentially with distance, so that the shift of the recording electrode must be such that the muscle spike is delayed and yet the e. p. p. does not disappear on increasing the distance (Eccles and O'Connor 1939, Eccles and Kuffler 1941).

The e. p. p. has the highest amplitude in the end-plate zone. It decreases exponentially with increasing distance. This fact may be utilised for the spatial mapping of end-plate zones and the points with a maximum amplitude of e. p. p. thus obtained can then be correlated with those having a minimum latent period of the muscle potential in the same muscle before curarisation.

The Q_{10} of the different e. p. p. components permits insight into the processes occurring during the different phases of e. p. p.

Object: frog sartorius muscle with its nerve.

Apparatus: as in experiment IV F a. A D. C. preamplifier. A square-wave stimulator with two pulses at the output.

Other requirements: d-tubocurarine chloride in Ringer's solution in increasing concentrations from 1 μmol. Otherwise as in experiment IV F a.

Procedure: The muscle with its nerve is fixed as in the preceding experiment. First the end-plate zone is found using the method of minimal latent periods. In this position the e. p. p. for the normal muscle is first determined.

A stimulator giving paired pulses with variable intervals between them as well as variable amplitude is connected to the stimulating electrodes. Both stimuli are maximal and responses to them are first recorded with a distance of 10 msec. (Fig. 115b.) The interval between the stimuli is then shortened and the potentials produced are recorded from the end-plate area (Fig. 115c).

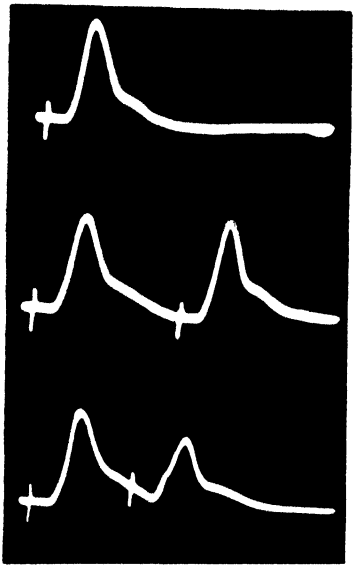


Fig. 115. Action potential of frog sartorius muscle stimulated indirectly by two stimuli with different time intervals. Note the hump in recording c) to the second stimulus.

The muscle is then immersed in Ringer solution containing 1 μmol or more curarine for 20—30 minutes.

After this the solution is sucked off and the response to one stimulus from the nerve is recorded. Care is taken to keep the recording electrode in the end-plate zone. In such a fashion the action potential from the end-plate zone is recorded after treatment of the muscle with different concentrations of d-tubocurarine (Fig. 116a, b).

If these tracings are projected with several fold enlargement and redrawn in the same time-response coordinates, the changes in slope of the ascending limbs may be determined during progressive curarisation.

As in the preceding experiment for determining the minimal latent period, the recording electrode is shifted along the whole muscle in one mm intervals and the e. p. p. is recorded. It can be seen that the amplitude varies at different points. There are two or more amplitude maxima along the muscle. Beyond those maxima no e. p. p. is observed in the direction towards the tendon, i. e. in those areas having no end-plates. The exponential decrement of e. p. p. in this zone is shown in Fig. 116c–e.

The Q_{10} of the end-plate potential is then determined. The e. p. p. is recorded at two different temperatures t_1 and t_2. The muscle temperature is registered with a thermocouple. Q_{10} is calculated according to equation:

$$\log . Q_{10} = \frac{10}{t_1 - t_2} (\log x_1 - \log x_2)$$

where x_1, x_2 are the values measured for temperatures t_1, t_2.

Conclusion:

1) The method of recording e. p. p.

It can be shown that an early second nerve volley produces a pure e. p. p. If the intervals between the stimuli are longer, a spike is also produced. The potential added by the second nerve impulse is obtained by subtracting the response to the first stimulus from the record of those to the first and second stimuli (Eccles and O'Connor 1939). The height of the e. p. p. at which spike activity occurs can also be determined in this way.

This pure e. p. p. thus obtained has the same course as the e. p. p. in a curarised muscle.

2) Spatial distribution.

A study of the amplitude of the e. p. p. along the muscle showed that there are 1—3 plate zones in this muscle and this was confirmed when determining the minimal latent period. Evaluation of the decrease in e. p. p. amplitude shows that the e. p. p. decays by half in 0·9 mm (fall to 1/e in 1·25 mm).

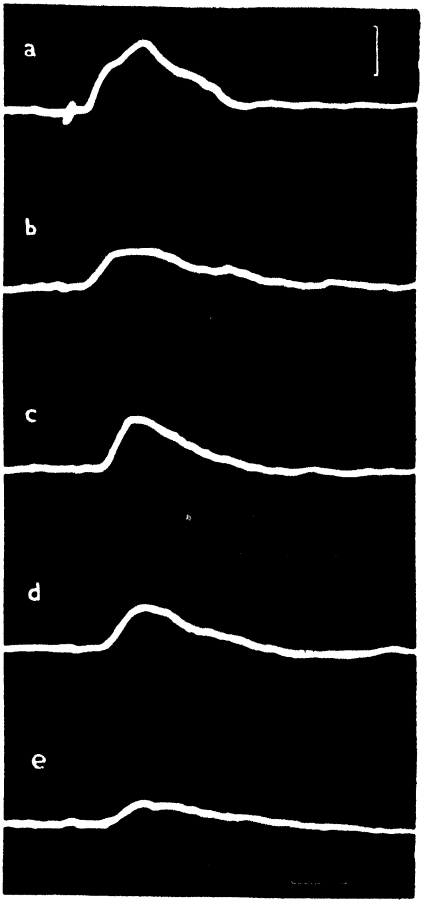


Fig. 116. End-plate potentials. a) during partial curarisation (3 μM d-tubocurarine). b) during complete curarisation (5 μM d-tubocurarine). c–e) spatial distribution of e. p. p. in curarised muscle. c) 7 mm, d) 6 mm, e) 5 mm from the pelvic end of the frog sartorius muscle. Time mark: 5 msec.; voltage calibration: 0·2 mV.

The characteristic exponential decrement of the e. p. p. along the muscle and the accompanying slowing down of its temporal course is similar to the spread of an electric charge along a leaky capacitative cable (Cremer 1909, Rushton 1934).

The extent of the spread of the e. p. p. along the muscle fibre depends on the resistance of the fibre, chiefly on the transverse impedance of its surface membrane. Since the surface membrane has a large distributed capacity (Cole and Curtis 1939), the electrotonic potential does not rise suddenly

but gradually, depending upon the rate at which local membrane capacitances are charged. Hence the larger the distance from the end-plate focus the slower the rise in the recorded potential (Eccles, Katz and Kuffler, 1941). It may be objected that the spatial decrement of the e. p. p. is due to diffuse distribution of motor endings rather than to spread of extrinsic current from the depolarisation focus. In such a case, however, the time course of e. p. p. would be unalterable and would not have the characteristic slowing related to electrotonic spread.

Sometimes it is possible to observe, particularly when working with homeotherms, hyperpolarisation preceding the e. p. p. at some distances from the point of depolarisation. This is due to a shunt across excess fluid or tissue as was demonstrated by Bishop (1937) and Bishop and Gilson (1929).

3) The effect of temperature on the e. p. p.

The experiment showed that the rate of decay of the e. p. p. is very little affected by temperature changes ($Q_{10} = 1{\cdot}3$), while the ascending phase is prolonged nearly 3× when decreasing the temperature by 10°C. It is evident that different processes are responsible for the build up and decay of e. p. p. It is assumed that the decay is mainly due to passive dissipation of the electrotonic charge along and across the muscle membrane. On the other hand, the onset of the e. p. p. is the main period during which the mediator acts.

c) *Repetitive stimulation of the end-plate. Recruitment. Wedensky inhibition*

Problem: Record the e. p. p. set up by two and more nerve volleys of different frequency. Demonstrate facilitation and Wedensky block in frog neuromuscular junction.

Principle: Depending upon the stimulation frequency, it is possible to restore transmission in a blocked neuromuscular junction or block transmission in a normal muscle.

The fact that a neuromuscular block can be overcome by repeated stimulation is termed facilitation or recruitment (Adrian and Lucas 1912, Bremer 1927). The e. p. p. acts on the muscle fibre as a subthreshold catelectrotonic potential and can add its excitatory effect to that of the subthreshold current spreading in front of an action potential (Eccles, Katz and Kuffler, 1941). It follows that two e. p. p. produced by two successive nervous impulses will summate as do successive catelectrotonic potentials (Schaefer and Haas 1939). When the e. p. p. attains a threshold amplitude, an action potential in the muscle fibre is produced. If the neuromuscular synapse is in a critical state of curare block, neuromuscular transmission may be renewed by summation of successive e. p. p.

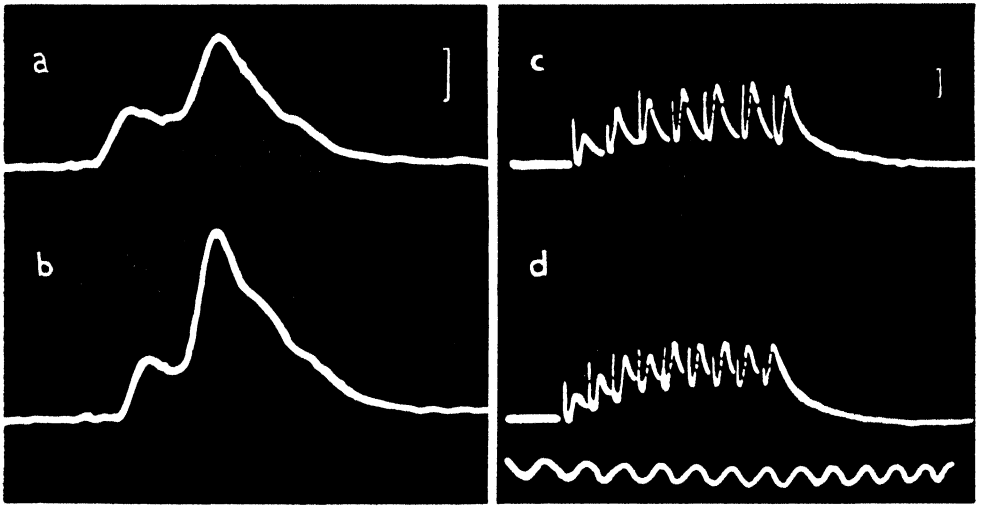


Fig. 117. Summation of e. p. p. in the frog sartorius muscle. a–b) summation of two e. p. p. s for different intervals between stimuli. c–d) summation in response to a train of impulses. Time mark: 100 c/sec. (for c, d). Voltage calibration: 2 mV.

Facilitation in the neuromuscular junction of frog sartorius muscle can be demonstrated by using a critical curare block of neuromuscular transmission to a single nerve volley. The nerve must then be stimulated by two stimuli varying the interval between them in order to determine the optimal interval for facilitation.

The following is Wedensky inhibition: If the stimulation frequency from the nerve exceeds a certain value, muscular tension decreases until the muscle relaxes and muscular electric activity disappears. This is not due to fatigue, since as soon as the stimulation frequency is decreased the muscle immediately responds normally or even supranormally. Wedensky attributed this phenomenon to differences in lability of the nerve, muscle and neuromuscular plate (Wedensky, 1892).

Demonstration of this fact is achieved as for facilitation, but the normal end-plate is stimulated with repetitive nerve volleys at different high frequencies.

Procedure: The sartorius muscle together with its nerve are prepared as in the preceding experiment. The muscle is immersed in 6 μM-d-tubocurarine chloride for 20—30 min until there is complete block of neuromuscular transmission.

1) Summation of two e. p. p.

The nerve is stimulated by two maximal stimuli with different time intervals. First longer intervals (50 msec.) are used. The e. p. p. are separated

from each other. The interval is then gradually shortened (Fig. 117a, b). The oscillograms show that, commencing with a certain interval, the potentials begin to summate. With the shortest time intervals the e. p. p. to the second stimulus has a higher amplitude than with longer intervals. This is called potentiation.

If the experiment is repeated under weaker curarisation, which just produces a block, the summated potentials reach a critical level for the occurrence of muscle spikes, the block is overcome and "neuromuscular facilitation" or "recruitment" occurs.

2) Summation in response to repetitive nerve volleys.

Transmission is completely blocked with d-tubocurarine and the nerve is stimulated with a volley of maximal stimuli at 100 c/second. The time base is slowed down so that all stimuli are visible on the oscilloscope screen.

It can be seen from the tracing that the first end-plate potentials which summate are larger and larger (Fig. 117c, d). Curarisation, however, is fairly strong, so that the critical level for spike formation is not attained. Then, however, both the amplitude and the total summated level decrease. Finally both stabilise at a certain level. After discontinuing stimulation the potential only slowly returns to zero line.

3) Wedensky inhibition.

The nerve of a normal nerve-muscle preparation is stimulated with repetitive nerve volleys at frequencies from 30/sec. upwards. The action potential is recorded from the end-plate free region of the sartorius muscle.

The muscle stimulated indirectly responds faithfully to frequencies up to 80 per sec. At higher frequencies the spike from the muscle decreases progressively with time and transformation of the rhythm occurs. If the frequency of stimulation is reduced, the action potentials rapidly reach the original height again.

Conclusion: Potentiation and the subsequent decline may be ascribed to corresponding changes in acetylcholine release (Eccles 1948, Eccles and Macfarlane 1949). Mobilisation of preformed acetylcholine is probably responsible for the potentation. Partial exhaustion of acetylcholine reserves may be reflected in subsequent decrease of potential. The plateau might indicate that both formation and secretion of acetylcholine have become stabilised.

Mammalian muscles do not show this potentiation phase. In them a decrease in successive e. p. p. can be seen right from the beginning (Liley and North 1953). This might indicate that mammalian nerve endings contain much lower acetylcholine reserves.

d) Specific excitation of the end plate by acetylcholine

Problem: Compare the sensitivity to acetylcholine of an end-plate region with that of a muscle region without end-plates in the frog sartorius muscle.

Principle: The chemical hypothesis explaining transmission of an impulse from a nerve to a muscle is generally accepted. The course of events on the end-plate is the following, according to contemporary opinion (Eccles 1953):

I. Synthesis of acetylcholine by choline-acetylase and formation of reserves in the nerve endings,
II. release of acetylcholine by the nervous impulse,
III. diffusion of acetylcholine, its temporary combination with membrane receptor and formation of e. p. p.,
IV. Destruction of acetylcholine by cholinesterase, which occurs simultaneously with III.
V. Depolarisation due to e. p. p. of the adjacent regions of the muscle fibre to a critical value at which a muscle impulse occurs.

There are many papers reviewing experiments designed to verify this hypothesis (Dale 1937, Kuffler 1948, Eccles 1936, 1953, Castillo and Katz 1956 — giving the newest quantitative tests).

One of the tests of this hypothesis involves direct application of Ach in small concentrations (Kuffler 1943, 1945, Buchthal and Lindhard 1939). These specifically depolarise the end-plate region. Other regions are at least 1000× less sensitive to the depolarising effect of Ach. The single nerve-muscle preparation gives the most convincing experimental results (Kuffler, 1943).

The whole sartorius muscle is also suitable for demonstrating the specific action of Ach on the end plate region. If the pelvic end of this muscle (without the end-plates) is immersed in a solution containing a threshold concentration of Ach, no potential is produced and no contraction is observed. If also the end-plate region is now also immersed, fibrillations occur. These last for several seconds.

Object: The frog's sartorius muscle.

Apparatus: A cathode-ray oscilloscope. An A. C. and D. C. preamplifier. Over-all sensitivity of the recording system is about 1 mV/cm for the D. C. and 100 μV/cm for the A. C. channel.

Other requirements: Arrangement for recording and drug application similar as in Fig. 81d. Acetylcholine and paraffin-oil.

Procedure:

1) Initiation of muscle action potentials due to acetylcholine acting on the end-plate region.

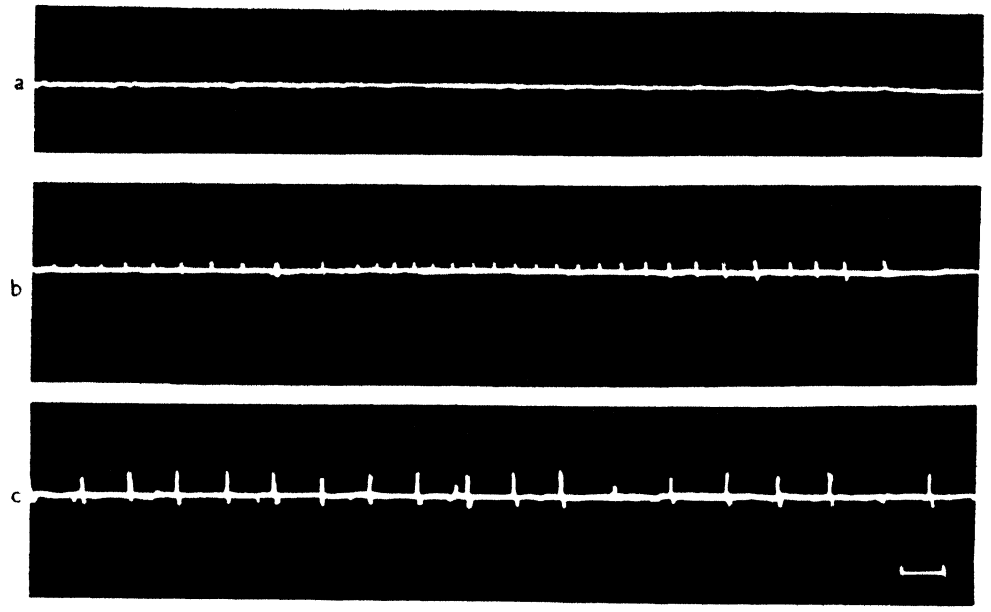


Fig. 118. The effect of Ach on the end-plate zone. a) sartorius muscle immersed 3 mm from the pelvic end in frog Ringer with Ach. b) 5 mm, c) 7 mm. Time mark 200 msec.

The sartorius muscle is prepared in the usual way. The nerve leading to it is not required and is therefore cut where it enters the muscle. Frog Ringer solution containing Ach is poured into a small vessel. This vessel is placed into a larger one containing paraffin-oil. The muscle is held with a forceps and is wholly immersed in paraffin-oil. It is slowly lowered into the vessel with Ach. One recording electrode is on the muscle in paraffin-oil, the other in the Ach solution. This electrode records changes at the paraffin-saline interface. Nonpolarisable Ag-AgCl electrodes are used. The pelvic end of the muscle is immersed in the test solution. Recordings are made for every mm of immersion. No activity on the muscle is seen down to 4 mm. After immersing 6 mm, fibrillations occur. These last several seconds (Fig. 118). The plate zone has been reached.

2) Depolarisation of the end-plate zone by Ach.

If the depolarising effect of Ach on the end-plate zone is to be demonstrated, the experiment is performed as above but a D. C. amplifier is used. It can be thus shown that at the point where the end-plates are situated and where immersion produces spike activity in the muscle, a negative potential of 10—20 mV occurs, while in the areas without end-plates this depolarisation in absent or reaches only 1—2 mV.

In this way the effect of other substances having an effect on neuromuscular transmission may also be tested (Kuffler 1943).

Conclusion: The experiment shows that the end-plate zone is more sensitive to Ach than the remainder of the muscle not containing end-plates. Kuffler showed that it is about 1000× more sensitive. Today it is assumed (Fatt and Katz 1951, 1952) that Ach released from the nerve endings causes depolarisation of the end-plate membrane, whereby it becomes highly permeable to all ions present.

G. Synaptic transmission in a sympathetic ganglion

Synapses in a sympathetic ganglion assume an intermediate position between neuromuscular transmission and synapses of the central nervous system.

Elementary integration, facilitation and occlusion can be demonstrated, but there is no true inhibitory mechanism.

Histologically they represent a connection between preganglionic fibres and ganglionic cells. As in neuromuscular junctions, there is no continuity but only close contact, probably with interposed intercellular material.

Transmission from preganglionic to postganglionic fibres is chemical, with the aid of Ach (Kibjakov 1933).

Electrophysiologically, transmission is also characterised by the occurrence of a synaptic potential.

a) Action potentials from the superior cervical ganglion

Problem: Record the action potential from the isolated superior cervical ganglion of the rabbit.

Principle: In order to determine the responses which a preganglionic nerve volley evokes in the presynaptic and postsynaptic elements of the sympathetic ganglion, various lead positions are used as shown diagramaticaly in Fig. 119.

In analysing the responses obtained with these lead combinations it is necessary to keep in mind that the cross section of the preparation is not uniform. The cross-section of the ganglion is about ten times larger than the nerve trunks. Hence both resistance per unit length and the density of longitudinal current produced by potentials in preganglionic terminals are much lower than in the nerve trunks. Consequently, the recorded potential is attenuated and the discrimination of recording is lessened. This means that electrode

R_5 will record preganglionic potentials as if it were at R_2 rather than potentials of the terminals in the ganglion.

In this experiment only action potentials recorded with R_1R_2 and R_3R_5 leads are described. Electrodes in position R_1R_2 record action potentials from the preganglionic nerve fibres. With leads R_3R_5, potentials generated in the activated ganglion cells are recorded rather then those of postganglionic nerve fibres.

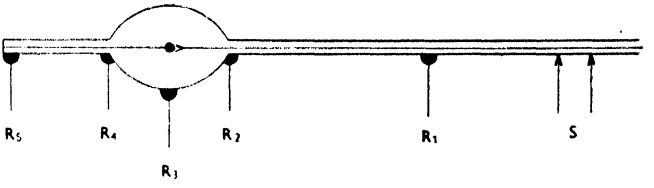


Fig. 119. The usual arrangement of recording electrodes when recording from the superior cervical ganglion.

For a detailed interpretation and description of records with other leads, the reader is referred to the papers of Bishop (1936), Eccles (1935a, b), R. Eccles (1952ab).

Object: The isolated superior cervical ganglion of the rabbit.

Preparation: The rabbit is anaesthetised with dial. The preganglionic trunk is exposed in the neck and cut several cm from the ganglion. The postganglionic trunk is separated from the internal carotid artery, the surrounding connective tissue is removed and the trunk is cut where it enters the skull base. Care is taken to preserve the blood supply to the ganglion. Finally the ganglion is rapidly removed after tying silk loops to both its ends and immersed in Krebs solution at room temperature. In the solution the sheaths of the ganglion are carefully removed so that ions can diffuse into the ganglion and the nerve.

Apparatus: A cathode-ray oscilloscope with a preamplifier, total sensitivity 100 μV/cm. D. C. amplification or a capacity coupled amplifier with a large time constant (about 8 sec.).

Other requirements: A recording chamber made of perspex with openings for the electrodes, for admitting gases and for exchanging solutions.

Procedure: The ganglion is fixed by the loops to the chamber and the stimulating and recording electrodes are placed on it. The chamber is filled with Krebs solution aerated with 95% O_2, 5% CO_2. The chamber is put into a Magnus bath warmed to 37°C. When recording, the ganglion must be taken out of the solution. This is achieved by running the solution into another vessel in the same bath connected to the chamber by a rubber tube.

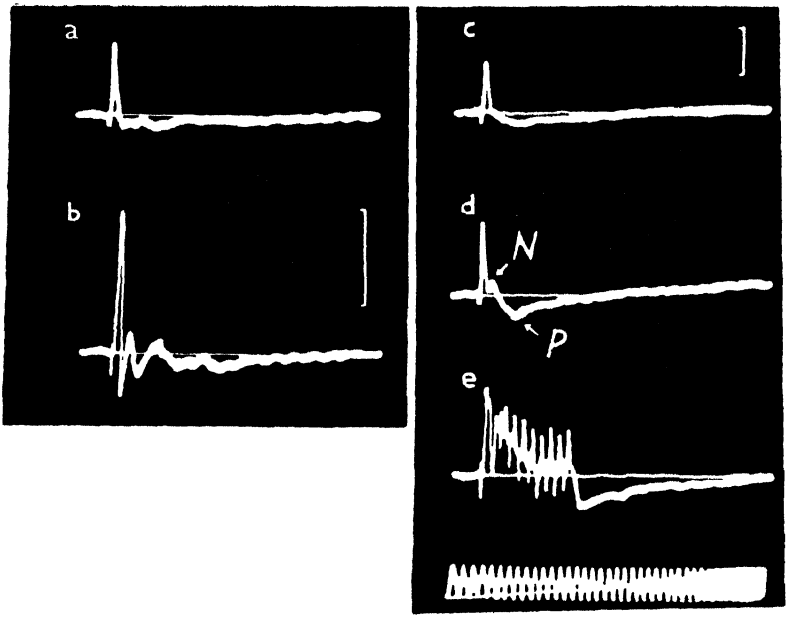


Fig. 120. Excised superior cervical ganglion of the rabbit. a) R_1, R_2 leads for a lower, b) for a higher stimulus strength applied to the preganglionic trunk. c–d) recordings (R_3, R_5 leads) for gradually increasing stimulating pulse strength. e) response to a train of impulses. N — slow negative, P — slow positive potential. Time mark: 100 c/sec. Voltage calibration: 0·5 mV.

Leads R_3R_5: As in the ganglion in vivo, a relatively sharp and synchronous spike is produced by a weak stimulus. This is followed by slow negative (N) and slow positive (P) waves (Fig. 120c, d). With stronger stimuli, the initial spike which represents a discharge of ganglion cells splits into components S_a and S_b (Eccles 1952). The S_a spike is compounded of the S_1 and S_2 waves in the cat.

When stimulating rhythmically (Fig. 120e) with a volley of impulses, it can be seen that on cessation of the stimulation the N and P waves are much larger than after a single preganglionic volley (Bronk et. al. 1938, Eccles 1944).

Lead R_1R_2: Fig 120a,b shows a tracing from these leads. A weak stimulus produces a synchronous spike with a conduction velocity of 20—25 m/sec. (Fig. 120a). On applying stronger stimuli more irregular activity appears after the first spike (Fig. 120b). The large preganglionic spike forms the S_a component in lead R_3R_5, and the small one the S_b component.

Conclusion: The action potential recorded from the superior cervical ganglion (lead R_3R_5) consists of a spike that is produced by impulses arising in ganglion cells. In addition there is a large negative and positive after-

potential which is several times larger than any recorded from a peripheral nerve.

Both the negative and the positive potentials are larger and more prolonged after repetitive stimulation.

b) Synaptic potentials in the superior cervical ganglion

Problem: Record the synaptic potentials and the late negative wave in the isolated superior cervical ganglion of the rabbit and determine the effect of d-tubocurarine and prostigmine.

Principle: As in the neuromuscular junction, it is possible to prevent the initiation of spikes in the postsynaptic neurone with relatively low concentrations of d-tubocurarine. This substance acts as a competitive inhibitor of the depolarising effect of acetylcholine. Under such conditions, the action potential disappears in the ganglion cells and only a prolonged synaptic potential termed postsynaptic (Eccles 1943) remains, since this occurs on the postsynaptic parts of the synapse.

The summation of postsynaptic potentials may be obtained if the time interval between the stimuli is suitably chosen. If the blockade with d-tubocurarine is not very deep, the critical level for spike initiation in the postsynaptic neurone may be attained by summation. If recordings are made using a very slow time base it is found that the postsynaptic potential (N) is followed by a prolonged late negative wave (LN). The LN wave cannot be observed at the neuromuscular junction. Obviously the transmission in the sympathetic ganglion is much more complicated.

Object: The isolated superior cervical ganglion of the rabbit. Preparation as in the precending experiment.

Apparatus: A D. C. oscilloscope with a D. C. preamplifier, total sensitivity 100 μV/cm. A stimulator giving one pulse or a volley with a variable number of pulses. The pulse or the beginning of the volley is synchronised with the sweep of the time base. Time marker 1000 c/sec. and 10 c/sec.

Other requirements: As in the preceding experiment. Krebs solution with different concentrations of d-tubocurarine.

Procedure:

1) Synaptic potential.

The electrode arrangement R_3—R_5 is used. With them the action potential is recorded (Fig. 121a). The solution in the chamber is then exchanged for Krebs solution with $1{\cdot}5 \cdot 10^{-5}$ M d-tubocurarine. The preparation is left in this solution for 20 minutes and the response to the same stimulus applied to the preganglionic trunk is recorded (Fig. 121b). A synaptic potential is

produced. This still has a spike on its peak. Curarisation is not complete. The preparation is then placed in a more concentrated d-tubocurarine solution ($3 \cdot 10^{-5}$ M). This produces complete curarisation and a pure synaptic potential is recorded (Fig. 121c).

If the sweep speed of the oscilloscope is slowed down it is seen that the synaptic potential is followed by a late negative wave (Fig. 121d). This is usually preceded by a small positivity.

2) Response to repeated stimulation.

If the preganglionic trunk is stimulated with a train of impulses summation of synaptic potentials is obtained (Fig. 121d). This results in the block being overcome, with a discharge of

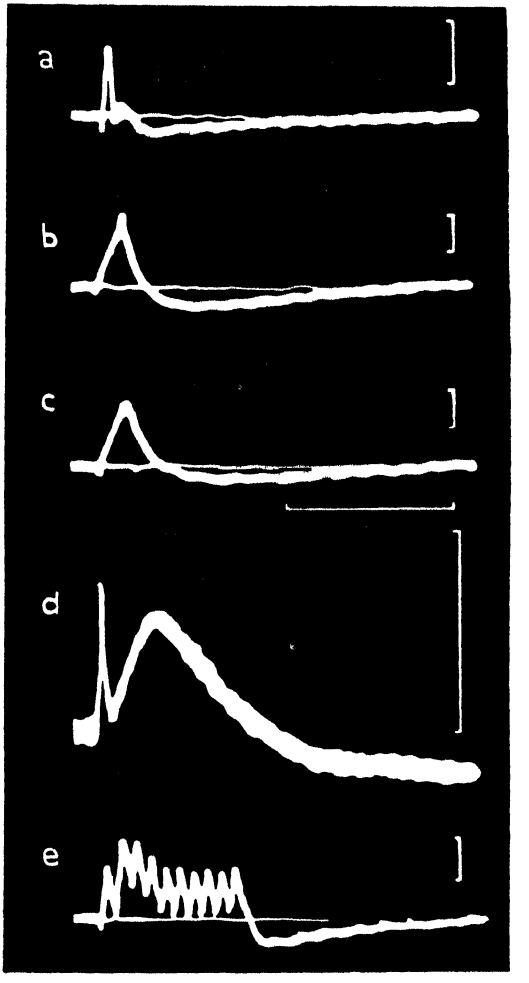


Fig. 121. The effect of d-tubocurarine on transmission in the superior cervical ganglion of the rabbit. R_3, R_5 leads. a) action potential before curarisation. b) partial curarisation ($1{\cdot}5 \cdot 10^{-5}$M). c) complete curarisation ($2{\cdot}85 \cdot 10^{-5}$M). d) synaptic potential and late negative wave. e) response to repetitive preganglionic nerve volleys. (60 c/sec). Voltage calibration for a) 2 mV, for b) and c) 0.25 mV, for d) 0.2 mV, for e) 0.25 mV. Time mark for a), b), c) 500 msec, for d) 2 sec.

impulses arising in the ganglion cells if a concentration that just blocks the spikes is used. This is facilitation similar to that in neuromuscular transmission.

3) The effect of anticholinesterases on synaptic processes.

The action potential in a synaptic ganglion is recorded with leads R_3R_5. After blocking with d-tubocurarine ($2{\cdot}8 \cdot 10^{-5}$ M), a typical synaptic potential with waves P and LN is recorded. After increasing the curarine concentration to $8 \cdot 10^{-5}$ M, the N wave decreases but the P wave increases. If $3 \cdot 10^{-6}$ M prostigmine are added, waves N and LN are decreased and wave P is considerably increased.

Conclusion: It can be seen from the tracings that the postsynaptic potential in the superior cervical ganglion has characteristics similar to e. p. p. Theoretically there are as yet no proofs that acetylcholine has a depolarising effect by shortcircuiting the membrane as is the case for the end-plates.

The experiment with different curare doses showed differences in the effect of deeper curarisation on the N wave on the one hand (decrease), and the P and LN waves on the other hand (increase).

Anticholinesterase also has different effects on the P and LN waves. The P wave is increased and the LN wave decreased. It is assumed that the LN wave is due to substances other than Ach, since it increases on curarisation and decreases under the effect of anticholinesterase. The P wave is probably due to the effect of acetylcholine, since it is increased and prolonged by anticholinesterase. Since large doses of d-tubocurarine produce a rise of the P wave it may be assumed that these doses change the depolarising effect of Ach to a hyperpolarising one (Eccles 1953, R. Eccles 1952, Laporte and Lorente de Nó 1950).

c) Occlusion and facilitation in the superior cervical ganglion

Problem: Demonstrate occlusion and spatial facilitation in the isolated superior cervical ganglion of the rabbit.

Principle: These phenomena, observable in the sympathetic ganglion, follow from the principle of convergence. If the preganglionic nerve is divided into two parts, both bundles may be stimulated separately or together. From the amplitude of the action potentials from the postganglionic trunk it may be determined that the resultant amplitude is not a simple arithmetic sum of amplitudes obtained when separately stimulating the nerve bundles. It can be larger than their sum (spatial facilitation is produced) or smaller (occlusion occurs).

Object: The isolated superior cervical ganglion of the rabbit.

Apparatus: A cathode-ray oscilloscope with an A. C. amplifier, total sensitivity 100 μV/cm. A stimulator with two independent outputs giving pulses with changeable amplitudes.

Procedure: The ganglion is prepared as before. The preganglionic trunk, however, is carefully divided along its longitudinal axis into two approximate halves of about 15 mm length. The preparation is then fixed in the chamber so that each trunk lies on a separate stimulating electrode. The recording electrodes are situated on the postganglionic trunk.

Facilitation: The response to maximal stimulation for S_a fibres of one and the other preganglionic bundle is recorded (Fig. 122a, c). Then

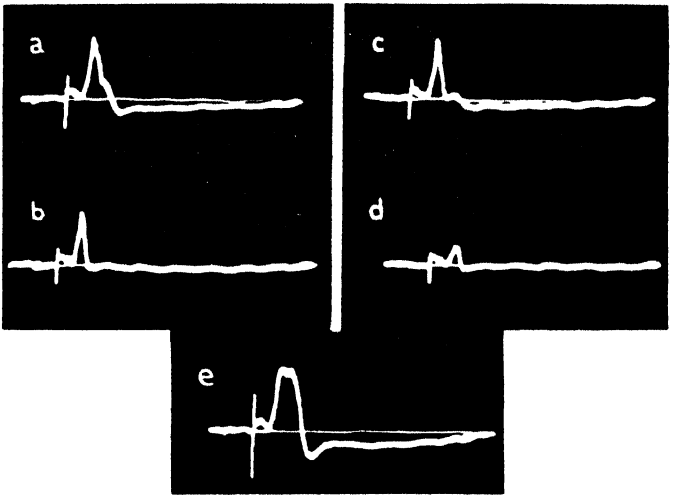


Fig. 122. Spatial facilitation in the superior cervical ganglion of the rabbit. R_4, R_5 leads. a) response to a maximal stimulus in one preganglionic bundle. c) response to a maximal stimulus in the other one. b, d) responses to submaximal stimuli in corresponding preganglionic bundles. e) response to simultaneous submaximal stimulation of both bundles.

the stimulus is decreased to submaximal until the amplitude is half the maximum value (Fig. 122b d). The preganglionic trunks are then stimulated together (Fig. 122e). If now the action potentials for the 1st and 2nd stimulus are subtracted from the common tracing, a certain negativity remains. This indicates that the potential due to simultaneous stimulation of both bundles is greater than the sum of those potentials. This is spatial facilitation.

Occlusion: Again both nerves are stimulated maximally. If both trunks are stimulated simultaneously with a maximal stimulus it can be shown that the recorded potential is smaller than the sum of the amplitudes of the action potentials obtained on separate stimulation. This is occlusion.

Conclusion: Fig. 123 best explains occlusion and facilitation. Fig. 123a shows the mechanism of occlusion. Each nerve bundle (n_1 or n_2) "supplies" a certain number of ganglion cells and these become active on maximal stimulation. Since some fibres of trunk-n_1-converge onto ganglion cells innervated also from trunk-n_2, the resultant effect of simultaneous stimulation of both bundles must be less than the sum of the amplitudes obtained when stimulating each bundle separately. Ganglion cells supplied from both trunks do not partake in the addition.

Spatial facilitation is shown in Fig. 123b. This was produced by weak stimuli. Each preganglionic fibre on submaximal stimulation produces activat-

ion of several ganglion cells, some of which are stimulated only subliminally. The region of cells stimulated is termed the discharge zone, the region of subliminally stimulated cells is termed the subliminal fringe. Some cells of the subliminal fringe are supplied from both n_1 and n_2 bundle. If the latter are stimulated separately, these cells are not activated, but if they are stimulated

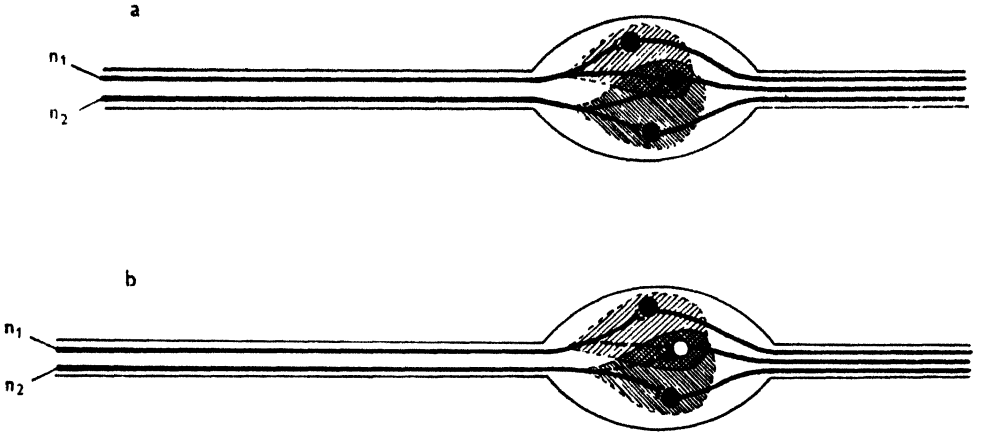


Fig. 123. Diagrammatic representation of the mechanism of occlusion (b) and spatial facilitation (a).

together a discharge is produced in the cells. Hence the resultant amplitude of the postganglionic trunk is higher than the sum of amplitudes when stimulating separately.

d) Recovery in the sympathetic ganglion

Problem: Determine the course of changes in excitability occurring in the superior cervical ganglion of the rabbit after maximal stimulation of preganglionic fibres and compare their course with that of the negative and positive after-potential.

Principle: The tracing of a complete action potential from the superior cervical ganglion shows that the latter is accompanied by a negative and positive after-potential, the amplitude of which is large in comparison of that of the after-potential in peripheral nerves. These after-potentials are accompanied by changes in excitability similar to those in the peripheral nerves.

Principally these changes in excitability are determined as in the peripheral nerve. Maximal conditioning and submaximal test stimuli are used. The time interval between the two stimuli is changed. The increase in amplitude in the postganglionic fibre in a certain time interval following the first stimulus

is termed temporal facilitation, its decrease after a longer time interval — inhibition.

Object: The isolated superior cervical ganglion. Preparation as before.

Apparatus: A stimulator — one output with a pair of stimuli — a variable time interval and amplitude; duration of pulse 0·2 msec. Otherwise as in the preceding experiment.

Other requirements: as before.

Procedure: The preparation is fixed in the chamber in the usual manner. The recording electrodes are on the postganglionic trunk. The nerve is stimula-

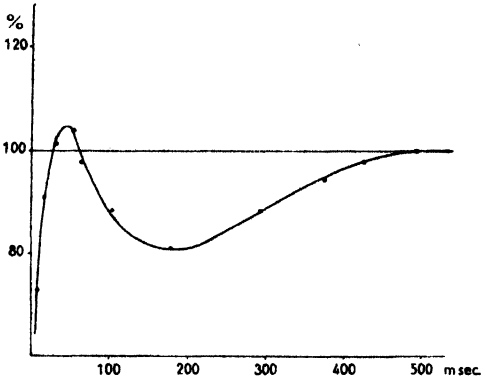


Fig. 124. Changes in excitability after a maximal stimulus in a sympathetic ganglion.

ted in such a way that the first impulse will produce a maximal response, the second one a submaximal one. First, a 60 msec. interval is chosen. The interval is then gradually increased and again decreased. The action potential changes its amplitude according to whether the second impulse reaches the nerve in the facilitation or inhibition interval of the first action potential. If the amplitude of the second submaximal potential is taken as 100% and the other amplitudes are expressed as percentage of this, a curve as shown in Fig. 124 is obtained. From simultaneity to 5 msec. is the absolute refractory period. This is followed by the relative refractory period and this changes to temporal facilitation and then subnormality.

Conclusion: Facilitation may be explained on the basis of the diagrams given in the preceding experiment. The preganglionic influx does not change and consequently, facilitation can only be explained by assuming that the cells in the subliminal fringe enter the discharge zone under the influence of increasing excitability. Inhibition, on the other hand, can be explained by assuming that cells from the discharge zone enter the subliminal fringe because of increased thresholds. It is understandable that inhibition of the postsynaptic response cannot be obtained without the existence of the subliminal fringe.

V

Electrophysiology of peripheral excitable structures in situ

Electrophysiology of peripheral excitable structures (muscle, nerve) in situ is based mainly on the recording of action potentials. These may be produced by normal reflex activity or by electrical stimulation.

Fundamentally two methods are used to record action potentials under such conditions: recording from organs made accessible surgically or without surgical preparation. Surgical exposure may be acute or may consist in implanting electrodes for a chronic experiment.

Electrophysiological recording from excitable structures made accessible surgically does not differ from that from the same structures in vitro. Usually no special modification of these methods is necessary for the latter, and thus the only new problem involved is a surgical one.

When recording from structures in the normal organism a new factor must be considered: registration from a nerve or muscle situated in a volume conductor. The fundamentals of the distribution of electric current in a volume conductor must be known if recordings are to be correctly interpreted.

Many electrophysiological methods of recording in situ have become independent clinical methods: electrocardiography, electromyography etc. The literature concerned with these methods is extensive and will not be considered here.

Experiments given in this section are examples of recording action potentials from nerves and muscles in situ.

a) Recording of an impulse in a volume conductor

Problem: Record the action potential from the frog sciatic nerve in a volume conductor in situ and in a model experiment.

Principle: When a nerve conductor is situated in an electrically insulating medium (a narrow film of extracellular fluid or Ringer solution surrounding the nerve, otherwise surrounded by air, paraffin oil etc.) a recording instrument will register changes in electrical potential only at the point of electrode

contact. If the nerve conductor is in an electrically conducting medium the manifestations of the nerve impulse are more complex.

The external current field of the nerve impulse acts on the volume lead for as long as the impulse passes through the nerve located in the volume conductor.

The nerve impulse forms in the nerve a mobile, double dipole connected by negative poles. Negativity is represented by depolarisation of the membrane

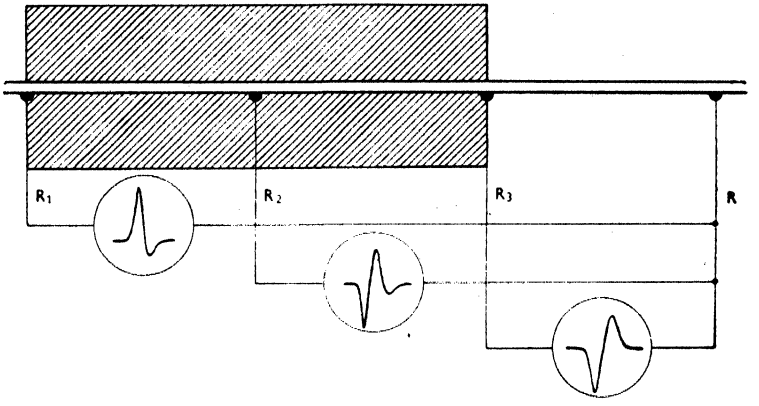


Fig. 125. Diagrammatic representation of nerve impulse recording in a volume conductor. R_1, R_2, R_3 — recording electrodes in the volume conductor (shaded area). R — indifferent recording electrode. The circles contain diagrams of action potentials recorded with the corresponding electrodes.

during the nerve impulse, the positive poles by segments proximal and distal to that point. The current flows first from the interior of the axon across the membrane to the exterior and then in the opposite direction. Finally it again flows towards the exterior. The external current field in a volume conductor is not limited to a narrow film of the conducting medium, as is the case in an insulating medium, but spreads a long way into the conducting surroundings.

The current flow from the axon to the exterior acts as a source, and in the opposite direction as a sink. From that aspect a nervous impulse is characterised by the sequence: source — sink — source.

Depending upon what part of this sequence passes the volume lead, corresponding electric changes will be recorded.

When the nerve impulse enters that part of the nerve which lies in the volume conductor (Fig. 125) the recording electrode (R_1) situated here will record a negative wave and later a positive one. At R_2 a triphasic positive-negative-positive wave is recorded. At the point where the nerve impulse leaves the volume conductor a positive followed by a negative wave is recorded.

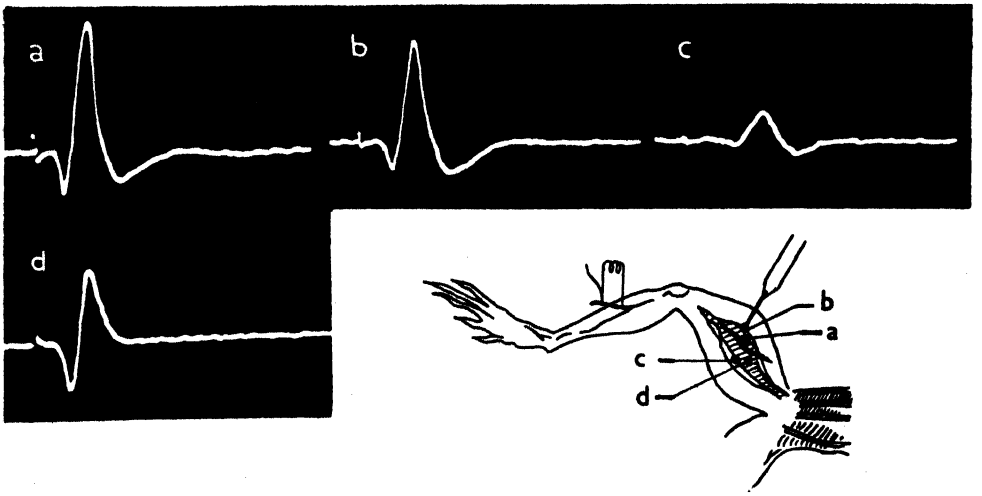


Fig. 126. Recording of action potential of the frog sciatic nerve with a volume lead in situ at the site marked on the drawing. Letters a–d in the diagram denote the number of the oscillogram. Stimulating electrodes are on the peroneal nerve. The indifferent earthed electrode is on the opposite limb.

Action potentials may be recorded in a volume conductor from a nerve in situ, or even better, in an artificial volume conductor which can easily be made by placing the nerve on filter paper soaked in Ringer solution. Such a model, which is also very instructive for understanding more complex three-dimensional volume conductors, such as structures in the brain (see p. 386) may be used to map out the electric field of a travelling nerve impulse.

The principle of such mapping is as follows. The nerve is placed on filter paper soaked in Ringer solution. One recording electrode is at the edge of the paper at a sufficient distance to prevent the external current field of the impulse from acting on it. The other electrode records responses at different distances from the axis of the nerve.

The values of the potential at some instant after the application of the stimulus are used to reconstruct graphically the equipotential contours and lines of current determining the sources and sinks of current flow. For the theory and practical procedure of such mapping see the papers by Lorente de Nó (1947), Lloyd (1952), Kostyuk (1956).

Object: The sciatic and peroneal nerve of the frog *Rana esculenta* or *Rana temporaria*.

Apparatus: A cathode-ray oscilloscope with a preamplifier. Total sensitivity 100 μV/cm. A voltage calibrator. Time marker 1000 c/sec.

Other requirements: A glass capillary semi micro-electrode with an opening 20—30 μ in diameter, filled with frog physiological saline and connec-

ted to the input via an AgCl coated silver wire immersed into the broader end of the tube. Filter paper 30 × 30 cm.

Procedure: The preparation (lower half of the trunk with both hind limbs of a frog) is skinned and fixed in a Petri dish having a cork bottom. The left limb is earthed. The peroneal nerve of the right limb is exposed, cut distally and placed on the stimulating electrodes. The semi micro-electrode is

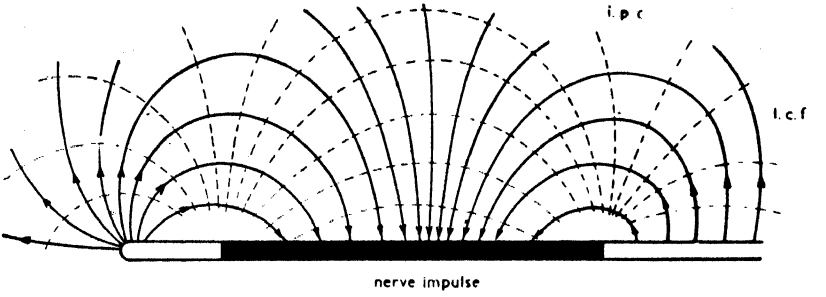


Fig. 127. Diagramatic representation of the distribution of the external current field in the nerve at the moment when the impulse wave is at the marked point, i. p. c. — isopotential contours, l. c. f. — lines of current flow, (diagramatically, according to Lorente de Nó, 1947 and Kostyuk, 1960).

used to record action potentials from the points marked by full circles in Fig. 126.

The responses recorded near the entry of the nerve impulse into the volume conductor are diphasic. Those recorded from more distant parts are triphasic and their amplitude decreases with increasing distance from the nerve.

The nerve is then freed from surrounding tissues for a length of about 25 mm and placed on filter paper. The nerve lies in an horizontal ridge that permits the surface of the nerve to be at the same level as the surface of the paper. The paper is soaked in frog saline. One end of the nerve is in a small bath containing paraffin oil. The stimulating electrodes are located here. Then the whole preparation is covered with paraffin oil to prevent drying of the paper and thereby a change in its conductivity.

As before, the action potential registered when the nerve impulse enters the volume conductor is diphasic, then triphasic and the amplitude decreases with increasing distance from the nerve.

Several hundred action potentials must be recorded for mapping the external current field. Then, at a certain time interval from the beginning of the stimulus, which represents a certain position of the nerve impulse in the nerve conductor, the corresponding potential amplitude is measured. Points of equal amplitude are connected thus forming isopotential contours. The

lines of current flow and the isopotential lines intersect one another at right angles. Zero isopotential lines separate zones of opposite polarity and determine the direction of the lines of current flow and thus the sinks and sources of the external current field of the nervous impulse at the chosen time.

The field diagram in Figure 127 is characterised by the sequence: source — sink — source. In a similar way it is possible to map the external current field from the same oscillograms for any position of the nerve impulse in the nerve conductor.

Conclusion: The volume distribution of the external current field of the nerve impulse is obtained by rotating the two dimensional current field drawn in this way through 360° about the axis formed by the nerve. The field strength will decrease more rapidly, however, with the distance.

When recording with volume lead, it is important to keep in mind that this lead records changes in the voltage drop caused by membrane current, while the monophasic lead in an insulating environment records changes in membrane potential. This explains the fact that recording with a volume lead depends upon the duration of the registered process. A potential change having a certain amplitude and a shorter space extent produces a greater current density than a change due to the same amplitude but of greater space extent. Hence it is difficult to record after potentials (their wave length is several m and they have a small amplitude), in a volume conductor.

Recording in a volume conductor is important when registering and interpreting electric activity in the brain (see chapter VIII K).

b) Impulse activity of somatic and vegetative nerves

Problem: Record impulse activity from a somatic and autonomic nerve in situ in the rabbit.

a) from the nerve leading to the gastrocnemius muscle when the latter is stretched,
b) from the cervical sympathetic trunk,
c) from the cervical sympathetic trunk together with respiration,
d) from the cervical sympathetic trunk together with discharges from the phrenic nerve.

Principle: Electrophysiological recording from whole nerve in the organism is simple, if recordings are not made in a volume conductor. The point of the nerve from which impulses are to be recorded is made accessible surgically and an electrode is placed on it. This must not touch the surrounding tissues.

If electric stimulation of such a nerve is used the method is the same as for nerves in vitro. In situ this method, however, is only rarely used.

Usually, impulse activity due to reflex activity of the organism is studied.

Electrical activity of a mixed peripheral nerve evoked by reflex activity gives a very complex picture. It is similar to spontaneous activity produced in a nerve in vitro, e. g. by decalcification. The physiological stimulus does not activate all fibres at the same time, so that the recording is characterised by asynchronous activity of nerve fibres. Such tracing may be used to deter-

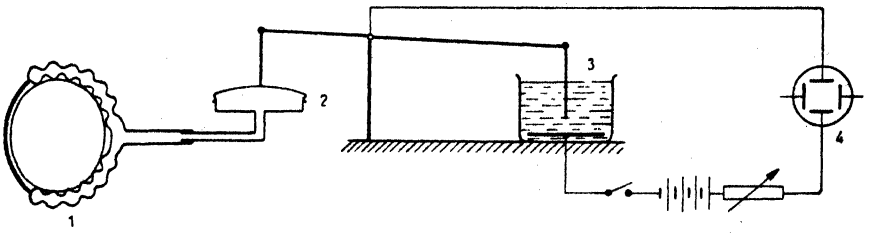


Fig. 128. Diagram of the oscilloscopic recording of respiratory movements from the thorax. 1 — pneumograph, 2 — Marey's drum, 3 — liquid rheostat, 4 — oscilloscope.

mine the presence or absence of stimulation. They are not suitable for quantitative evaluation.

For such purposes it is best to use a nerve bundle with a small number of fibres, or even better a single nerve fibre isolated for a certain length. This is very difficult and time consuming but permits quantitative evaluation.

If the direction of the impulses is to be determined the nerve must be severed on the corresponding side of the recording electrode since the nerve trunk contains both afferent and efferent fibres.

Sources of error during recording.

1) Artifacts due to movement occur if the nerve shifts along the recording electrodes during movement of the animal. Such movement along the metal electrodes may also produce mechanical stimulation of the nerve and thus an interfering impulse acitivity. This artifact is avoided if the nerve hangs freely on the electrodes and if the part of the nerve near the recording electrodes is supported by glass hooks.

2) Drying of the nerve and disturbances of circulation. Drying of the nerve may either produce automatic activity or, on the contrary, decrease of excitability. The same holds true for impairment of the circulation. This may arise as the result of incorrect preparation. Drying is prevented by having the animal in a thermostat with adequate temperature and moisture, or by covering the nerve with a layer of paraffin oil at an appropriate temperature.

3) Electric activity of other organs: Most frequently this is the heart, particularly when working in its vicinity. If assymetrical amplifiers are used, this is prevented by a treble electrode. The grid electrode is in the middle,

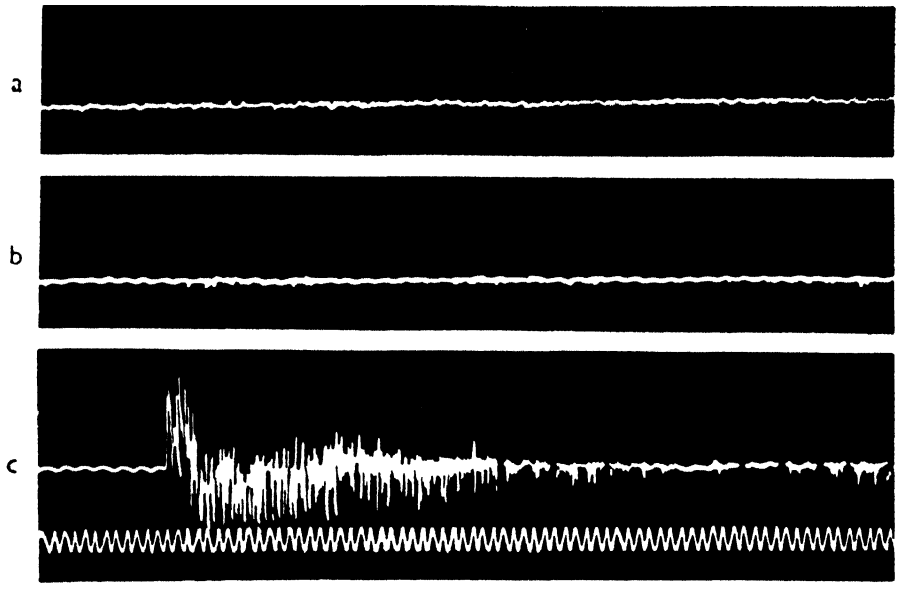


Fig. 129. Discharge from the gastrocnemius muscle of the rabbit. a), b) discharge during rest. c) after stretching the tendon. Time mark: 25 c/sec.

the other two are earthed. Symmetrical amplifiers should have a high discrimination coefficient (see page 118).

4) Finally external interference may be more important when registering in situ than when working with isolated preparations.

Object: A rabbit under urethane anaesthesia with the required nerves exposed.

Apparatus: A double beam cathode-ray oscilloscope with A. C. preamplifiers. Total sensitivity 100 μV/cm. Time marker 1000 and 50 c/sec. Respiratory movements are recorded oscillographically as shown in Fig. 128. Movements of the thorax are recorded in the same way as when using a kymograph. The movements of drum 2, however, are transformed to electric potentials by a liquid rheostat - 3 — from which they are lead to the input of the D. C. amplifier of the oscilloscope. A power amplifier with a loudspeaker.

Other requirements: Surgical instruments. Adrenaline. Nicotine.

Procedure:

1) Impulse activity in the nerve leading from the gastrocnemius muscle, when the latter is stretched.

The gastrocnemius muscle of a rabbit under urethane anaesthesia is exposed, together with the nerve leading to one of its heads. The nerve is

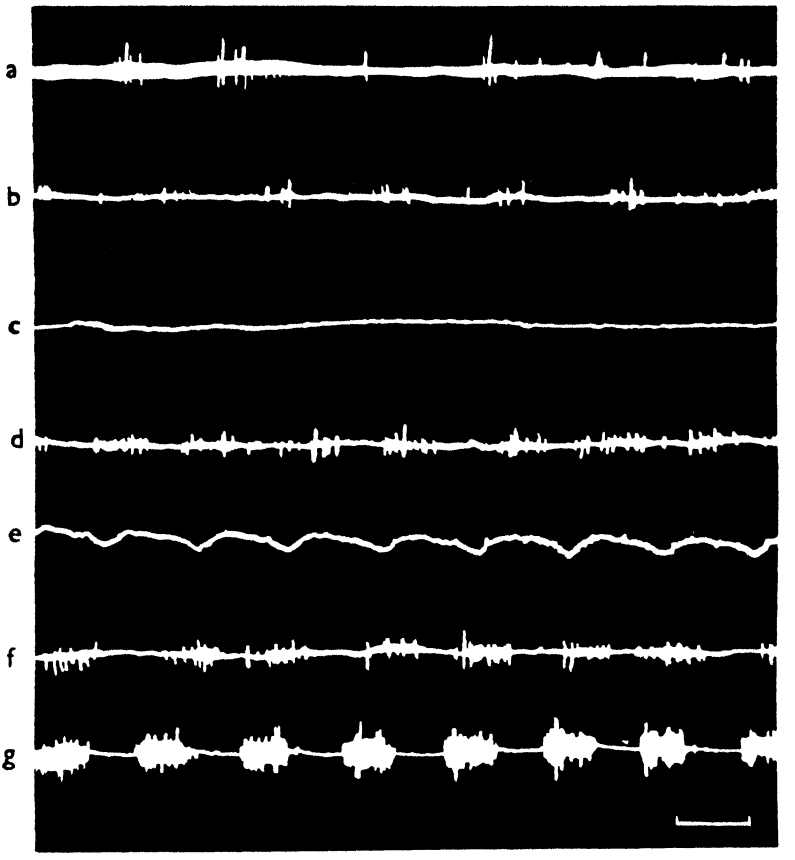


Fig. 130. Discharge from the cervical sympathetic nerve of the rabbit. a) spontaneous discharge. b) after treating the superior cervical ganglion with 0.5% nicotine. c) after i. v. adrenaline. d) discharge in the sympathetic nerve after cutting the vagi. e) respiration. f) discharge in sympathetic trunk. g) discharge in phrenic nerve. Time mark: 2 sec.

cut centrally and attached to a stand by a silk thread. The recording electrodes are placed on the nerve in such a way that they do not touch the rest of the preparation. On connecting them to the oscilloscope with attachements of the muscle intact, a continuous irregular discharge is observed on the screen (Fig. 129ab). This discharge is centripetal and is produced in muscle proprioceptors, since it considerably decreases on cutting the muscle tendon.

If a load is put on the muscle the discharge increases (Fig. 129c).

The figure shows that the response from the whole nerve is completely asynchronous and that a more detailed analysis of the discharge is not possible. The same experiment may be easier performed also in vitro with the frog sciatic-gastrocnemius muscle preparation.

2) Impulse activity from the cervical sympathetic trunk.

The cervical sympathetic trunk is exposed and cut distally. A silk loop is tied to its central end and the nerve trunk is placed on recording electrodes. A discharge is shown in fig. 130a. The figure shows that activity from this nerve is not so asynchronous as in the preceding experiment, when a somatic nerve was used.

The amplitudes of the recorded waves varie, but as a rule the majority do not vary much with respect to duration and shape of the action potential.

This is due to the fact that groups of nerve fibres become active synchronously. Activity is sometimes so well synchronised that it may be considered as a unit discharge.

Activity is exclusively due to centrifugal fibres since activity of centripetal fibres was excluded by cutting the nerve distally. Centrifugal fibres may by either pre- or postganglionic. The participation of both kinds of fibres may be determined using Langley's nicotine method. The superior cervical ganglion is treated with 0·5% nicotine and the discharge is recorded (Fig. 130b). It can be seen that activity decreases to only a small extent. This indicates that the spontaneous discharge in the sympathetic nerve is mainly preganglionic in origin.

If 0·5 mg adrenaline is given intravenously this natural discharge disappears (Fig. 130c.) for 5—10 minutes and then returns to normal levels only slowly. It has been shown that this natural sympathetic activity has a vasoregulatory effect (Adrian et al. 1932).

3) Simultaneous recording of impulse activity from the cervical sympathetic trunk and of respiration.

The impulse discharge in vegetative nerves is grouped in a more or less regular rhythm. In rabbits this grouping often coincides with respiratory movements.

This effect is present even with the vagus intact but is more evident when it is cut. Fig. 130d shows this grouping in a recording with very slowly moving film Fig. 130e is a recording of respiration.

4) Discharges from the sympathetic and phrenic nerves.

A double beam oscilloscope with two A. C. amplifiers is used. The electrodes from the cervical sympathetic nerve are connected to the input of one preamplifier. Electrodes placed on the distally cut branch of the phrenic nerve in the neck are connected to the other one. The vagi are cut. Fig. 130f, g shows tracings from such an experiment. The upper beam is a recording from the sympathetic trunk, the lower one from the phrenic nerve. The discharge from the phrenic nerve (Fig. 130g) starts before the actual sympathetic discharge and ends before the sympathetic discharge begins to decrease. It can be seen that this occurs at different frequencies of respiration.

Acoustic monitoring of nerve activity may be of advantage. The output of the preamplifier or of the oscilloscope amplifier is connected to the power amplifier with a loud-speaker. The coincidence between respiratory movements and the acoustically displayed nerve discharges can be easily followed in this way.

It follows that in the absence of sensory impulses from the vagi the sympatic centres in the rabbit are stimulated directly by every period of activity in the respiratory centre.

Conclusion: Recording of electric phenomena due to impulse activity from whole nerves gives only a very rough idea of the activity of these nerves. Vegetative nerves differ from somatic ones in that respect since during reflex activity the discharge is less asynchronous and has a smaller number of relatively larger potential waves. As shown above, this enables one to compare the function of these nerves with the activity of other systems in the organism.

In general whole nerves are not suitable for exact analytical work, for which smaller units must be used.

c) Impulse activity of muscle in situ. Electromyogram

Problem: Record the electrical phenomena accompanying muscular activity using surface and concentric electrodes:

a) during shivering evoked by cold,

b) during metrazol convulsions.

Principle: If recording electrodes are applied to a muscle, a very complex recording of electrical phenomena due to muscular activity is obtained when an asynchronous volley of nerve impulses enters the nerve, such as during reflex stimulation. Impulse activity obtained by this surface electromyography is then difficult to analyse.

It is, therefore, better, also in the case of muscle, to work with smaller units. This may be achieved in two ways, either by using an isolated muscle fibre, or with the aid of electrodes having a very small interelectrode distance. The first method is possible only in vitro and is very difficult. Only the second method is feasible for in situ work.

This was introduced by Adrian and Bronk (1929) and its principles are as follows.

When recording impulse activity from a muscle with two electrodes with a certain distance between them (Fig. 131A), the electrodes will record the algebraic sum of potential changes from several fibres, in addition to those in contact with the electrodes. The potential gradient in the fibre at a distance d produces a considerable potential difference between a and b.

If the electrodes are very close to each other (a', b', Fig. 131B) they can record the same potential gradient only from distance d'.

The small interelectrode distance is attained in a way suggested and used by Adrian. A very fine wire (about 100 μ) is used, covered with an insulating layer. This is inserted into an injection needle (Fig. 193). The needle forms one electrode and the wire in the centre, insulated from the needle, the second electrode. The needle also serves to pierce the muscle and to give stability

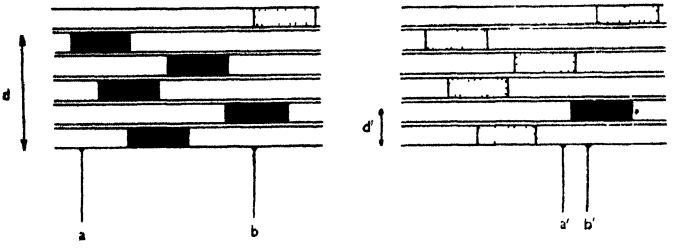


Fig. 131. Recording of muscle activity with needle electrodes, with a large (a) and small (b) interelectrode distance.

to the electrode. In addition the needle serves as the screen for the grid lead. This electrode is termed Adrian's concentric needle electrode.

In addition to this concentric needle, a bipolar needle electrode may also be used. This differs from the above only by the fact that two fine wires are inserted into the needle.

Object: rat.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier, total sensitivity 100 μV/cm. An amplitude and time marker.

Other requirements: Plate silver electrodes (3 × 3 mm.). Adrian's concentric needle. An ice bath. Arrangement for mechanical recording of muscular activity.

Procedure:

1) EMG during shivering produced by cold.

The rat is anaesthetised with ether. Plate electrodes covered with ECG paste are placed above the previously depilated region of the gastrocnemius muscle, using collodium. The rat is tied on a board on which a polyethylene bag containing ground ice is placed. At first continuous muscle activity is not observed. Only occasionally does a discharge of irregular activity appear. As the animal begins to lose heat, shivering sets in as a compensating mechanism and gradually more and more intense muscular activity can be seen.

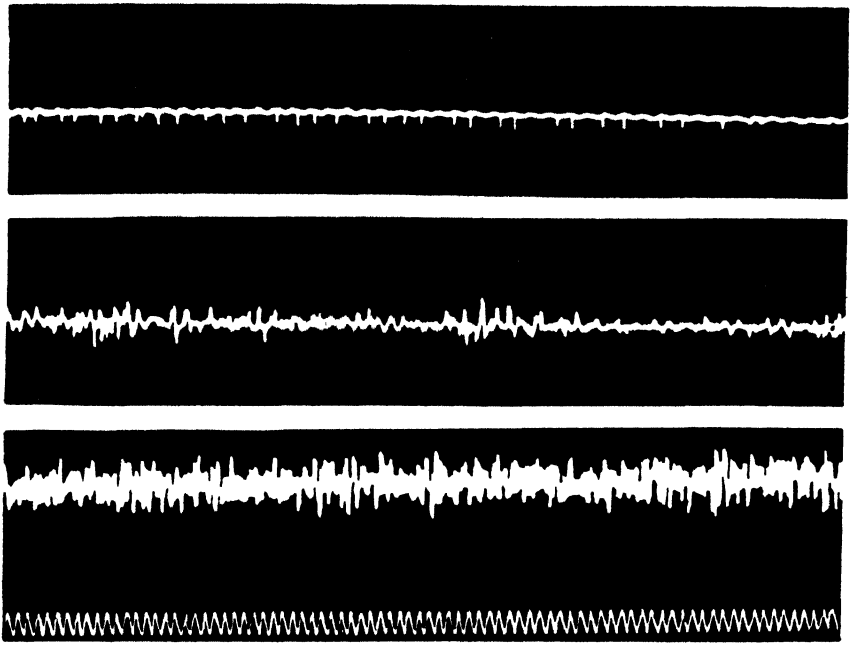


Fig. 132. Electromyogram during shivering due to cold recorded with Adrian's concentric electrode. Time mark: 25 c/sec.

The recording shows a very complex, intense, asynchronous activity.

If Adrian's concentric needle is inserted into the muscle the picture becomes much simpler. During weak shivering, provided the electrode is in a suitable position, unit muscular activity of low frequency may be recorded (Fig. 132a). This increases with increasing shivering. In addition, the electrode now records activity from more than one muscular unit (Fig. 132b, c).

2) EGM during cardiazol convulsions.

A rat under ether anaesthesia is tied to a special stand (Servít 1958). One hind limb is free, the other fixed. The free limb is connected to a liquid rheostat via a mechanical arrangement. The rheostat will record the muscular contraction of the limb. The fixed hind limb is depilated as above and either surface electrodes are attached to it or Adrian's concentric needle is inserted into the muscle. Metrazol (pentamethylentetrazol) is then given intraperitoneally (80 mg/kg).

During the first convulsion, the muscle contractions and the surface electromyogram are recorded. During the second seizure the electromyogram is registered using the concentric needle (Fig. 133).

The muscle recording shows typical tonic-clonic convulsions and the electromyogram intense muscular activity of the gastrocnemius muscle

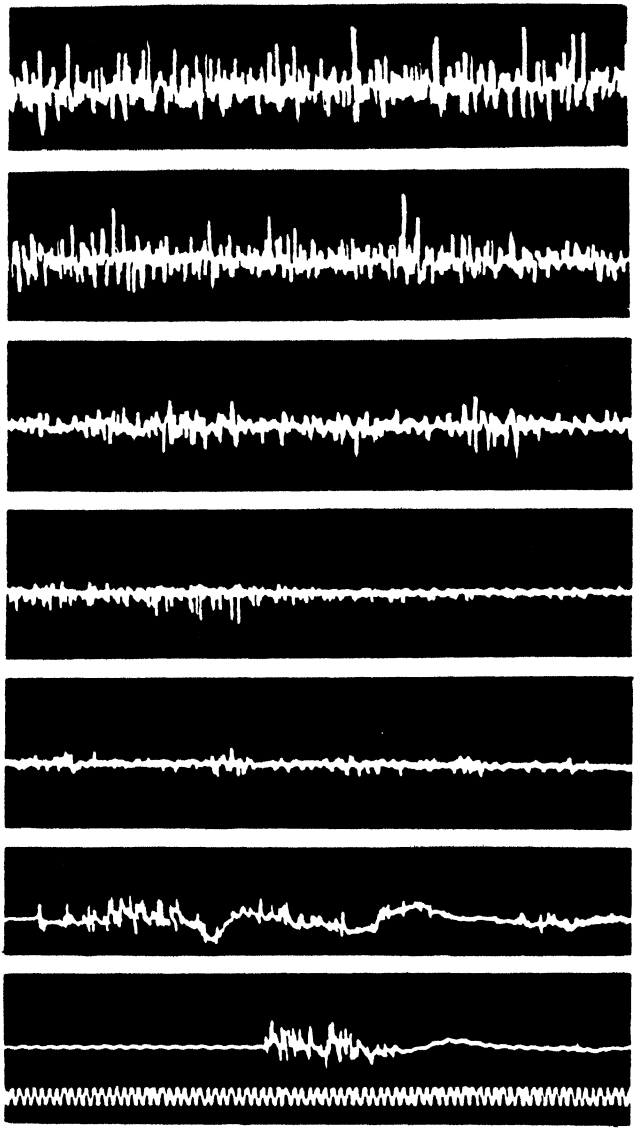


Fig. 133. EMG during metrazol seizures recorded with Adrian's concentric electrode. Time mark: 25 c/sec.

during the tonic phase and intermittent activity during clonus. Acoustical display (see p. 285) of EMG makes it possible to observe the behaviour of the animal and to follow simultaneously its muscle activity.

Conclusions: Electromyography has developed into an independent clinical discipline used as an aid for diagnosing neuromuscular disorders. It is often supplemented by EMG recording after percutaneous stimulation

of nerves. It makes it possible to distinguish between fasciculution (spontaneous contraction of muscle bundles) and fibrillation (spontaneous contractions of individual fibres), (Denny-Brown and Pennybacker 1938).

It is also possible to distinguish between neurogenic atrophies due to disorders of nerve conductors from those due to impairment of the anterior horn cells according to the degree of synchronisation (Buchthal and Clemmensen 1943, Buchthal and Madsen 1950).

The amplitude may be used for determining disturbances in neuromuscular transmission (Harvey and Masland 1941, Hodes 1948, Buchthal and Höncke 1944).

Multilead electromyography gives a broader picture of peripheral changes due to disturbances in the CNS, such as disturbances of reciprocal innervation (Hoefer 1941).

VI

The electrophysiology of sensory receptors

Electrophysiological methods for studying the peripheral end of sensory receptors are described in this chapter.

By means of this type of studies the nature of informations sent from the receptors to the central nervous system was elucidated for the first time.

These methods may be divided into two large groups: Recording of nervous impulses from nerve fibres leaving the receptors, and recording of electrical manifestations of processes in the receptor occurring during application of a specific stimulus. Such recordings are difficult in an anatomically complex receptor organ such as the eye. Recently progress has been made in this direction by the use of ultramicroelectrodes. It has thus become possible to analyse the activity of individual components of such complex receptor organs.

Electrophysiology has been used for studying many receptors. Results have been generalised to a certain extent and several monographs have been devoted to this subject (Adrian 1928, Granit 1955).

Some fundamental rules of receptor activity are demonstrated below for some receptors. The application of the two methodical approaches to other receptors can be studied in the monographs mentioned above. No fundamental modifications of methods described in this chapter are necessary.

A. Models of sensory organs

Characteristic discharges of nervous impulses in nerve fibres from specifically stimulated receptors can be demonstrated in the isolated nerve fibre to which a weak constant current is applied.

Nonmyelinated crustacean nerves are particularly suitable for this purpose. These respond to a D. C. current by repetitive discharge.

In such a manner the fundamental characteristics of a discharge in nerves in response to receptor stimulation may be demonstrated:

a) dependence of discharge frequency on the stimulus strength,

b) adaptation,

c) "on" and "off" effect,

d) spontaneous receptor activity.

a) The effect of D. C. current on the isolated nerve fibre

Problem: Determine the dependence of the discharge frequency on the intensity of a weak constant current pulse in the isolated inhibitory nerve in the claw of the crayfish *(Astacus fluviatilis)*.

Principle: The response to constant current varies considerably in different axons (Fessard 1936, Arvanitaki 1938, Hodgkin 1948). Hodgkin studied several kinds of axons in *Carcinus meanas* and divided them into 3 groups according to the kind of response.

His first group of axons is most interesting. They have no significant supernormal phase in the recovery cycle and are capable of responding within a large frequency range.

Zacharová (1957, 1960) found that the inhibitory nerve of *Astacus fluviatilis* belongs to this group.

Responses of this group of fibres to constant current are similar to those of sensory receptors.

In principle, then, the isolated inhibitory fibre of *Astacus fluviatilis* is stimulated with constant currents of different strengths and the resulting activity is recorded.

Object: The isolated double fibre (inhibitory and excitatory nerve fibre) leading to the abductor muscle of the claw of *Astacus fluviatilis*. The nerve is prepared as in experiment IVBb.

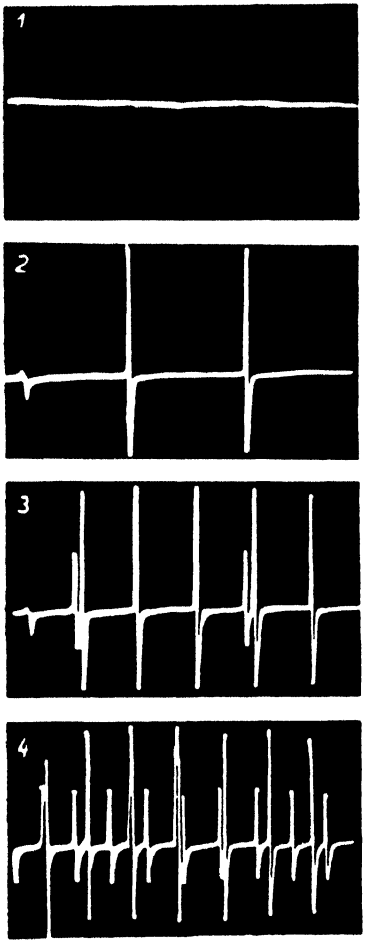
Apparatus: A cathode-ray oscilloscope with an A. C. amplifier; total sensitivity 100 μV/cm. A source of D. C. current. A microampermeter. A time marker.

Other requirements: As in experiment IVAb. Non-polarisable Ag-AgCl electrodes.

Procedure: The preparation is fixed to the recording arrangement as in experiment IVAb. The double-fibre to which the recording electrodes are applied is under paraffin oil. The nerve trunk with the nonpolarisable electrodes is above the paraffin oil. The distance between the nonpolarisable electrodes is 2 cm.

That strength of the stimulating pulse is chosen at which one action potential appears. This current strength is taken as unity. The number of spikes increases with the strength of the constant current (Fig. 134).

At a certain current strength the rheobase of the excitatory fibre is reached and its spike is registered (smaller spike — see experiment IVBb).



Conclusion: The recordings show that the discharge frequency depends on the strength of the stimulus. If the stimulus continues to act, the discharge frequency decreases. This is accomodation of the nerve.

There is a general similarity between this recording and the response from a receptor (Adrian 1932, Matthews 1931a, b, Hartline and Graham 1932).

A graphical evaluation of the relationship between current strength and repetition interval, on the one hand, and the response time from the beginning of the stimulus, on the other hand, gives curves as shown in Fig. 135.

These curves are very similar, indicating that both are determined by the same process.

This model experiment led Hodgkin to assume that for the frequency of a repetitive discharge, the response time of a sensory organ to a constant stimulus may be as important as its refractory period.

Fig. 134. The effect of a weak D.C. current on the inhibitory fibre of *Astacus fluviatilis*. Time interval between the spikes in recording 2) is 25 msec.

Several explanations have been offered for the repetition interval between pulses in a volley produced by a constant current. The first was given by Adrian 1928. This is based on the recovery cycle of nervous impulses. It is assumed that a given stimulus strength produces a constant degree of excitation in the axon and that the second response appears only when the nerve has sufficiently recovered from the effect of the preceding impulse. Thus a weak stimulus will excite late in the refractory period and the repetition interval

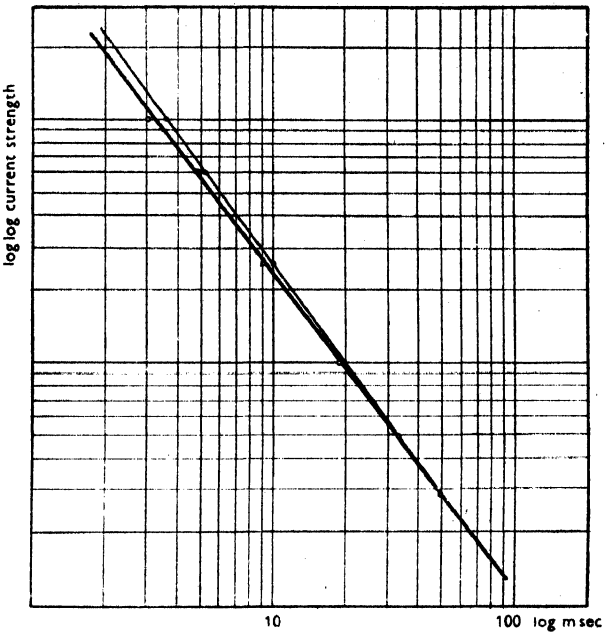


Fig. 135. The effect of current strength on the response time and repetition interval in a single axon of *Astacus fluviatilis*. Filled circles: relation between curent strength and response time (interval between make of current and the first spike). Open circles relation between current strength and repetition interval (interval between first and second spike). Abscissa: interval in msec. Ordinate: log . $\log_{10}$ of current strength, rheobasic strength taken as unity.

will be great. Strong stimuli excite sooner at the beginning of the refractory period and the interval is short. The upper frequency limit depends on the absolute refractory period.

This hypothesis requires an extremely long refractory period for receptors in order to explain the long intervals between natural impulses.

This is partly overcome by Hodgkin's hypothesis as supported by experiments of the same kind on invertebrates (Hodgkin, 1948).

B. Impulse activity in sensory nerve fibres

Afferent impulses from muscles. Stretch responses

Problem: Record the response from the nerve bundle, leading to the m. extensor brevis profundus dig. III. in the frog, to stretching of this muscle.

Principle: In muscle, the muscle spindles and Golgi tendon organs form the proprioceptive sense organs. The muscle spindle consists of a certain

number of intrafusal striated muscular fibres. The intrafusal fibre has two polar parts that have transverse striations and are separated by a noncontractile segment — nuclear bag. The transitional part — myotube — looses its striations towards the centre of the segment. The contractile parts are innervated by small motor fibres.

There are two afferent systems:

1) Primary endings (also annulospiral endings) in the noncontractile segment and
2) secondary endings (also flower-spray) in the myotube.

The tendon organ of Golgi, cooperating closely with the muscle spindle, is situated where the tendons are attached to the muscle.

Golgi's tendon organs are in series with the extrafusal fibres. They register activity both on stretching and contraction.

This anatomical organisation makes it possible to identify fibres and to state whether they belong to the spindles or tendon organs (Matthews 1933).

Such an exact analysis, however, is of course, only possible when working with a muscle unit.

In principle this may be achieved in two ways: isolation of one end organ or recording from single nerve fibre.

The first method may be used with some species of animals. In frogs it is possible to find small muscles with one spindle and one sensory nerve ending. In mammals this is practically impossible. They have many spindles and their muscles contain also many other nerve endings (tendon-organs, small nerve fibres, etc. — Matthews 1933).

The second method is very tedious and a large sample must be used so that the receptors of nerve fibre activity may be identified.

Electrophysiologically this is not difficult since only the standard amplifying apparatus and oscillograph and recording electrodes placed on an exposed segment of the isolated nerve fibre are required. The problem that is difficult and tedious is preparing the fibre.

Here the first method as used by Matthews (1931), has been chosen.

Object: The small muscle on the superior lateral aspect of the middle toe of the frog is used (m. ext. brev. prof. dig. III). This muscle extends the toe and produces adduction. It is supplied by the peroneal nerve. Only the lateral branch is exposed. On the average two out of three such preparations give unit discharges. The other either does not respond, or more than one unit is recorded.

The muscle is then freed from the rest of the body and only the proximal end remains attached to the bone.

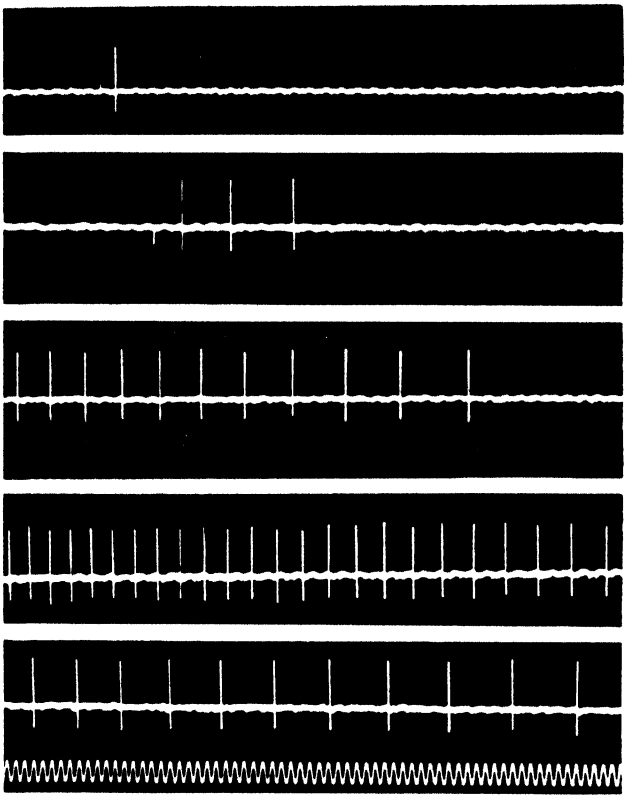


Fig. 136. Discharge in sensory nerve fibre after proprioceptive stimulation. The muscle is loaded with increasing weights at 5 minute intervals. The last recording is continuation of the previous one. M. extensor brevis profundus digiti III. Time mark: 25 c/sec.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier, total sensitivity 100 μV/cm. Time marker. A camera for the oscilloscope with continuous movement of the film. A set of weights.

Other requirements: A binocular dissecting microscope. A preparation dish, dissecting instruments, threads.

Procedure: The preparation is fixed in a moist chamber. The bone to which the muscle is attached is fixed. A loop of thread is tied to the other tendon and is threaded through the bottom of the chamber. A metal hook is fixed to the other end of the thread. Weights will be hung on it (1—10 g). The nerve is placed on platinum recording electrodes in such a way that it hangs freely between its entry into the muscle and the first recording electrode. This prevents movement artifacts during muscular contraction.

The part of the chamber containing the muscle is filled with frog Ringer solution so that the muscle is completely submerged.

Results: A 2 g load is suddenly put on the muscle. A frequent discharge is obtained. This gradually decreases as shown in Fig. 136d, e.

If the experiment is repeated with a smaller load, a slow random discharge continues for several minutes. With heavier loads the discharge stops much sooner. With very large loads it ceases after several seconds; probably the end organs have become permanently injured. After several such large loads, no response is obtained.

If the muscle is not loaded supramaximally the experiment can be repeated very exactly as long as at least 5-minute intervals are observed between stretchings.

The dependence of the frequency of discharges on the size of the stimulus.

The response of a sensory nerve to stimuli of different strengths is recorded. At 5-minute intervals the muscle is loaded with increasing weights. A linear relationship is obtained if the discharge frequency within the first two seconds after application of the stimulus is plotted against the logarithm of the load.

This relationship is a concrete example of Fechner's rule which states that a geometric increase in stimulus strength results in an arithmetic increase in sensation.

Adaptation.

The number of impulses per second not only depends on the stimulus strength but also on the time during which the stimulus acts. At first the discharge frequency is high, then slows until a certain constant frequency is attained. This is called adaptation.

Adaptation is not the same in all sense organs. The muscle spindle belongs to the group of slowly adapting sense organs as do other stretch receptors, i. e. receptors in the lungs, cold receptors, nociceptive receptors in the cornea, pressure receptors of the carotid sinus. Rapidly adapting receptors include cutaneous touch receptors, especially those connected to fibres with much encapsulated Paccini bodies.

Conclusion: The above experiments demonstrate, in addition to the fundamental characteristics of receptors, the fact that the sensory end apparatus complies to the "all or none" rule, as has been known since the first experiments with isolated sensory fibres were performed (Adrian 1932). Stronger stimulation does not produce a larger action potential. Sense organs signalise changes in stimulus strength by changing the frequency of discharges. Weaker stimulation produces a slower discharge of impulses, a stronger one a more frequent discharge.

Stimulus strength is also signalised in another way — by so called recruitment of end organs. This is activation of additional end sensory organs, with production of discharges in further sensory fibres leaving them.

In the receptor chosen for the above experiment the efferent system of so-called small fibres (Kuffler and Gerard 1947, Kuffler and Hunt 1952, Kuffler, Hunt and Quilliam 1951) was discovered, which influences the response of the end organ to a stimulus.

C. Receptor potentials

A receptor potential is a potential change which depends upon the size or the rate of increase of the stimulus and is produced in the receptor. The discharge frequency in sensory axons leaving the receptor is dependent upon the size of the receptor potential.

This potential is thus an intermediary link in the process of transforming external energy into a nervous impulse.

The receptor potential is similar to local processes in nerves or synapses, i. e. to a local or a synaptic potential. It is localised at the point at which it arises, it spreads electrotonically with a decrement for a small distance only, its amplitude is gradual, depending on the stimulus strength. The nervous impulse arises in the sensory axon when the receptor potential attains a certain amplitude. Finally the receptor potential is a depolarising potential.

This potential was found in different receptors, e. g. the muscle spindle (Katz 1950), the eye of *Limulus* and *Dytiscus* (Hartline and Graham 1932, Bernhard 1942, Granit 1947), the cones (Svaetichin 1954), the Paccini body and the lobster stretch receptors (Gray and Sato 1953a, b, Kuffler and Eyzaguire 1955, Alvarez-Buylla and Ramirez de Arleano 1953).

Other local changes in receptors are also known, e. g. the electroretinogram of vertebrates, microphonic potentials, electro-olfactogram. Further research will show whether they also belong into the category of receptor potentials.

The existence of receptor potentials does not exclude the possibility that some end organs may be activated by a chemical mechanism without receptor potentials as an intermediate link.

The experiments described below are intended to show some fundamental characteristics of receptor potentials (muscle spindle). They also give some examples of other local changes in complex receptors, the basis of which is not yet clear (ERG, EOG).

a) The receptor potential of a muscle spindle

Problem: Record the receptor potential from a muscle spindle of the frog for different stimulus strengths.

Principle: When a muscle is stretched mechanical energy is transformed into nervous impulses through local potential changes which can be recorded from the sensory axon at a point near the muscle spindle. This potential change has all the characteristics of a receptor potential: it increases with stimulus strength, it is local and spreads with a decrement. When a certain level is attained, impulses are produced in the sensory nerve.

Using local anaesthetics it is possible to separate these two processes, i. e. local depolarisation and impulse discharge, and thus to study the course of this receptor potential undisturbed by spike activity.

Object: The M. extensor longus digiti IV of the frog with its nerve branch. This muscle is supplied by a branch from the peroneal nerve. The nerve divides into several bundles before entering the muscle. The most proximal part often contains one motor and two sensory axons.

The muscle and the nerve are isolated, one of the proximal axons is selected and the remaining nerve branches are cut.

Apparatus: A double beam cathode-ray oscilloscope with a D. C. amplifier and a total sensitivity of 100 μV/cm. A time marker, a stimulator giving one pulse at the output. A fixing arrangement for the muscle and nerve. Recording electrodes: silver wires connected to the preparation with the aid of capillary tubes filled with Agar-Ringer solution.

Procedure: The isolated axon together with its sheaths is about 30—60 μ in diameter and several mm long (Katz 1950). The resistance of this preparation is very high and may increase the time constant of the input circuit and thus slow down and attenuate the recorded response.

In this preparation terminal structures remain enclosed in the spindle capsule and thus a change occurring in them is only recorded after electrotonic spreading along fine terminal branches, i. e. with a decrement, so that actual values of registered potentials are not obtained.

Every potential change in the muscle lying between the electrodes may be a serious source of artifacts. These errors may be tested by shortening the axon or by squeezing it where it enters the muscle. The electrodes are situated as shown in Fig. 137. The stimulating electrodes are placed on the sartorius muscle which is in series with M. extensor longus dig. IV. This latter is stretched by the contraction of the former. First a so called resting discharge, when the muscle is not stimulated and is under physiological tension, is registered. The stimulus intensity for the sartorius muscle is then gradually increased and the corresponding responses are recorded. The same procedure is repeated using

monophasic recording after the sensory axon has been squeezed below the recording electrode.

The recordings show that during stretching a volley of impulses is produced. The frequency of this discharge is the higher the greater the stimulus strength — the greater stretching. In addition it can be seen that the level shifts upwards during a discharge of impulses. This is the receptor potential. With stronger stimuli the discharge frequency rises as does the level of depolarisation (Fig. 138).

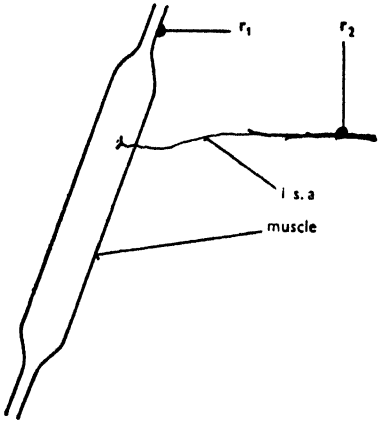


Fig. 137. Arrangement of electrodes for recording receptor potentials from the m. ext. long. dig. IV. i.s.a. — isolated sensory axon, r_1, r_2 — recording electrodes.

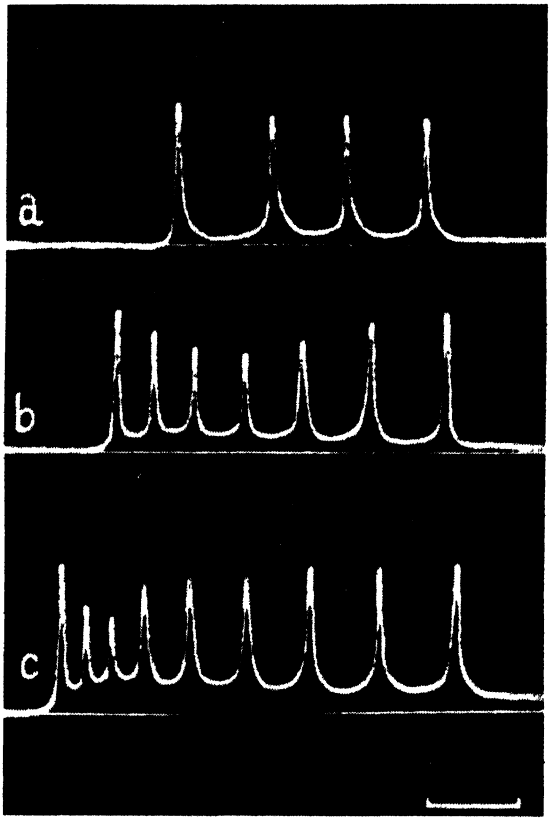


Fig. 138. Receptor potentials and discharge in a sensory axon after stretching m. ext. long. dig. IV of the frog. Stretch was produced by contraction of the sartorius muscle. a–c) discharge at different stretch intensities. Time scale: 20 msec.

The effect of local anaesthetics.

The preparation is then soaked for 10 minutes in a 0·3% buffered procaine-Ringer solution and the experiment is repeated. The impulse discharge from the muscle spindle disappears but the receptor potential remains unchanged.

If now the nerve is crushed just before it enters the muscle, no response

is obtained from the muscle spindle. This indicates that the recorded depolarisation is not an artifact.

Conclusion: The experiment showed that the origin of impulses in the muscle spindle is preceeded and accompanied by a depolarising potential. These spindle potentials may be explained as a local link between the stimulus and the nerve response. This explanation is supported by the fact that there is a correlation between the amplitude of this depolarisation — receptor potential — and the impulse frequency in the sensory nerve (Katz 1950). A similar depolarisation may be found also in other mechanoreceptors: Paccini's bodies (Gray and Sato 1953a, b) or the stretch receptors of the crayfish (Kuffler and Eyzaguirre 1955).

b) The electroretinogram

Problem: Record the electric response of the frog's eye adapted to light and darkness to continuous stimulation.

Principle: The production of impulses in this receptor is accompanied by (a) changes in steady potential, (b) the electroretinogram and (c) a discharge of impulses in the optic nerve.

The potential change arising between the cornea and the back of the eye bulb on illuminating the eye is termed the electroretinogram. Different ways of recording the electrical activity from the eye are shown diagramatically in Fig. 139 (Granit and Therman 1938). Lead I is termed the standard lead in which electroretinograms are usually recorded.

The formation of the electroretinogram (ERG) depends upon the favourable orientation of some retinal structures capable of conducting current in one direction. Such structures are the layers of receptors and bipolar cells. Both these structures are thought to give rise to the electroretinogram.

The shape of the ERG depends upon the kind of optic receptor (a retina containing only rods or cones or a mixed one) and on previous adaptation to light or darkness.

Four components are described in the retinogram. An initial negative *a*-wave, a positive *b*-wave followed by a slow *c*-wave and a positive *d*-wave which appears on cessation of light stimulation (Fig. 141). With the exception of the *c*-wave the rods and cones of the eye show the same components, although their relative size may vary from one animal to another.

Object: Large frogs *(Rana esculenta* or *temporaria)*. Recording the ERG from the frog's eye is of adavantage for two reasons:

a) the retina has about the same number of rods and cones,

b) excised bulbs can be used. These are easy to prepare.

Frogs are adapted by keeping them for 1 or more hours in a completely dark room. Under dark red light the bulb is then excised and placed into an arrangement for recording the ERG.

In curarised frog the eye may be left in situ. The cornea, lens and corpus vitreum are removed and the different electrode is applied directly to the exposed retina.

Adaptation to light is carried out during the experiment by exposure to light for a long period.

Apparatus: A cathode-ray oscilloscope with a D. C. amplifier. An A. C. amplifier is satisfactory if the registration of c-wave is not required. Total sensitivity 100 μV/cm. A photostimulator. A time marker.

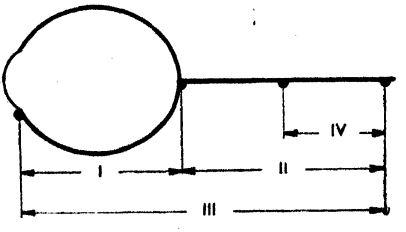


Fig. 139. Four ways of recording electrical activity from the eye (I, II, III, IV). I is the standard lead.

Other requirements: An arrangement for fixing the bulb and applying the recording electrode (Fig. 140). Nonpolarisable wick Ag-AgCl electrodes.

Procedure: The bulb is placed into a short glass tube. The space behind the bulb is filled with cotton wool soaked in frog Ringer solution. The tube is placed into a small dark chamber so that the front surface of the cornea is close to the iris diaphragm. The recording electrodes are placed as shown in

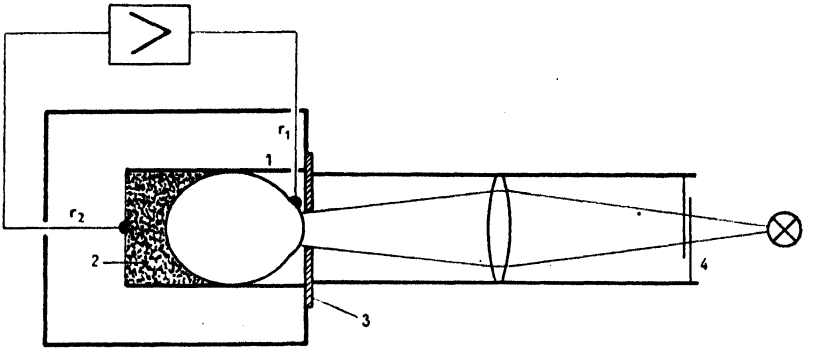


Fig. 140. Arrangement for ERG recording in vitro. 1 — glass tube, 2 — cotton wool soaked in frog Ringer solution, 3 — iris diaphragm, 4 — shutter, r_1, r_2 — recording Ag-AgCl wick electrodes.

fig. 140. One is on the anterior, the other on the posterior border of the bulb where it touches the cotton wool. Care must be taken to prevent light from acting directly on them in order to avoid photochemical electrode artifacts. The recording electrodes are connected to the input of a D. C. amplifier.

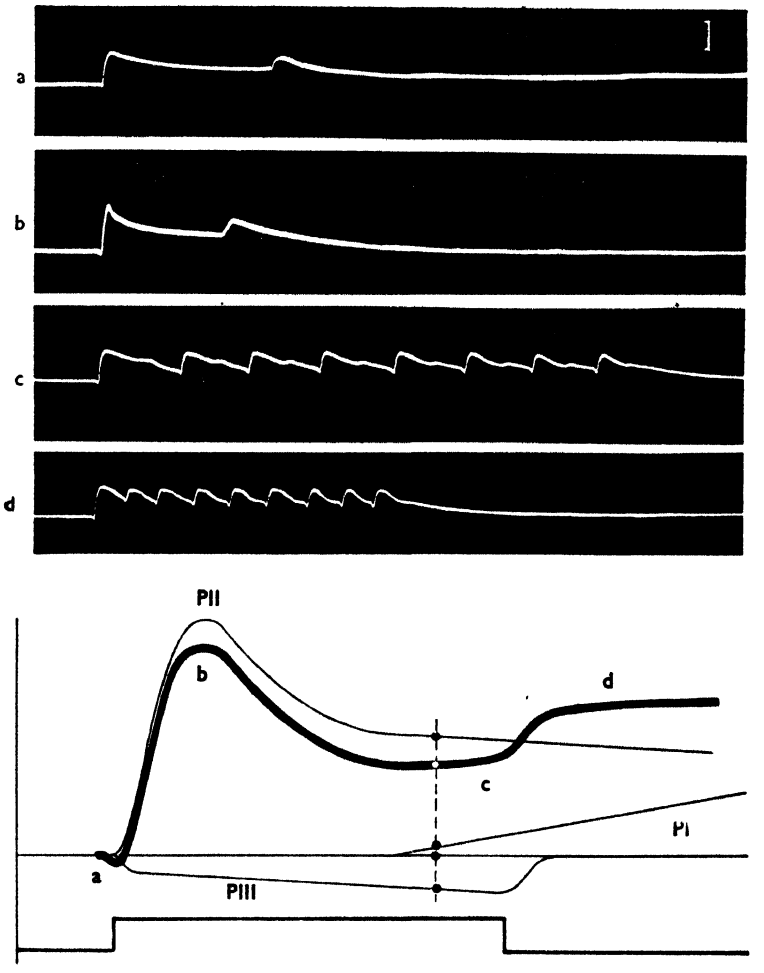


Fig. 141. Electroretinogram of excised frog's eye adapted to the dark (a — b). c) repetitive stimulation at 0,5/sec. and d) 1/sec. Voltage scale: 1 mV. Diagram below: schematic representation of various components of ERG.

The shutter is opened admitting a light beam lasting 2 seconds. The intensity of the beam is gradually increased and the bulb is stimulated at 3 min. intervals. Fig. 141a, b shows the response to such stimulation. The shutter is then left open and light is permitted to fall on the bulb for 10 min. The shutter is again closed for 3 sec. and a beam of the same intenisty is admitted for 2 sec. The response is recorded. The retinal response to dark includes all the components: a small negative *a*-wave, followed by a rapidly increasing *b*-wave, which falls relatively quickly and gradually changes into the *c*-wave (not shown in Fig, 141). After switching off the light a positive off-effect or *d*-wave appears. The response of a retina adapted to light differs from the above by a decreased

b-wave, and a more rapid increase and a higher amplitude of the off-effect which also has a shorter latent period. In addition, the *c*-component is absent in an eye adapted to light.

Conclusion: The ERG is the final result of several processes occurring in the retina. It has been possible to separate it into several components (PI, PII, PIII).

The figure 141 (diagram below) shows that the cornea-negative *a*-wave is due to the early appearance of wave PIII. This is interrupted by the cornea-positive PII component which forms wave *b*. Wave *c* is formed by component PI.

The ERG may be separated into its components by different methods (anaesthesia, asphyxia, adaptation to light, chemical means — Granit 1933, Noell 1951, 1952a, b).

c) The electro-olfactogram

Problem: Record the electro-olfactogram in the frog.

Principle: The electric potential change occurring in the olfactory receptor in response to specific stimulation is termed the electro-olfactogram (Ottoson 1956). Most probably this is an electrophysiological effect of reactions between substances in the olfactory epithelium and particles of the stimulating agent.

The electro-olfactogram is a negative monophasic potential which can be recorded with an electrode placed on the olfactory part of the nasal mucosa.

Object: The olfactory epithelium of the frog is very suitable since it is easily accessible and since recordings from both the olfactory epithelium and the olfactory bulb can be made simultaneously.

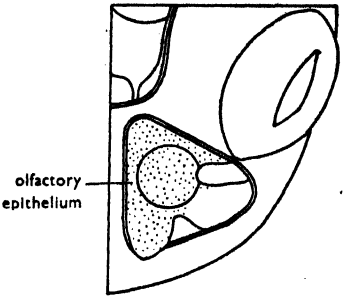


Fig. 142. Diagramatic representation of the anatomy of the frog's olfactory organ.

Fig. 142 is a diagramatic representation of the anatomy of parts of the olfactory organ. In comparison to the same organs in higher vertebrates the nasal cavities of the frog are very simple. The external nasal aperture is connected through the vestibulum to the cavum principale nasi covered with sensory epithelium. The areas containing the sensory epithelium have a slightly yellow tinge and thus differ from the respiratory tract which is pink in colour.

It is not difficult to reach the cavum principale in the frog. The animal

is decapitated and the olfactory epithelium is exposed by removing the dorsal wall of the cavity of the olfactory organ.

Apparatus: A cathode-ray-oscilloscope with a D. C. amplifier, total sensitivity 1 mV/cm. An amplitude marker.

Other requirements: a) Agar Ag-AgCl electrodes. The electrode situated on the sensory epithelium has a tube tip diameter of 0·1—0·2 mm.

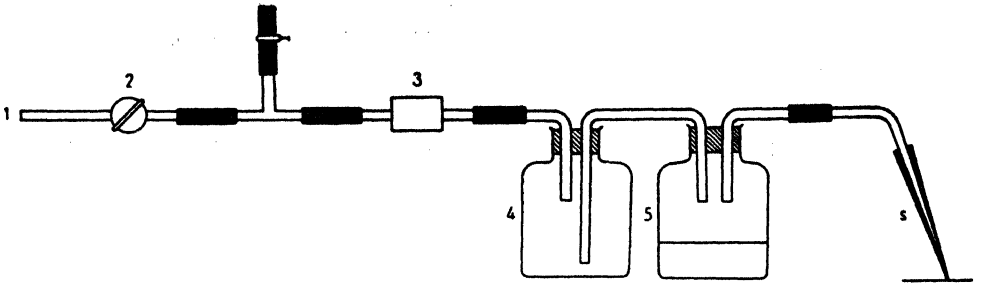


Fig. 143. Arrangement for olfactory stimulation of the olfactory epithelium. 1 — air pump, 2 — cock, 3—4 — filter with active charcoal, 5 — test vessel, s — stimulating pipette.

b) An arrangement for olfactory stimulation as shown in Fig. 143. The principal equipment is an air pump. At every turn the pump expells about 50 ml. of air into the air line. The major part of the air leaves through the cock (2), the rest passes through activated charcoal (3, 4) and enters a 150 ml. test vessel (5) containing 10—15 ml. of an aqueous solution of a volatile substance. The odorised air is blown onto the nasal mucosa through a glass pipette (s) connected to the outflow of the test vessel. The tip of the pipette is at a distance of 5—8 mm from the epithelial surface. The volume of air expelled for each blow depends on the tip diameter of the pipette.

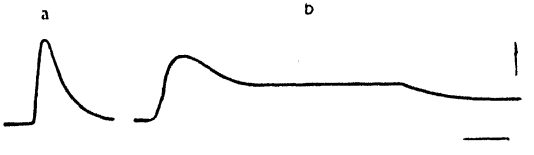


Fig. 144. Electro-olfactogram from the olfactory epithelium of the frog. a) EOG with 0·001M butanol. b) EOG on continuous stimulation (0·005 butanol). Time mark: 3 sec; voltage calibration: 1 mV.

c) n-butanol in concentrations of 0·001M, 0·005M, 0·025M, 0·05M, 0·25M is used as the odorising substance. 15 ml. of each concentration are placed into the 150 ml. vessels.

Procedure: The preparation is placed on cotton wool soaked in frog Ringer solution. One agar Ag-AgCl electrode is applied to the cotton wool, the other to the centre of the eminentia olfactoria. The stimulating pipette is situated 5 mm above the point at which the recording electrode is applied.

After connecting the electrode to the D. C. amplifier and preparing the camera, the odorised air is blown onto the olfactory epithelium. The electric response to this is shown in fig. 144a. If the electrode and pipette are moved by 1—2 mm to different points of the mucosal surface, the spatial amplitude distribution of the EOG can be obtained.

If a 0.5% cocaine solution is dropped onto the mucosa the amplitude is partly decreased but does not disappear.

This insensitivity to cocaine also applies to receptor potentials of other organs (Katz 1950, Gray and Sato 1953a, b, Kuffler and Eyzaguirre 1955 etc.). This supports the assumption that the EOG originates in olfactory sensory cells.

Distilled water, ether and chloroform, on the other hand, rapidly eliminate the EOG.

The stimulating arrangement is changed for continuous stimulation. The air current has a speed of 1 ml./sec. and lasts for 15 sec. The recordings in Fig. 144b show that the potential in response to continuous stimulation rapidly attains a maximum, returns to a certain level and remains at the plateau. This decrease to a certain plateau indicates adaptation of the olfactory receptors.

Olfactory receptors may consequently be termed slowly adapting end organs.

Conclusion: The literature concerning the EOG is new and sparse. The nature of EOG has by far not yet been elucidated.

VII

The electrophysiology of the spinal cord

The basic functional unit of the central nervous system is the reflex. The fundamental knowledge concerning reflex activity of the spinal cord was obtained by classical methods of stimulating the afferent part of a reflex arc and recording the reaction of the effector, usually the muscle. A classical summary of this work is given by Sherrington in his "Integrative Action of the Nervous System" (1906) and later by the Oxford school (Creed, Denny-Brown, Eccles, Liddel and Sherrington "Reflex Activity of the Spinal Cord", 1932).

With classical methods it was only possible to draw indirect conclusions concerning processes in the cord itself (the central component of spinal reflexes). Refinement of electrophysiological methods has made it possible to study this component directly.

Two periods are evident in the development of electrophysiological research into spinal activity. During the first period previous myographic analysis of reflex activity was defined more exactly using action potential recordings from efferent and afferent pathways of the reflex arc. Transmission of nervous impulses from afferent to efferent neurones was studied by electrotonic recording of synaptic potentials generated within the spinal cord from the ventral and dorsal spinal roots.

The second period (since 1952) was made possible by the development of a new electrophysiological technique (Brock et al. 1952). Introduction of intracellular microelectrodes enabled direct observation of those processes occurring during transmission of impulses from afferent fibre terminals to motoneurones. The most significant contribution of this period is the discovery of hyperpolarisation of the subsynaptic membrane during direct spinal inhibition.

In experiments described below a survey of the electrophysiology of the spinal cord during the first period is given.

a) Electrophysiological manifestations of a monosynaptic and polysynaptic reflex arc

Problem: Record the action potential from the ventral roots of the cat in response to afferent stimulation of a cutaneous nerve and a nerve from a muscle.

Principle: Cajal (1909) simplified the anatomical complexities of the reflex spinal system to two fundamental types as shown in Fig. 145. Fig. 145a illustrates the circumscribed reflex mechanisms. The afferent fibre terminates directly on the motoneurone in a certain narrow segment. This reflex arc is termed monosynaptic. Fig. 145b shows a disynaptic reflex as the most simple example of a polysynaptic reflex. An interneurone is inserted between the afferent terminals and the motoneurone. This causes diffusion of impulse activity from the afferent neurone to a large number of efferent fibres along the spinal cord.

The functional picture of Cajal's diagram may be obtained by stimulating the posterior roots with an electric pulse and recording from the ventral root of the same segment. Thus a recording of a segmental reflex discharge is obtained.

All afferent influxes, however, reach the cord via the dorsal root. A rough separation of this influx may be obtained if instead of the dorsal root the muscle and cutaneous nerve are stimulated separately (Lloyd, 1943).

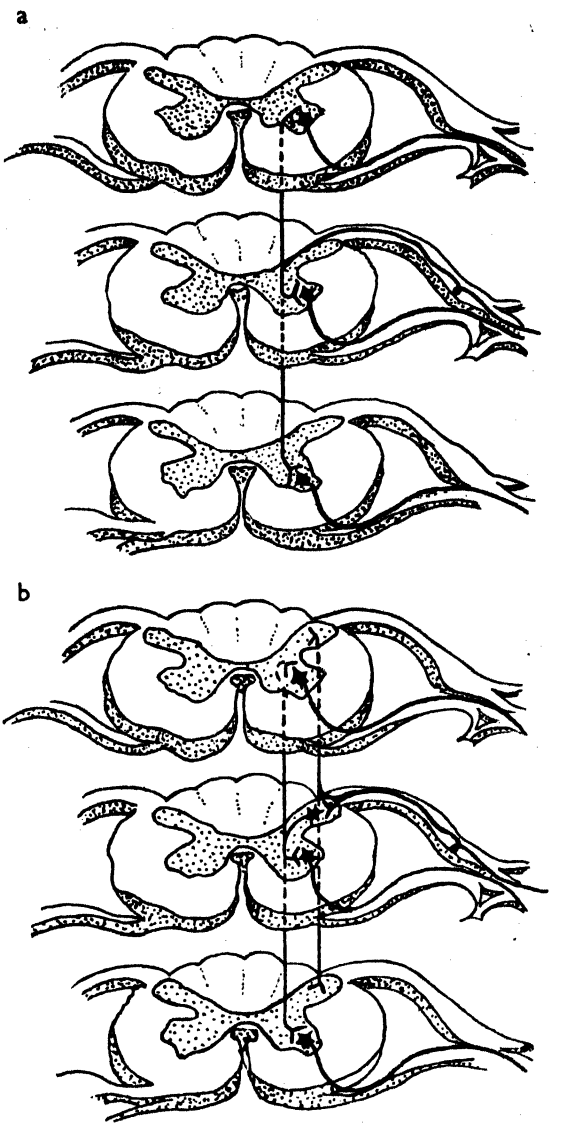


Fig. 145. Diagramatic representation of the basic types of connections in the spinal cord, according to Cajal. a) circumscribed reflex mechanism. The afferent neurone terminates directly on the motoneurone. b) diffuse reflex mechanism. One interneurone is inserted between the afferent neurone and the motorneurone.

Object: The decerebrate cat with the lumbar cord exposed and the ventral roots cut peripherally (7th or 8th post-thoracic).

Preparation: The cat is decerebrated during brief ether anaesthesia. A trephine opening is made above one hemisphere and is then rapidly widened with rongeurs, until the whole dorsal surface of the hemispheres is exposed. Bleeding from the bones is stopped with bone wax or plasticine. The brain is lifted with a spatula introduced along the tentorium and rapidly removed in parts. A small amount of brain tissue is left in place at the base to prevent injury to the circulus Willisi. The cavity thus formed is filled with cotton wool soaked in warm Ringer solution. Then both carotid arteries, which were compressed during decerebration, are released. The animal breathes spontaneously and no respiratory pump is necessary.

The skin is incised in the lumbar region, the fasciae are loosened and the paravertebral musculature pulled aside so that a small enclosure is formed over the cord. The spines of the vertebrae are removed with plyers and then the dorsal and lateral parts of the vertebrae forming the osteous covering of the spinal cord are removed. The dura mater is then opened by cutting it longitudinally. Its edges are fixed with silk threads to the edges of the laterally displaced paravertebral muscles.

The cord is divided between the 2nd and 3rd lumbal level. All ventral and dorsal roots are cut on one side. Recordings will be made from the other. This permits turning of the spinal cord and thus makes it possible to identify the roots. Now the ventral roots (7th and 8th posthoracic) of the ipsilateral side are found and are carefully cut peripherally so that at least 20 mm. of them are preserved. The remaining ventral roots are cut nearer the cord.

Then the nerve leading to the gastrocnemius muscle and the sural nerve are exposed.

Apparatus: A cathode-ray oscilloscope with an A. C. preamplifier (100 μV/cm). A simple stimulator giving rectangular pulses with variable amplitude.

Other requirements: Platinum electrodes: 2 pairs of stimulating and one pair of recording electrodes.

Procedure:

1) Segmental reflex discharge.

The stimulating electrodes are placed on the dorsal root S_1. The recording electrodes are on the ventral root of the same segment at a sufficient distance from the cord. Recordings are monophasic so that the more distal recording electrode is on the cut end of the ventral root. The spinal cord and the region of stimulation are covered with paraffin oil, filling the trough formed by the retracted muscle and skin edges. The preparation is allowed to recover from the effects of anaesthesia so that reflex discharges may be recorded.

Fig. 146 a) shows a response obtained in these conditions. The tracing is composed of a sharp spike at the beginning and of diffuse activity with lower and different conduction velocities.

2) Response of the ventral roots to stimulation of the muscular and cutaneous nerves.

The stimulating electrodes are placed on the sural nerve [S_1] and on the nerve leading to the gastrocnemius muscle [S_2]. The former is a cutaneous nerve, the latter contains fibres with a maximal diameter for mixed nerves.

A response as shown in Fig. 146b is obtained on stimulating the nerve leading to the muscle, and that shown in Fig. 146c on stimulating the cutaneous nerve.

Conclusion: The response to stimulation of the nerve from the muscle is a typical monosynaptic response. It can be calculated that the interval between the stimulus artifact and the onset of the action potential includes, in addition to the time necessary for conduction in the nerve fibres, only a single synaptic delay.

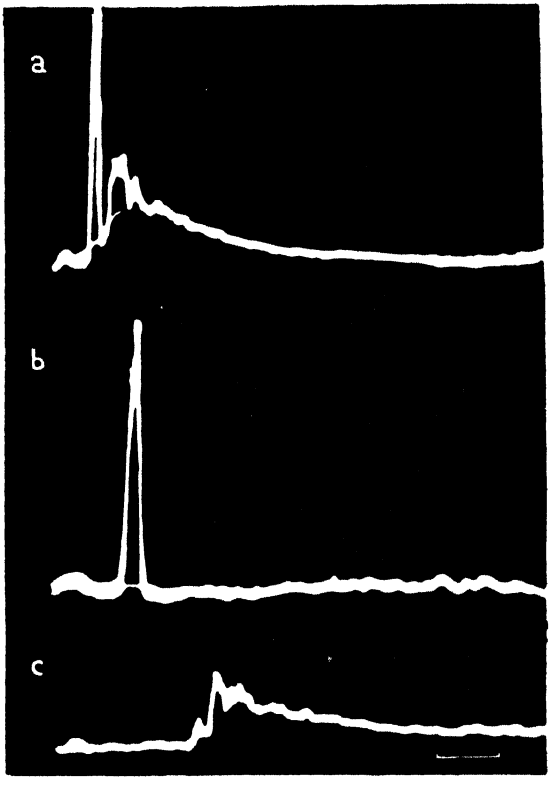


Fig. 146. Action potentials from the ventral root S_1. a) produced by stimulating the dorsal root of the same segment. b) action potentials recorded from the ventral root evoked by stimulating the nerve leading to one head of the gastrocnemius muscle. c) in response to stimulation of the cutaneous nerve. Time mark: 4 msec.

Transmission through several synapses must be expected in the response to stimulation of the cutaneous nerve. This is also indicated by its irregulatity lasting for more than 10 msec.

b) Facilitation and inhibition in the motoneurone

Problem: Demonstrate facilitation and inhibition of response in a monosynaptic path of the spinal cord.

Principle: Facilitation in a monosynaptic reflex arc (Lloyd 1946) is due to convergence of presynaptic neurones onto the body of the motoneurone.

Two afferent nerves from two synergistic muscles are to be stimulated at different stimulus intervals. The amplitude of the conditioning volley is maximal; that of the testing one submaximal. The increase in the amplitude of the latter is the expression of the magnitude of facilitation.

Direct inhibition (Lloyd, 1941) exists because of the presence of direct inhibitory synapses on the body of the motoneurone. Acording to contemporary opinion, direct inhibition occurs in a disynaptic reflex arc. An inhibitory neurone is inserted between the afferent and efferent neurone (Eccles 1957).

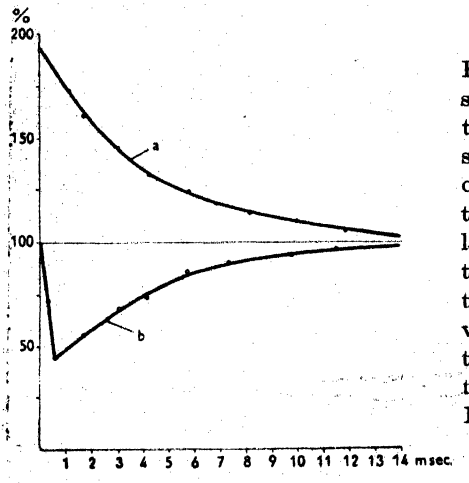


Fig. 147. Facilitation and inhibition in a monosynaptic reflex arc. a) facilitation curve. Abscissa: time interval between conditioning stimulus and submaximal test stimulus. Nerves to both heads of the gastrocnemius muscle. Ordinate: Percentage increase in test stimulus. Response to isolated test stimulus was taken as 100%. b) inhibition curve in a monosynaptic reflex arc. Abscissa: time interval between maximal conditioning volley to nerve from gastrocnemius muscle and testing volley applied to the nerve leading from the antagonist (anterior tibial muscle). Ordinate: Percentage decrease in amplitude of the response to the test stimulus.

The existence of direct inhibition can be demonstrated in the same way as facilitation, but by stimulating afferent nerves of two antagonistic muscles (e. g. the anterior tibial and gastrocnemius muscles).

Object: The spinal cat prepared as in the preceding experiment. The nervous branches leading to both heads of the gastrocnemius muscle and to the anterior tibial muscles are exposed. Recordings are made from the ventral roots L_7 or S_1.

Apparatus: As in the preceding experiment. A stimulator with two independent outputs is, however, needed in which the time interval between the two stimuli can be changed continuously.

Procedure:

1) Demonstration of facilitation.

The recording electrodes are placed on L_7 or S_1. One pair of stimulating electrodes is situated on one nerve leading to the gastrocnemius muscle, the other pair on the other nerve leading to the same muscle. The first stimulus is of maximal strength, the second submaximal. First a time interval of about 20 msec. is used. Then it is gradually shortened. It can be seen that the amplitude begins to increase starting from a certain time interval until a maximum value is obtained when both impulses coincide.

2) Demonstration of direct inhibition.

The recording electrodes remain in the same position. One pair of electrodes is left on the nerve leading to the gastrocnemius muscle, the other pair lies on the nerve to the anterior tibial muscle. A maximal conditioning stimulus is applied to one of these muscles. The test stimulus is applied to the antagonist. From a certain limit, the amplitude of the response to the test stimulus decreases with the time interval between the stimuli.

Conclusion: Curves as shown in Fig. 147 are obtained if the increase (facilitation) —a— or decrease (inhibition) —b— of the amplitude of the test stimuli is plotted against time. Inhibition, save for an initial increment phase, takes the same course.

A similar time course and configuration is seen for postsynaptic excitatory and inhibitory potentials, which may be recorded intracellularly from motoneurone cells.

c) The relation between afferent influx and efferent efflux in the spinal monosynaptic arc

Problem: Demonstrate the extent of the subliminal fringe and of the discharge zone in the motoneurone pool of the gastrocnemius muscle.

Principle: The existence of facilitation in the motoneurone indicates that a subliminal fringe is present in the motoneurone pool (Lloyd, 1943). This means that in addition to motoneurones discharged by a presynaptic volley, a number of motoneurones are activated only subliminally. If the extent of this area is to be estimated, first the discharge zone, i. e. the number of activated motoneurones must be known. This may be estimated from a correlation between the amplitude of the presynaptic spike and that of the postsynaptic one. The curve obtained in this way represents the limit between the discharge zone and the sublimal fringe. The other limit of the subliminal fringe is given by the facilitation curve (see the preceding experiment).

In principle this experiment requires recording from the ventral and dorsal roots and stimulation of two afferent nerves from two synergistic muscles. The conditioning volley applied to one of these nerves is maximal. The strength of the testing one is an independant variable.

Object: The spinal cat with the ventral and dorsal roots of L_7 and S_1 exposed together with the nerves leading to the gastrocnemius muscle.

Apparatus: A double beam cathode-ray oscilloscope with A. C. amplifier, total sensitivity 100 μV/cm. A stimulator with two independent outputs.

Other requirements: As in the preceding experiment.

Procedure: The stimulating electrodes are placed on the nerve leading to one head of the gastrocnemius muscle. One pair of recording electrodes is situated on the dorsal and one pair on the ventral root.

The stimulus strength is gradually increased and the response from both recording electrodes is traced. The first postsynaptic discharge appears only when the presynaptic spike attains a certain value. On further increasing the stimulus strength, the postsynaptic response increases rapidly with increase in the presynaptic spike until the latter spike reaches 50% of its maximum value. The amplitude of the postsynaptic spike increases only slightly with further increases in the presynaptic response.

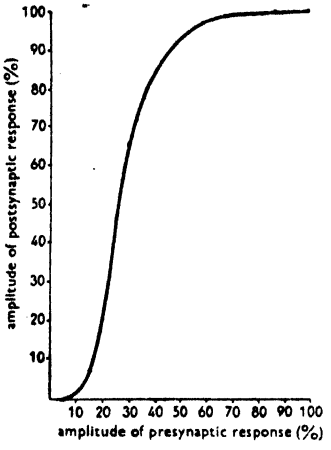


Fig. 148. Relationship between the size of the presynaptic influx and the postsynaptic efflux. Abscissa: amplitude of the presynaptic spike in percent. Response to maximal stimulation of afferent nerve from gastrocnemius muscle taken as 100%. Ordinate: amplitude of postsynaptic response in percent. Maximal amplitude to orthodromic stimulation taken as 100%.

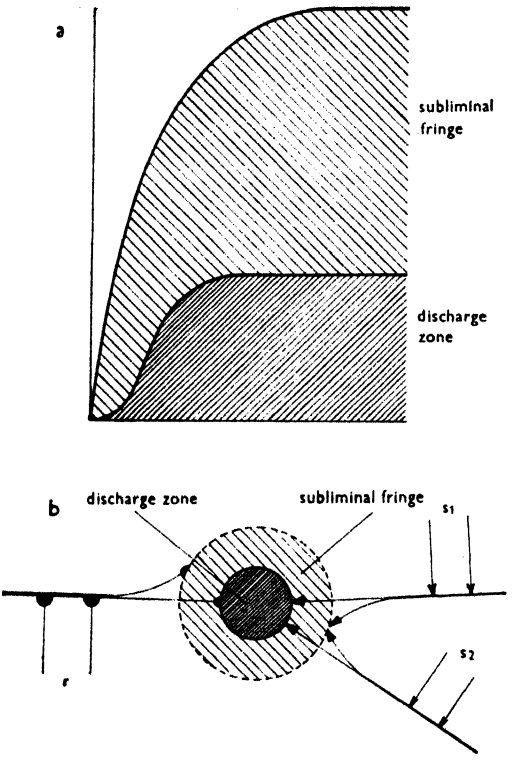


Fig. 149. a) Relationship between subliminal fringe and discharge zone. b) diagramatic representation of determination of the discharge zone and of the subliminal fringe (for detail see text).

The experiment is then repeated, but in addition a constant submaximal stimulus is applied to the afferent nerve from the second head of the gastrocnemius muscle. Both nerves are stimulated simultaneously and again responses from ventral and dorsal roots are recorded. This procedure is repeated several times using different stimulus strengths on electrodes S_1 with the stimulus intensity on S_2 remaining unchanged.

Both experiments are expressed in graphic form. If the size of the presynaptic influx is expressed as the percentage of the maximal amplitude

in response to stimulus S_1 on the abscissa, and the size of the postsynaptic response plotted on the ordinate (also as the percentage of the maximum postsynaptic response), a curve as shown in Fig. 148 is obtained. This shows the development of the discharge zone.

In the second experiment the size of the spike-amplitude recorded from the ventral root in response to separate stimulation of the two nerves is subtracted from the amplitude obtained on simultaneous stimulation, and thus the value for the subliminal fringe is obtained for each value of the presynaptic pulse at S_1. The curve obtained shows the limit of the subliminal fringe.

Finally both curves are plotted into the same coordinates.

Conclusion: The diagram in Fig. 149 shows that the subliminal fringe is large in comparison to the discharge zone of the monosynaptic reflex arc studied. This diagram, of course, shows synchronous afferent excitation and the two-neurone-arc discharges. As excitation is realised in the motoneurone pool as the result of a diffuse influx of impulses, the changes shown in the figure as a function of the intensity will occur with time as an additional parameter. At first they will move to the right along the abscissa as the impulse activity increases, and then to the left as this influx decreases.

d) The recovery cycle in the motoneurone

Problem: Determine changes in excitability of the motoneurone following transmission of the nerve impulse.

Principle: Since the motoneurone pool has a large subliminal fringe, it is not possible to determine the course in excitability changes following a maximal orthodromic volley. Consequently, antidromic excitation is used to determine such changes. This causes excitation simultaneously in all motor cells, that are to be tested orthodromically.

In principle, this is application of a maximal antidromic stimulus to a ventral root (the conditioning stimulus) and a submaximal orthodromic stimulus to the corresponding dorsal spinal root — the test stimulus (Lloyd, 1951).

Apparatus: A cathode-ray oscilloscope with an A. C. amplifier, total sensitivity 100 μV/cm. A stimulator with two outputs and a variable interval between the stimulating pulses.

Other requirements: As in the preceding experiments.

Procedure: The arrangement of the experiment is shown in Fig. 150b. The stimulating electrodes S_2 for antidromic stimulation are on the ventral root, and the stimulating electrodes S_1 for orthodromic stimulation are on the dorsal root S_2. The recording electrodes are situated on the ventral root.

First, the maximum antidromic stimulus is determined from the height of the action potential amplitude in the ventral roots by stimulating at S_2. The maximal spike value for orthodromic stimulation is then determined.

The interval between the conditioning and test stimulus is then changed and the size of the action potential in the ventral roots is observed.

If the amplitude of the action potentials as the percentage of that of the maximal orthodromic spike is plotted on the ordinate and the interval between the stimuli on the abscissa, changes in excitability in the motoneurone pool are obtained (Fig. 150a).

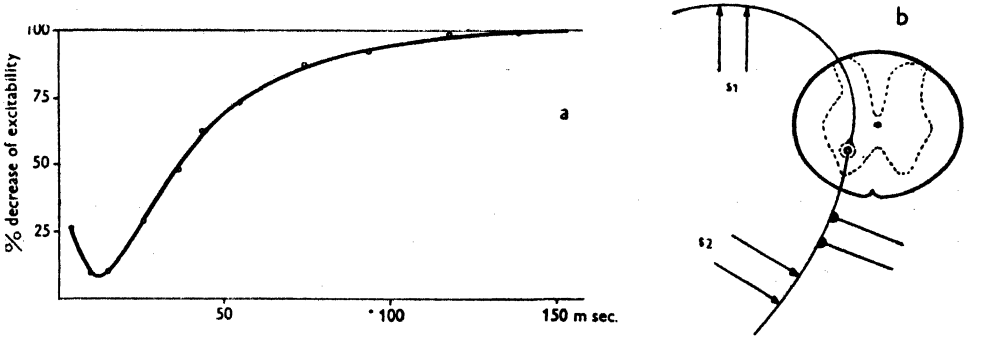


Fig. 150. a) Changes in excitability in the motoneurone pool following maximal antidromic activation. Abscissa: time interval between conditioning and test stimuli. Ordinate: percentage decrease of the spike amplitude due to orthodromic stimulation. b) Arrangement of electrodes for determining the recovery cycle in the motoneurone. S_1, S_2 — stimulating electrodes, R — recording electrodes.

Conclusion: Fig. 150a shows that the motoneurone remains subnormal for 120 msec. after the first response. This subnormality is one factor determining the discharge frequency during reflex or voluntary contractions (Adrian and Bronk, 1929).

e) *Synaptic potentials in a monosynaptic reflex arc*

Problem: Record synaptic potentials from the ventral spinal roots in the cat.

Principle: As in peripheral synaptic junctions, transmission in a monosynaptic reflex arc is characterised electrophysiologically by a synaptic potential, in this case referred to as excitatory postsynaptic potential. This is more difficult to record than at the periphery, since the synaptic region is situated in the spinal grey matter and, therefore, can be approached experimentally only with difficulty. Introduction of intracellular micro-electrodes has made direct tracing of postsynaptic potentials possible.

Before this method was introduced, these potentials were recorded with an electrode placed directly at the point where the ventral root leaves the cord. Electrotonic spreading of these potentials was utilised. They spread from the synaptic area along the ventral roots. Since this synaptic region is close to the point where the ventral roots leave the cord, the potentials can still be recorded, although with a decrement (Eccles 1946, Eccles and Malcolm 1946).

In order to record a pure e. p. s. p. the occurrence of spike discharges must be avoided. Many more ways may be used to achieve this than in peripheral synapses. The following means are suitable:

1) block using curare,

2) block with deep barbiturate anaesthesia,

3) a very small presynaptic influx. This utilises the large subliminal fringe of the motoneurone pool.

4) inhibition produced by stimulating antagonist nerves.

Interneurone discharges may interfere when recording e. p. s. p. from the spinal cord. These may be prevented by using a monosynaptic discharge — stimulating afferent fibres from muscular nerves.

Object: A decerebrate cat, spinal preparation. The ventral roots L_7 and S_1 and nerves to both heads of the gastrocnemius muscle.

Apparatus: A cathode-ray oscilloscope with a D. C. amplifier or an A. C. amplifier with a long time constant. A stimulator with the following possibilities: 1 pulse, 2 pulses with variable intervals, 2 independent outputs with variable intervals between pulses.

Other requirements: d-tubocurarine, nembutal.

Procedure:

1) Synaptic potential.

The stimulating electrodes are placed on one branch of the nerve leading to the gastrocnemius muscle. The recording electrodes are on the ventral root.

The nerve is stimulated and the response from the ventral roots is recorded (Fig. 151a). Under light anaesthesia a monosynaptic reflex discharge is obtained. Then 70 mg./kg. of nembutal are given intravenously. From time to time the nerve is stimulated and changes in the recordings are observed. The spike gradually disappears and in its stead a long negative potential finally appears (Fig. 151b). When 90 mg./kg. are given the potential decreases and the descending phase of the potential is steeper. This is a typical synaptic potential. It arises about 0·7 msec. after the beginning of the small spike, which is recorded by the electrode by direct electric spread from the dorsal root action potential.

2) Spatial decrement of synaptic potentials.

If the recording electrodes are moved further away from the spinal cord, and if the synaptic potentials are recorded at different distances from

it using a fast sweep, it is found that the rate of rise in the synaptic potential decreases together with its amplitude. If the amplitudes of the synaptic potentials are plotted in a logarithmic scale on the ordinate against the distance in mm from the cord, an approximately straight line is obtained, indicating an exponential relationship for the spatial decrement (Fig. 152).

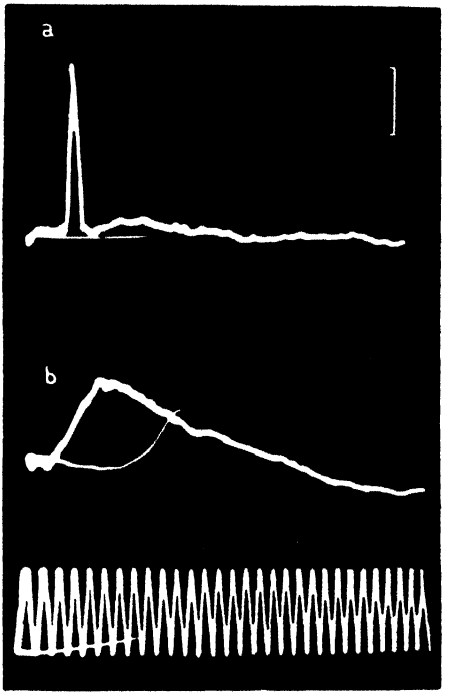


Fig. 151. a) action potential from the ventral roots evoked by stimulating of the afferent nerve from the gastrocnemius muscle. b) postsynaptic potential. Time mark: 1000 c/sec. Voltage calibratoin: 1 mV for a), 0·1 mV for b).

3) Summation of synaptic potentials.

If two successive stimuli are applied to the afferent nerve, summation of synaptic potentials may be obtained.

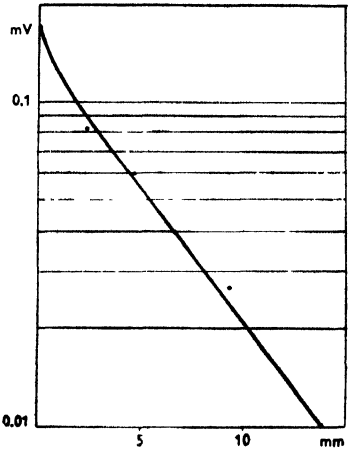


Fig. 152. Logarithm of the amplitude (ordinate) of the synaptic potential plotted against the distance (abscissa) from the spinal cord.

It can be seen from the recordings (Fig. 153a—c), that the second potential is the same or slightly smaller than the first one. The sum of the synaptic potentials exceeds the height of the single potential if the interval is shorter than 16 msec. The absence of the second synaptic potential with intervals shorter than 1—2 msec. is due to the fact that the second stimulus falls into the refractory period of the afferent nerve.

If the afferent nerve is stimulated with a volley of several tens of impulses with different frequencies recordings as shown in Fig. 153d—f are obtained. The after-positivity observed for one synaptic potential prevents the formation

of a plateau of synaptic potentials. There is summation of the first few responses, and then this after-positivity causes a rapid fall to a low plateau.

On cessation of stimulation, the synaptic potential decreases with a slope very similar to that of a single response.

Conclusion: Synaptic potentials are produced when a synaptic area is bombarded with afferent impulses. They reach the ventral root electrotonically. The fibres of these roots lead very selectively since, if synaptic transmission is blocked, the adjacent roots have much smaller potentials than the ventral roots corresponding to those dorsal ones, and the contralateral roots show no potentials at all. It may, therefore, be concluded that the central site of catelectrotonus is in the motoneurone bodies belonging to the corresponding ventral roots.

These potentials have characteristics similar to peripheral synaptic potentials; they can be summated, spread with a decrement and their time course is fundamentally the same. They represent the average response of the whole motoneurone pool.

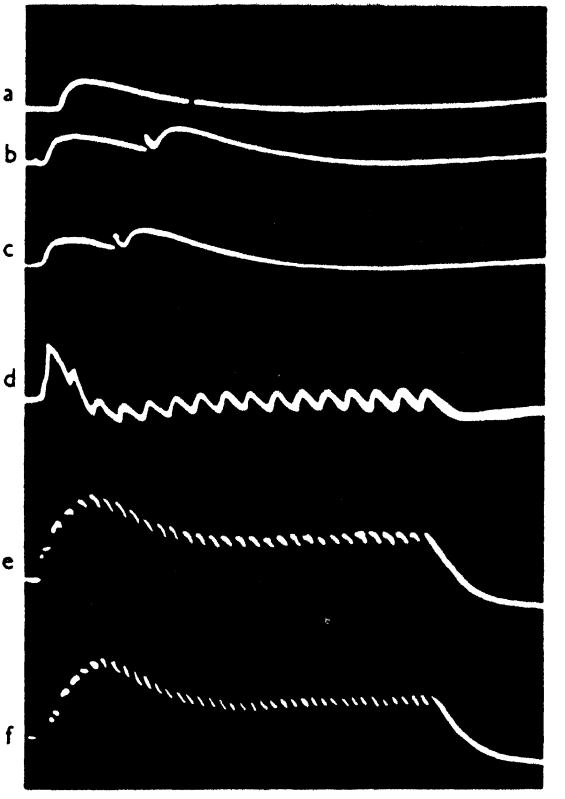


Fig. 153. Summation of synaptic potentials in response to two stimuli (b, c) and a train of stimuli (d, e, f) (after Eccles 1946).

f) Post-tetanic potentiation

Problem: Demonstrate potentiation in the monosynaptic reflex arc after tetanic stimulation.

Principle: In addition to changes in excitability lasting for several miliseconds, changes lasting for minutes may also be observed in synaptic structures. Such a change is the phenomenon of post-tetanic potentiation (Lloyd 1949). This is an increased postsynaptic response lasting for several minutes following intense repetitive synaptic stimulation.

Post-tetanic potentiation occurs in peripheral synaptic junctions, but has been most often studied in the monosynaptic reflex arc. (Lloyd 1949, Eccles and Rall 1951).

In principle, changes in amplitude height of the action potential from ventral roots, evoked by orthodromic activation of the monosynaptic reflex arc lasting several seconds are studied.

Object: The spinal cat with exposed ventral root L_7 or S_1 and the nerve leading to the gastrocnemius muscle.

Apparatus: A cathode-ray oscilloscope with an A. C. amplifier, overall sensitivity 100 μV/cm. A stimulator giving pulses synchronised with the time base at 3 sec. intervals and a generator of rectangular pulses with a variable frequency from 1—500 c/sec. Two pairs of stimulating and one pair recording electrodes.

Other requirements: as in experiment VII a).

Procedure: The recording electrodes are placed on the ventral root further away from the spinal cord, in order to avoid electrotonic spreading of postsynaptic potentials. Both pairs of stimulating electrodes are placed on the nerve to the gastrocnemius muscle with the electrodes for tetanic stimulation situated more peripherally. First the action potential maximal for *Ia* fibres from the ventral root is recorded. Then the stimulator for repetitive stimulation is switched on with a frequency of 500 c/sec. On cessation of the tetanic stimulation, the action potential from the ventral roots evoked by single pulses is recorded at regular 3 sec. intervals for 6 minutes. Figure 154 shows that the amplitude increases rapidly to a maximum. It remains thus for some time and then decreases gradually and very slowly.

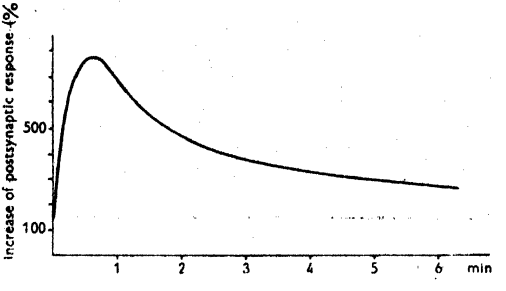


Fig. 154. Post-tetanic potentiation. Abscissa: time in minutes after tetanic stimulation of the afferent nerve from the muscle. Duration of tetanic stimulation 50 sec. Ordinate: increase in postsynaptic response as compared with normal values.

The experiment may be repeated several times in succession and it may thus be determined how different durations or frequencies of tetanic stimulation affect post-tetanic potentiation.

It can be shown that the longer the duration of stimulation the longer post-tetanic potentiation. During potentiation two phases may be observed. The first is shorter, the second prolonged.

Conclusion: It can been demonstrated that postsynaptic elements do not change during the period of potentiation, and that changes which occur are present only in those fibres stimulated tetanically. It is generally accepted

that post-tetanic potentiation is related to some change in specific presynaptic fibres. The basis of this change has not been fully elucidated. Several hypotheses have been put forward. It has been attributed to the effect of post-tetanic positive after-potentials, morphological changes in synaptic knobs and to release of larger amounts of mediator.

Potentiation is related in some way to restoration of synaptic activity which has become ineffective because of prolonged disuse (Eccles and McIntyre 1951).

Post-tetanic potentiation and restoration of action impaired by disuse, by repetitive stimulation are examples of plastic changes which occur in the central nervous system.

VIII

Electrophysiology of the Cerebral Cortex

In higher mammals the cerebral cortex is by far the largest and most accessible part of the central nervous system. The most important analytical and synthetic processes, forming the basis of the higher nervous activity (Pavlov 1926), occur here. It is consequently not surprising that in electrophysiological research on the brain, electrical characteristics of the cerebral cortex play an important part. The majority of electrophysiological experiments on the cerebral cortex can be realised using relatively simple means, and this organ will, therefore, be used as a suitable object for many experiments in this chapter.

A. Electroencephalography and Electrocorticography in General

Registration of potentials arising in the brain and especially in the cerebral cortex, using electrodes placed on the cortical surface or introduced into the cortex, is called electrocorticography — ECoG. Recording of potentials from the surface of the head through the skin and bone or by means of subcutaneously situated needles is called electroencephalography — EEG. In man and higher mammals potentials recorded in the latter way are also due to activity of the cerebral cortex, their amplitude, however, is considerably decreased. This is not due to the series resistance of dura mater, bone and skin, which need not be taken into account because of the high input impedance of the EEG amplifiers, but is mainly caused by parallel tissue resistance, especially of the galea, short circuiting the electrodes and distorting the distribution of equipotential lines. The EEG has usually an amplitude only 1/10 of that of the ECoG (Tönnies 1933). According to Umbach and Bauer (1955) the amplitudes registered from the skin surface, bone, dura mater and intracortically are proportionally 1 : 2·5 : 4 : 7. According to Beritov et al. (1942) skull bones reduce the ECoG amplitude 2—3 times. In electroencephalography

there is an increased possibility of interference by electrical potentials from lower parts of the brain, especially when using occipital and temporal leads. This is best demonstrated in experiments after hemispherectomy, when it is possible to record considerable EEG activity from the surface of the head above the extirpated hemisphere (Cobb, Sears 1956).

In both acute and chronic animal experiments electrocorticography is preferable, because, among other things, muscle potentials of the large subcutaneous muscles of the scalp or adjacent temporal muscles (cat, dog) are easily eliminated.

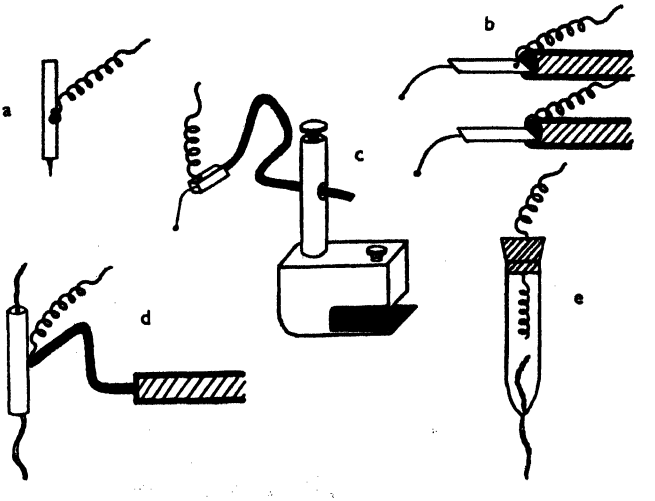


Fig. 155. Electrodes used for recording ECoG in an acute experiment. a) steel needle electrode for fixing into the skull bone. b) silver wire mounted on bronze spring fixed to an insulating holder. c) silver wire on flexible lead bar with holder permitting direct fixation to bone. d) wick Ag-AgCl tube electrode on a flexible lead rod with an insulated holder. e) wick Ag-AgCl electrode with saline in the electrode vessel.

The ECoG can be picked up either from exposed parts of the brain after extensive craniotomy or using needle electrodes introduced through the bone to the surface of the dura mater (epidural registration) or the cortex. Other methods — e. g. recording from the cortical surface exposed by only small trephine holes can also be used. Suitable recording electrodes are used:

a) When recording from the exposed cortical surface we usually use electrodes with wick contacts (non-polarisable Ag-AgCl or Hg-Hg_2Cl_2 electrodes — Fig. 55b, c, e and 155d, e) or ball tipped silver or platinum wires — Fig. 155b, c — fixed to steel springs. The contact must be as reliable as possible but must not cause mechanical stimulation or injury of the brain. In larger animals it is of advantage to fix the electrode holder directly to the bones of the skull with a screw or by sealing wax or phosphate cement.

b) In corticography in the closed skull, needle electrodes fixed directly into bone are advantageous. Stainless steel needles have a stop, limiting the insertion of the tip to the thickness of the skull bones of the species used. The needle held with a special holder is hammered or screwed into place

(Fig. 156). If more secure fixation is required silver or steel screws (Fig. 157a) or plexiglass screws, with one or more silver, platinum or tungsten electrodes (Fig. 157b), are used. The screws are fixed into prepared trephine holes that have a diameter a little smaller than that of the electrodes. In soft bone the electrode will thread its own way. In hard bone it is better to use a tap beforehand. If the bone surface is sufficiently dry neither needles nor screws need be insulated.

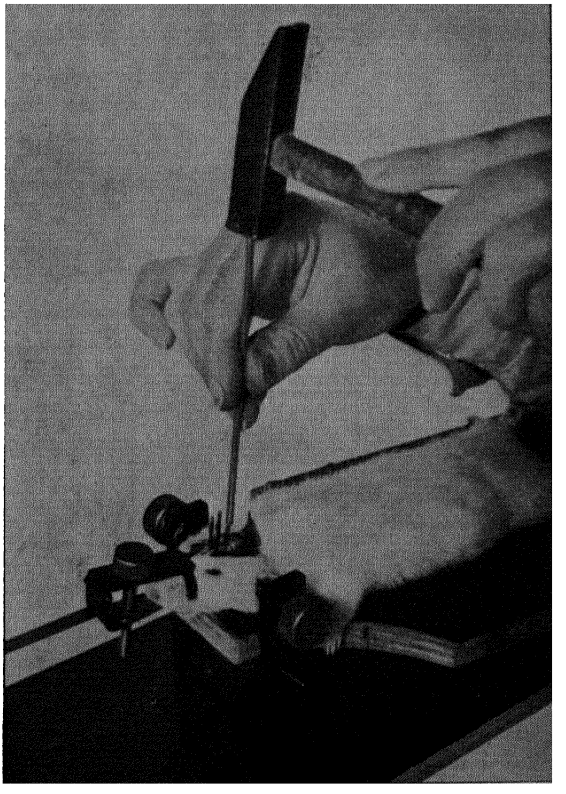


Fig. 156. Fixation of needle electrodes in the rat.

Finally, it is possible to place freely into small trephine openings platinum, silver, tungsten or steel foil, or wires of appropriate size, that are fixed to the surrounding dried and roughened bone with a drop of dental cement or acrylate. An example of such a fixation is shown in Fig. 157c. In acute experiments, in which the animals are anaesthetised or fixed to a head holder, leads may be connected directly to electrodes fixed in the above manner. In freely moving animals in which the connecting wires may be pulled due to movements of the animals, the former must be well insulated (polyethylene tubing) and anchored firmly to cranial bone (Delgado 1955, Batrak 1958). This is best achieved by suturing the insulated leads with heavy steel wire to the occipital bone (occipital protuberance in the dog and cat, the edge of the occipital bone in rabbit and rat) and soldering them to a similarly fixed multiple miniature socket. This may also be fixed to a collar or a special harness placed round the shoulders.

A more detailed description of electrodes and their fixation is given in the individual experiments. For review of literature see Delgado (1955) and Kogan (1949, 1952).

Arrangement of electrodes is a more complicated problem. Potentials recorded from the surface of the cerebral cortex are the sum of potentials

arising in ganglion cells, dendrites and axones. Their amplitude depends on the distance of the electrode from active area, on the resistance of the tissues and on the localisation of the reference electrode. Here too, as elsewhere, two fundamental methods of recording are used — monopolar and bipolar.

a) Monopolar recording. Potentials from individual electrodes are recorded against a common reference (indifferent, non-active) electrode. It is assumed that in this way activity of a region below the active electrode is recorded. This assumtion, however, is only justified if the amplitude of potentials below the active electrodes is much higher than that below the reference electrode. It is therefore important to choose the correct position of the reference electrode in electrically inactive regions. In electroencephalography the following sites are most frequently used for the reference electrode: the ear lobe, skin of the forehead, the nape of the neck or even the neck. In electrocorticography the cranial bones, ear lobes, injured parts of the brain, etc., are used.

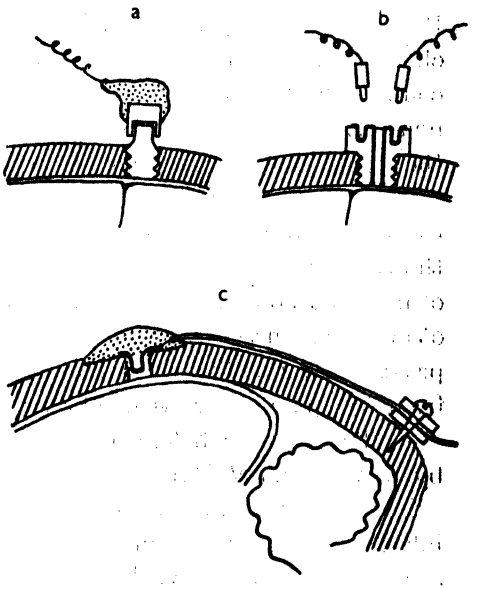


Fig. 157. Electrodes used for recording of ECoG in chronic experiments. a) silver screw. b) screw of insulating material with two silver electrodes. c) silver plate fixed to the bone with acrylate and clamp for fixing the insulated leads.

In distinction to monopolar stimulation, where an increase of the area of the indifferent electrode and resulting decreased density of stimulating current in the indifferent electrode is desirable, in monopolar recording an increase in the area of the reference electrode only slightly improves its "indifference" (potentials of opposed polarity from different asynchronous foci below the electrode may cancel each other).

Earthing of the indifferent electrode also in no way changes conditions of recording and only helps to remove electrostatic interference. Care must be taken to earth the object only via the reference electrode, as otherwise the latter will receive potentials from other earthed parts of the body. When using push-pull preamplifiers the indifferent electrode should not be earthed.

The so called "average" electrode is a special solution of the problem of the indifferent electrode (Offner 1950, Goldman 1950). This is the EEG alternative to Wilson's ECG electrode. The average electrode is a point to which all electrodes are connected through 0·5—2·0MΩ resistances (R). Any

pair of electrodes is thus short circuited by a resistance of $2R$. The average electrode in a system of n electrodes has a resistance of

$$R_a = \frac{R}{n}$$

Potentials arising below only one electrode will also appear at the average electrode but with reversed polarity and n times smaller if n electrodes are connected. The average electrode is useful especially when recording asynchronous activity and when using a large number of electrodes (12—16), as is the case with modern multi-lead EEG apparatus.

b) Bipolar recording. Potential difference between two electrodes relatively close to each other are recorded. The closer the electrode the smaller the area from which potentials are recorded, and the better limited sources of potentials can be uncovered. The differential character of recording, on the other hand, may leave even potentials of high amplitude unrevealed. Interpretation of results obtained with bipolar registration is usually more difficult from the point of view of electrical fields than with monopolar recordings.

The relation between monopolar and bipolar registration is illustrated by experiment VIIIB.

Various apparatus may be used to register EEG and ECoG. Today, ink writing apparatus predominates. Cathode-ray oscillographs are used for special purposes, especially for recording of short-lasting potentials or if an exact picture of the shape of cortical potentials is required. The awkward loop oscillographs are used more and more infrequently. The different types of apparatus, their characteristics and use, have been described in more detail in the IInd part of this book. Here only a few instructions as to the correct procedure necessary for the majority of apparatus used are given:

1) switch on the apparatus, wait the necessary interval for the valves to warm up,

2) control batteries, power supplies and operational voltages of the amplifiers,

3) calibrate and set amplification in all channels to the desired value,

4) choose filters of low and high frequency adequate to the phenomenon registered,

5) connect electrodes to the individual channels and measure their resistance.

Only after having carried out the above can actual registration be started.

B. Spontaneous*) EEG and ECoG in animals

Problem: Record spontaneous electrical activity of the cerebral cortex of the dog, rabbit and rat using the monopolar and bipolar methods.

Principle: The cerebral cortex is source of rhythmical potentials with an amplitude of tens or hundreds of μV and a characteristic frequency that can be recorded with suitably placed electrodes using the bipolar or monopolar method of recording. The shape of the EEG (ECoG) depends to a certain extent on the recording technique used, but mainly, however, on the functional state of the animal.

Object: rat 150—200 g., rabbit 2—3 kg., dog 5—10 kg.

Apparatus: Ink writing multichannel EEG apparatus of conventional type or a cathode-ray oscillograph with A. C. amplifier, overall amplification at least 50 μV/cm.

Further requirements: Screw and needle electrodes of suitable size and shape, surgical instruments, a series of trephines and a screw tap, electrode holder, hammer, a fixation board with a stereotaxic head holder for the rat, an animal board for the rabbit, a solution of collodion, dish shaped silver electrodes for sticking to the skin, an electric fan, dental cement or acrylate, procaine penicillin.

Procedure: The rat is under light ether anaesthesia. After infiltration of subcutaneous tissue with novocaine the scalp is cut along the midline from the level of the eyes up to 1 cm. behind the level of the external auditory meatus. The skin is reflected towards the sides as far as possible and the exposed bone is cleaned of muscle and fascia so that it appears dry and the bone sutures are clearly visible. These serve as important reference points further on. The animal is then tied to the board with the head holder (Fig. 156). Firm fixation of the head is a necessary condition for a good recording. A holder ensuring fixation of the head in three points (the maxilla and both auditory meati) similar to those used in stereotaxic apparatus (cf. page 392) is best. According to a diagram prepared beforehand three needle electrodes (diameter 1 mm, 12 mm length, 1·5—2 mm length of the tip, shoulder 0·3 mm) are then placed above each hemisphere. They are either pinned or hammered into the bone using a special needle holder. A further, stronger needle electrode is hammered to a depth of about 3 mm into the frontal bone above the front edge of the olfactory bulbs (7—9 mm rostrally to the coronal suture). Flexible leads 20—30 cm long (thin high frequency cable is best) are fixed to the electrode input panel in a stand above the animal's head. The panel

*) The term "spontaneous" does not mean that EEG activity is independent of the influences of external and internal environment (Smirnov 1956).

is connected to the EEG apparatus by a shielded cable. After the electrodes are fixed, anaesthesia is interrupted. After a further 10—20 minutes, during which the fixation of the head is corrected, if necessary, registration is started. Depending on the number of channels of the EEG apparatus the following connections are tested simultaneously or one after the other:

1—2, 2—3, 4—5, 5—6
4—1, 5—2, 6—3
0—1, 0—2, 0—3, 0—4, 0—5, 0—6

The following leads are then recorded, simultaneously if possible:

0—1, 0—2, 1—2, 2—1

With each pair of electrodes activity is recorded for several minutes at a speed of 1·5 or 3 cm/sec.

In the rabbit, implanted electrodes and chronic scalping are used. The electrodes are put into place under short lasting barbiturate anaesthesia (i. v. evipan or pentothal). After a midline incision of the scalp and removal of the galea trephine holes (2·4 mm. diameter) are made into the skull bones reaching the dura. A 3 mm. tap with a ground off tip is used to thread the bone into which a silver or steel screw is finally placed. The whole procedure is carried out under semisterile conditions with sterile instruments and the use of formalin (37%), "Ajatin", "Zephiran" or alcohol (70%) for sterilising the skin, screws etc. The skin is then adapted to the dimensions of the exposed skull and sutured to the parieto-temporal bone edge where it easily heals. The leads from the electrodes are insulated with polyethylene tubing and are connected to a common socket carried on a harness fixed to the neck or back of the animal. In order to prevent a direct pull on the electrodes, the leads are fixed with stainless steel wire to the edge of the occipital bone perforated with a sharp needle. Both the anchoring wire and the leads are covered with acrylate. Procain penicillin (100,000 I. U.) is given 12 hrs. before the operation and immediately after it. The animal is placed in a separate box with smooth walls.

Registration itself is carried out in the freely moving animal only 1—3 days after fixation of the electrode. The animal is placed into a circular container with a diameter of about 50 cm and a height of 50 cm. Above the centre of the container a cable connecting the socket on the neck of the animal with the input panel of the EEG is elastically fixed on a pulley, a steel spring or a rubber band. Tension on the cable must be uniform during movement of the animal. Sometimes it is of advantage to connect the animal with the pulley via a special string that bears the main weight of the animal's movements and prevents the cable from being pulled. A suitable choice of the fixation string also prevents undesirable rotation of the animal to one side only.

Registration is fundamentally the same as in the rat. The distribution of the electrodes and their connections are shown in Fig. 158.

In the dog electroencephalography may be attempted. It is best to use an animal used to work with conditioned reflexes in a Pavlov stand. On the day before the experiment the scalp is clipped and the skin is shaven with a suit-

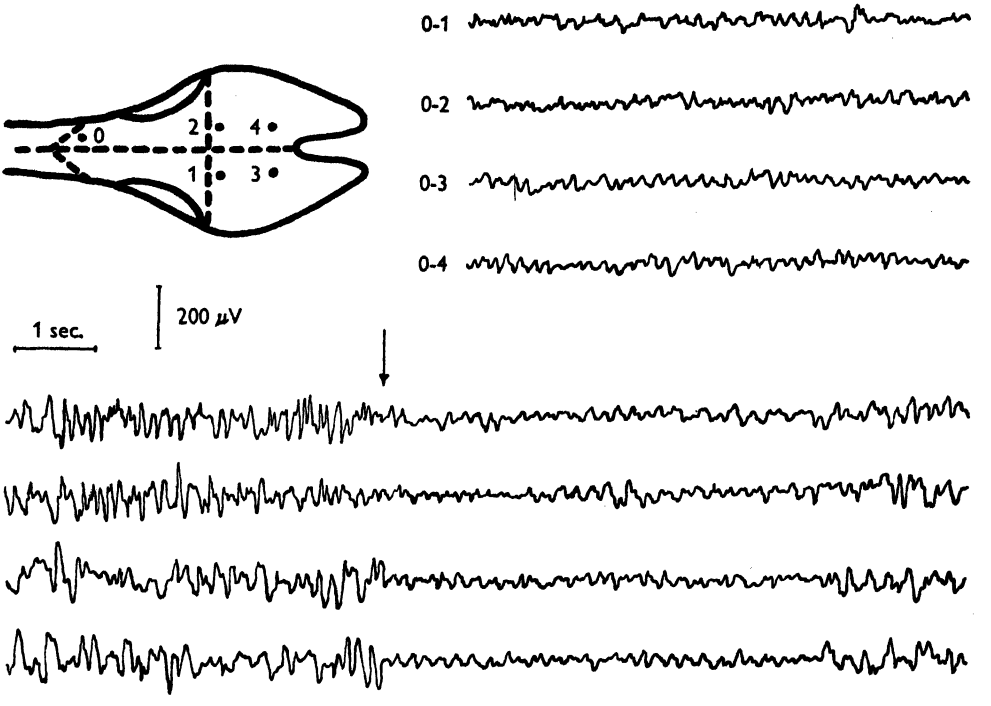


Fig. 158. Characteristic ECoG activity in an unanaesthetised unrestrained rabbit. Above: waking type of EEG activity. Below: sleep activity and arousal on whistle (arrow).

able instrument and then washed with soap and water. After thorough drying, the electrodes are stuck to the head. Fat at the sites of application of the electrodes is first removed from the skin by rubbing with cotton wool soaked in ether. Then a drop of EEG paste is applied to these points and is thoroughly rubbed into the skin. Rubbing is easily done with a smooth rotating axle of a small electro-motor. Only then is a dish-shaped electrode, also filled with EEG paste, applied. While holding the electrode with a glass rod placed into a small hollow on the upper surface, this and its nearest surroundings are covered with a few drops of collodion from a dropper. The assistant must hold the head of the dog firmly to prevent any movement. Drying of the collodion is speeded up using a current of warm air from an electric fan. The skin must be perfectly dry and without fat for the col-

lodion to hold in place firmly. The connecting wires from the electrodes are again fixed to a small socket on the collar. The dog is placed into a stand for experiments with conditioned reflexes and as soon as it settles down registration may commence. This is preferably bipolar. E. g.:

1—2, 2—3, 4—5, 11—12, 12—13, 14—15 — longitudinally
11—1, 12—2, 13—3, 14—4, 15—5 — transversally

Results: Fig. 158 shows a characteristic record of EEG activity in the rabbit. There are no fundamental differences between the EEG in different animals used, however, and individual leads also do not differ substantially from each other in the same animal. The record in the rabbit has the highest amplitude. This is due to the relatively large electrodes placed directly onto the dural surface. The amplitude of the EEG in the dog is smallest. This again is due to the large short circuit decrease in potential when recording across the skin. Fundamentally, two basic types of activity can be distinguished in the EEG of the unanaesthetised animal: a sleep pattern and a waking pattern.

Sleep activity is most frequently found in our experimental conditions in the rabbit and dog. This corresponds to the very natural conditions during registration, the animal being relaxed and often even falling into sleep. Sleep ECoG is characterised by high voltage slow waves (100—200 μV, 1—3/sec) of irregular shape. Occasionally spindles of waves 8—12/sec occur in the fronto-parietal region. (They are termed spindles because in a series of 10—20 such waves the amplitude first increases, reaches a maximum and then again decreases.) During deep sleep slow irregular activity preponderates.

If various external stimuli (clapping, shaking, touch, changes in illumination, olfactory stimuli etc.) act on an animal showing this type of EEG activity, irregular fast activity of low amplitude suddenly appears in the records from all cortical regions. This, again, is most apparent in the fronto-parietal leads. In the rabbit a regular activity 4—6/sec. (theta rhythm, see also p. 415) occurs sometimes in the limbic cortex during arousal. The change from sleep activity to waking activity is sudden and very obvious. The term "arousal reaction" (waking reaction, blockade, activation of EEG) is therefore used. The blockade of alpha rhythm in the EEG in man when opening the eyes or during psychic activity (Berger 1930) is also an arousal reaction. The EEG arousal may be accompanied by motor reactions corresponding to motor arousal of the animal. At other times, however, the animal remains motionless. As a slow, high EEG can be explained by synchronisation of potentials of individual cortical neurones, the transition to rapid, low voltage activity is related to desynchronisation of electrical activity of individual cortical elements (Adrian, Matthews 1934).

A high frequency due to muscle movements (especially the temporal

muscles) often occurs in the dog EEG. Sometimes it is not easy to differentiate between such muscle artefacts and high frequencies originating in the brain tissue. More abrupt movements of the head are further accompanied by so called movement artefacts in the record. These are irregular, high voltage

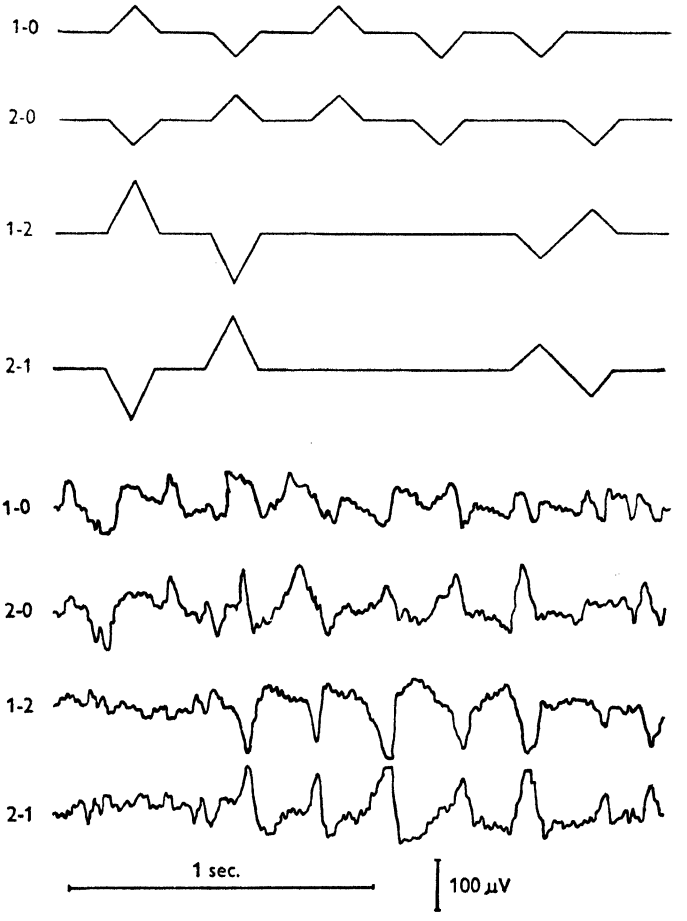


Fig. 159. Reconstruction of bipolar recording from two monopolar ones. Top: diagrammatically. Bottom: example from rabbit's ECoG.

changes, usually of long duration, blocking the amplifier because of the excessive input signal. The cause of such artefacts is usually a change in contact of poorly fixed electrodes, large changes in electrode resistance, skin potentials, capacitative influences on the moving cables, etc. If such artefacts that can usually be identified without difficulty appear, the electrode on which they occur must first be localised. Movement artefacts are most reliably prevented by reducing electrode resistance, by better fixation of the electrode, and by

decreasing the animals movements. A high electrode resistance is usually noticed even without movement artefacts by a frequency of 50 cycles/sec. occuring in the record (the lead is "noisy").

Records from the various electrodes usually differ from one another. Some characteristic waves, however, may be found simultaneously in several leads from the same or even both hemispheres (especially during sleep activity). In general it is true that the bipolar longitudinal record shows higher amplitudes than the bipolar transversal one, the distance between the electrodes being the same. This is so because connection of symmetrical points gives a smaller potential difference since the potentials are to a certain extent synchronous. In addition to these physiological conditions, purely physical factors influence the shape of the recording. Thus, for instance, a comparison of records 1—2 and 2—1 (fig. 159) shows that both tracings are identical but of opposite polarity (mirror images), of course assuming an equal function of amplifiers in both channels. It is important to bear in mind that the polarity of a record is determined by the way the electrodes are connected to the left and right half of the amplifier input. It is therefore necessary to pay special attention to this when using the multi-lead method.

A comparison of records 1—0, 2—0 and 1—2 (Fig. 159) shows that the latter is a curve of the difference between the first two. This also determines the relation between mono- and bipolar records, assuming, of course, that the 0 electrode contributes equally to records 1—0 and 2—0. The amplitude of the bipolar record is the lower the smaller the distance between the two electrodes, i. e. the greater the similarity between both monopolar records.

Conclusion: The ECoG and EEG of unanaesthetised animals is an expression of basic states of cerebral activity — inhibition (sleep) and excitation (alertness) and is fundamentally similar in different mammalian species.

C. Spontaneous EEG during Anaesthesia

Problem: Observe changes in EEG activity during increasing barbiturate or ether anaesthesia. Use EEG for controlling the depth of anaesthesia.

Principle: During anaesthesia characteristic changes occur in the EEG. These correspond to different depth of anaesthesia as defined by reflexes. According to initial changes anaesthetics may roughly be divided into two groups:

1) Barbiturates. Their effect is characterised by synchronisation and increased amplitude of cortical potentials (e. g. Forbes et al. 1956).

2) Ether. This, on the contrary, causes desynchronisation of the EEG and a decrease in its amplitude (e. g. Schlag and Brand 1958). The two main groups, corresponding to a certain extent to Pick's classification into cortical

and subcortical anaesthetics, show that anaesthesia is not the result of a simple inhibition of brain metabolism but is due to complex relations between various parts of the CNS, differently affected by the anaesthetic. The effect on the cortex is not primary (as was thought e. g. by Derbyshire, Rempel, Forbes and Lambert 1936). Rather does the EEG reflect changes in excitability of brain stem formations, especially of the reticular activating system (French, Verzeano, Magoun 1953, Bovet and Longo 1956, cf. also p. 409).

During the deeper stages of anaesthesia initial differences disappear and inhibition of the metabolism of all groups of neurones in the brain sets in. As a result of this, EEG activity decreases and gradually disappears altogether. These phases of anaesthesia, in particular, can be characterised exactly and quantitatively, e. g. by integration of EEG amplitude, and can thus be used to control the depth of anaesthesia.

Object: Rabbit weighing 2—3 kg.

Apparatus: An ink-writing multichannel EEG apparatus or a cathode-ray oscillograph with a pre-amplifier, total A. C. amplification of 50 μV/cm. Integrator of EEG activity (cf. p. 332).

Other requirements: Fixation board for rabbit, silver screw electrodes for leading off the ECoG, a mask for ether administration, a polyethylene cannula for infusion of anaesthetic, apparatus for automatic injection (Petráň 1952).

Procedure: The rabbit with chronically implanted electrodes as in the previous experiment is tied to the board. When using ether a gauze mask is fixed to its snout. After the animal has quietened down the actual experiment is started. First a normal record is made for 5—10 minutes characterised by alternating waking and sleep activity. Then ether is dropped onto the mask, rapidly at first (10—20 drops/min.), later more slowly. The assistant is ready to prevent artefacts due to obvious restlessness of the animal during the excitatory phase of anaesthesia. The depth of anaesthesia is controlled by reflexes, especially by the presence of corneal and pupillary reflexes. During the deeper phases of anaesthesia ether is added very carefully and the reflexes are controlled continuously together with respiration and heart activity, which can suitably be recorded in one of the EEG channels using needle electrodes located in the skin on the sides of the thorax. After EEG activity has been suppressed completely, ether administration is discontinued and arousal from anaesthesia is observed. Usually, of course, a certain rudimentary activity is preserved when using high amplification even during the deepest stages of ether anaesthesia.

When using barbiturate anaesthesia the anaesthetic is given i. p. (Nembutal, Pentothal 45 mg./kg. with a little heparin) after the control recording has been made. The development of anaesthesia during absorption of the anaesthetic from the peritoneal cavity is then studied. When surgical anaesthesia has been produced a polyethylene cannula is introduced into an ear

vein for further infusion of the anaesthetic i. v. The cannula is connected to a syringe, the plunger of which is pushed by a threaded block moved in guide bars by a screw (driven via an adjustable gear by an electric motor or by a stepping relay). One of the channels of the EEG is connected to an integrator — fundamentally a storage condenser charged by the rectified output current. After reaching a certain voltage this condenser is discharged through a thyratron or a sensitive relay. The thyratron discharge either directly activates the stepping relay or connects a circuit that for a certain period of time (1—2 sec.) switches on the motor for automatic injection. Fig. 160 shows a diagram of the whole circuit and gives a more detailed description of the apparatus. Other solutions of the same problem are given in the work of Bickford (1950, 1951), Verzeano (1951), Forbes et al. (1956), Degelman (1956).

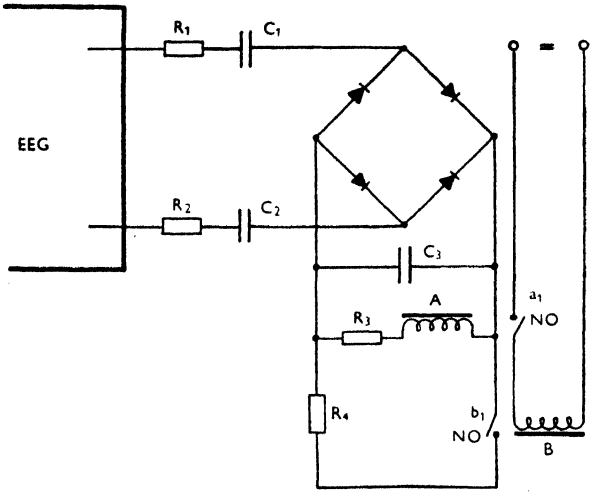


Fig. 160. A simple integrator of EEG activity. Condenser C_3 is continuously charged by the rectified output voltage of an EEG channel, and discharged across a large resistance R_3 and a sensitive relay A. After a certain voltage is reached at condensor C_3 relay A makes contact. This activates relay B, through the contact of which C_3 is rapidly discharged through a small resistance R_4. The arrangement for automatic injection is connected to further contacts of relay B.

Automatic regulation of the depth of anaesthesia is carried out as follows: when EEG activity, after having reached the maximum depth of anaesthesia, again reaches maximum amplitude, the amplifier connected to the integrator is regulated in such a way that the latter gives approximately 50 discharges per minute. Then the integrator is connected to the apparatus for automatic injection and the gear is so adjusted that the number of discharges (and thus the depth of anaesthesia) remains roughly the same (i. e. it neither increases, which would indicate a decrease in the depth of anaesthesia, nor does it decrease, indicating a deepening of anaesthesia). It is important to note that during light anaesthesia a decrement of ECoG amplitude and a corresponding reduction of integrator discharges may, on the contrary, indicate desynchronisation and arousal.

Large individual differences exist between animals and the control of anaesthesia must be adjusted to them. It is relatively easy to regulate deep anaesthesia. Maintenance of a medium level of anaesthesia, on the other

hand, requires exact adjustment of the rate of infusion to a certain type of EEG activity and occasional re-regulation.

Results: The development of ether anaesthesia is shown in Fig. 161. Immediately after the first drops of ether are given a rapid, low-voltage activity, typical for the aroused animal, replaces the EEG sleep pattern.

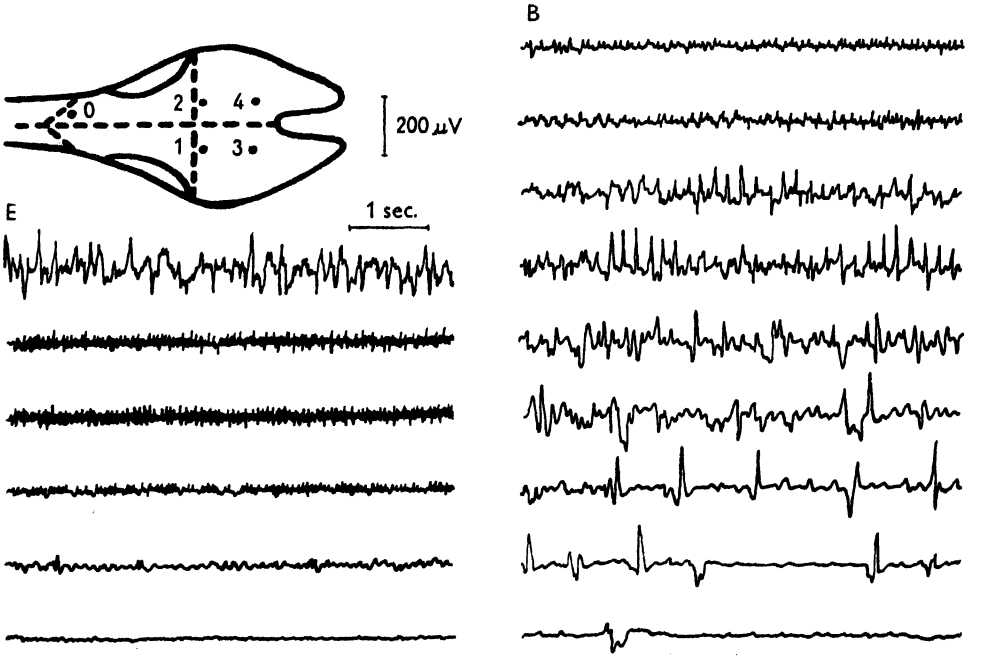


Fig. 161. ECoG activity during gradually increasing ether (E) or barbiturate (B) anaesthesia. Recording from electrodes 1—3. E: first row—control, further rows in 2 min. intervals while continuously adding ether. B: first row—control, further rows in 3 min. intervals after i. p. injection of 35 mg./kg. pentothal.

This type of activity is preserved up to the stage of deep anaesthesia even though it may be combined with slower, more or less regular waves. Only during deepest anaesthesia, when severe respiratory disturbances appear does EEG activity disappear completely. After application of ether is discontinued all changes recur in the opposite sequence.

Barbiturate anaesthesia develops quite differently. After the initial i. p. dose of Pentothal (45 mg./kg.) there is a short phase of waking activity due to the injection. Then sleep activity rapidly increases changing into high voltage discharges, synchronous in the whole cortex — so called spindles. These are separated by intervals of lower asynchronous activity. In the beginning it is still possible to wake the animal using sensory stimuli (cf. arousal reaction p. 328). Later on, however, peripheral stimuli lose their generalised effect

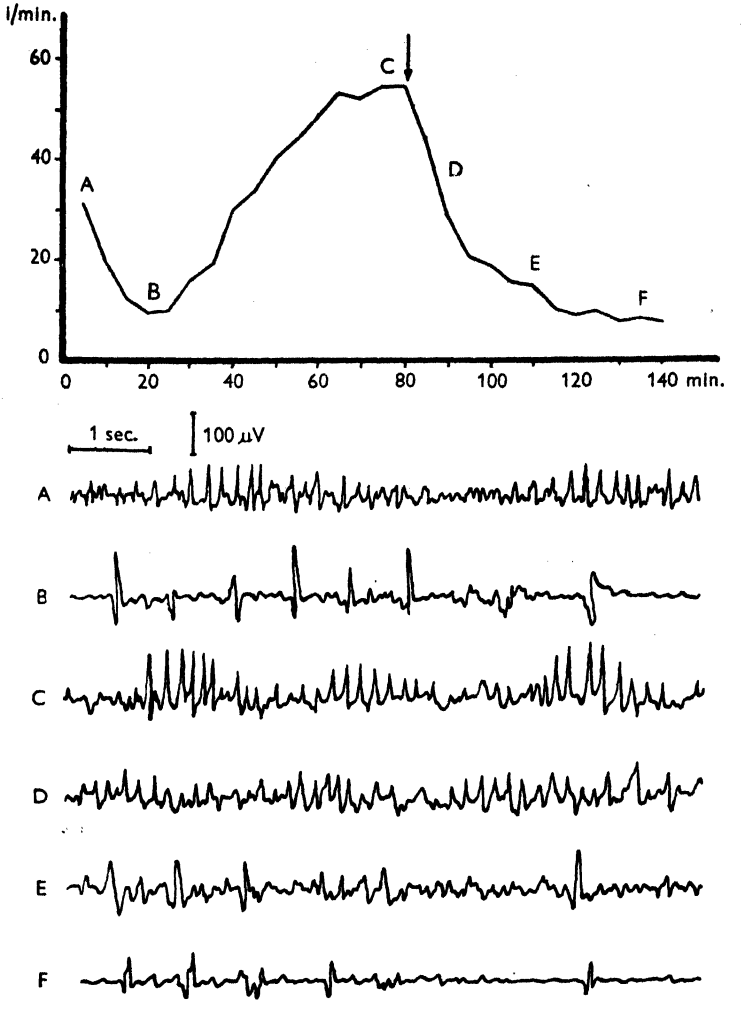


Fig. 162. Control of the depth of barbiturate anaesthesia by feedback between the EEG integrator and the injection system. Top: recording of integrator discharges. Bottom: examples of ECoG curves at corresponding stages of anaesthesia. A-B — deepening of anaesthesia after i. p. injection. B-C — gradual recovery of the animal. Arrow — connection of the automatic injection to the integrator. C-D-E-F — stabilisation of the system at a new anaesthesia level maintained by a strong feedback. Calibration: 1 sec, 100 μV.

and only cause primary responses in the corresponding cortical areas (cf. p. 335).

This phase is also rapidly displaced by a new one: irregular, synchronous, high voltage activity predominates. The frequency of this activity becomes less and less until finally individual high voltage waves or short series of such waves are separated by intervals of electrical silence (black-out). During most

profound anaesthesia electrical activity disappears completely and only an uninterrupted iso-electric line is recorded. Respiration during this phase of anaesthesia is considerably slowed down but is still regular and deep. In long lasting experiments it is important to prevent a larger decrease in body temperature of the animal, which might also influence the EEG picture.

Thus after an i. p. injection of Pentothal EEG activity reaches a stage characteristic for a certain degree of deep anaesthesia and then, as the concentration of the anaesthetic decreases in the blood it returns to a stage typical for moderate or slight anaesthesia. It is evident from the above that it is not easy do determine the different phases of anaesthesia with the help of EEG. It is even more difficult to find a quantitative expression for the depth of anaesthesia. Forbes et al. (1956) for instance, suggested a simple indicator: in one channel the amplitude of the highest waves seen on the record during increasing or decreasing anaesthesia is determined. At regular intervals all waves exceeding one third of this value are then counted on 10—20 sec. strips of the tracing. The average number of such waves/sec. during a given interval then serves as an index characterising the depth of anaesthesia. More exact results are obtained with an integrator. Even in that case, however, the result depends on many circumstances so that integrator data are also best related to a certain characteristic level of anaesthesia (e. g. to the maximum rate of the integrator discharges in the same channel). Absolute comparison between individual leads of different animals is possible only approximately.

Fig. 162 shows a characteristic EEG record together with integrator data during anaesthesia produced with Nembutal before and after introduction of automatic control of anaethesia.

Conclusion: During anaesthesia characteristic changes occur in the electrical activity of the cerebral cortex, making it possible to identify the type and depth of anaesthesia and to regulate it.

D. Primary Cortical Responses

Problem: Register electrical changes in the cerebral cortex due to short auditory, optic or somaesthetic stimuli. Determine the extent of the cortical projection of these afferent systems.

Principle: Receptors are usually a source of complex patterns of impulse activity in paths connecting them to higher centres. Electrical signs of a large number of asynchronous action potentials coming continuously, e. g. from the eye that is watching its surroundings, and entering the optic areas of the cortex, cancel each other and disappear during registration from the surface of the brain in the electrical activity of other neurones. A different situation arises if receptors are stimulated with a relatively strong and short lasting

stimulus eliciting a synchronous volley of impulses in a large number of fibres of the afferent system. Their nearly simultaneous arrival into different relays of the specific pathway and their consequent entry into a limited area of the cortex results in synchronous changes in membrane polarity in a large group of cortical ganglion cells and dendrites. Characteristic electrical potentials result that are very clear cut because of the regular radial structure of cortical elements and can easily be distinguished on the baseline of spontaneous EEG activity. Their localisation makes possible exact electrophysiological limitation of the extent of cortical projection of the analyser studied. In view of the fact that responses are the more apparent the lower the amplitude of spontaneous EEG activity, it is of advantage to use relatively deep barbiturate anaesthesia in order to demonstrate them, as during such anaesthesia spontaneous EEG is suppressed much sooner than primary responses.

Object: Cat, 2—3 kg; rat, 200 g.

Apparatus: Ink-writing multichannel EEG apparatus. A cathode-ray oscillograph with overall A. C. amplification of at least 50 μV/cm, linear for 1—5000 cycles/sec. A two channel apparatus is best. Needle electrodes for corticography in the rat (cf. p. 321). Electrodes for corticography from the exposed brain surface (silver wire ball-tipped electrodes mounted on springs in an electrode carrier fixed to the bone, or wick Ag-AgCl or $Hg\text{-}Hg_2Cl_2$ electrodes in separate carriers).

A power amplifier and a loudspeaker. A stimulator giving single square wave pulses or condenser discharges, paired stimuli for determining the refractory phase and rhythmic stimuli from 0·5—200 c/sec. Isolation output unit for the stimulator. A photostimulator (stroboscope) giving light flashes of high intensity singly or rhythmically.

Further requirements: Surgical instruments, a set of trephines, a dental drill a photographic camera for recording from the screen of the cathode-ray oscillograph, 10% Dial solution.

Procedure:

1) Localisation of primary responses in the rat.

The rat under dial anaesthesia (40 mg./kg.) is operated as in experiment VIIIB. In addition the whole lateral surface of the temporal bone is exposed. The temporal muscle is either cut off or deflected and fixed together with the skin to the head holder. The sciatic nerve is exposed in both thighs for a length of about 2 cm. The rat is tied to the animal board and the head is fixed to the holder as desribed in experiment VIIIB. If an auditory stimulus is to be used the screws in the external meati must have a central opening. Near the animal an electrically shielded loudspeaker is installed. The lamp of the stroboscope is aimed at the head of the rat from above and in front. One of the exposed sciatic nerves is placed on a pair of electrodes connected through an isolation transformer or a radiofrequency unit to the stimulator. A row of needle

electrodes, at a distance of 2 to 2·5 mm from each other, is placed above the expected projection area according to the diagram in Fig. 163. A stronger needle electrode pierces the frontal bones above the anterior edge of the olfactory bulbs to a depth of about 3 mm.

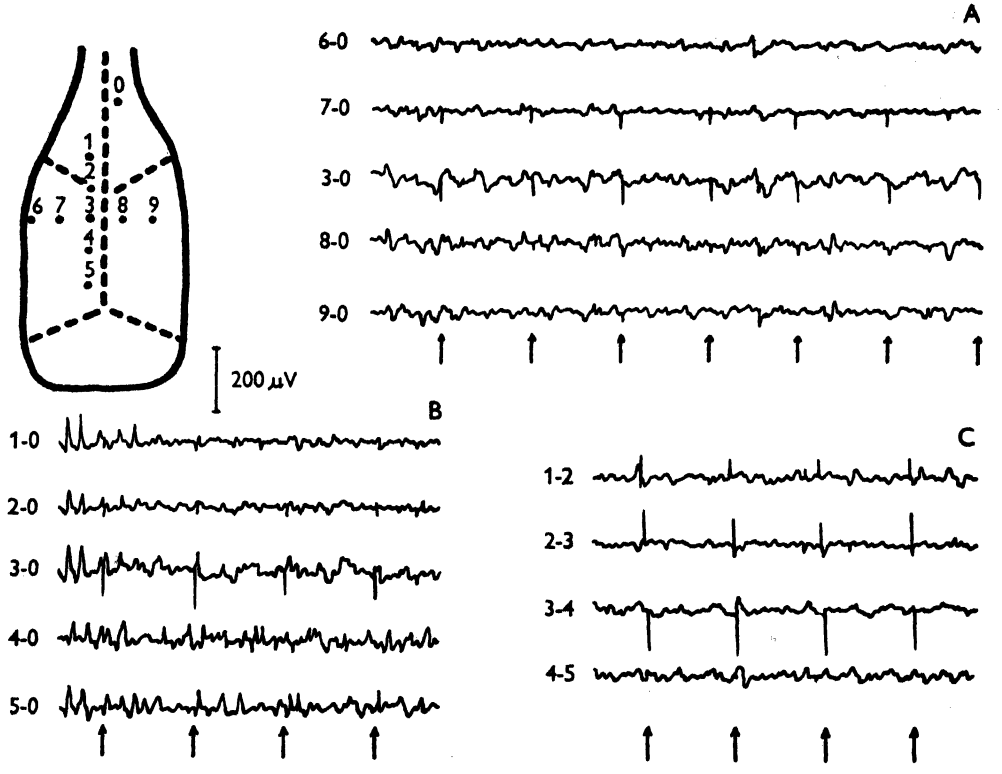


Fig. 163. Localisation of primary projection area of the left sciatic nerve (in the rat) in a monopolar (A, B) and bipolar (C) ECoG recording. Arrows indicate electrical stimulation of the sciatic nerve with single stimuli repeated at 1 sec. intervals. The downward deflection in the monopolar recording corresponds to positivity of the active electrode.

The arrangement shown in Fig. 163 can, for instance, be used to localise the projection area of the right sciatic nerve. Depending on the number of available channels of the EEG apparatus the following combinations of electrodes are connected to the individual amplifiers either simultaneously or consecutively:

Monopolar: 0—1, 0—2, 0—3, 0—4, 0—5
0—6, 0—7, 0—3, 0—8, 0—9

Bipolar: 1—2, 2—3, 2—4, 4—5
6—7, 7—3, 3—8, 8—9

The signal synchronous with the stimulus used is recorded in another channel.

A control recording lasting for 30—60 sec. is first made with each combination of the electrodes. Then stimulation is applied for 1 min. using the various stimuli at regular 2—3 sec. intervals. For acoustic stimulation pulses from the stimulator are fed into a power amplifier, the output of which is coupled with the loud speaker. The intensity of stimulation of the sciatic nerve is just sufficient to produce a slight twitch of the limb, visual and auditory stimuli may be maximal. When all stimuli have been tested with a certain arrangement of the electrodes a new combination is chosen according to the above table and the whole procedure is repeated. Depending on the results obtained in these recordings a further recording is taken if necessary with a different connection of the electrodes or a different combination of simultaneously recorded leads. Thus, for instance, for a better determination of the somaesthetic area so called triangulation is used — connection of 3 electrodes forming the tops of a triangle to three leads corresponding to its sides. E. g.:

2—3, 3—7, 7—2
3—7, 7—4, 4—3, etc.

If large responses are obtained from peripheral electrodes further electrodes are introduced located towards the periphery of the expected projection focus.

In order to decrease the stimulation artefact during stimulation of the sciatic nerve the animal is grounded. A clamp fixed to the cut skin of the thigh above the stimulating electrodes is best. Other methods for decreasing the stimulation artefact (described on p. 351) may also be applied here.

2) Localisation of primary responses in cats using a cathode-ray oscillograph.

The skin of the head of a cat under deep dial anaesthesia (40—50 mg./kg. i. p.) is cut in the midline for a length of 6—9 cm. The skin is reflected towards the sides as far as possible and fixed with haemostats. The temporal muscle is dissected away from the skull, retracted as far as possible and fixed. The cranial bones are thoroughly cleaned. An 8—10 m mopening is made with a trephine fixed to an electric drill about 1 cm laterally from the midline and 1 cm caudally to the coronal suture. This opening is widened with bone pincers anteriorly, dorsally and laterally. Only 2 mm from the midline a strip of bone is left in situ so as not to injure the sinus and thus cause haemorrhage that is difficult to stop. Haemorrhage from the cranial bones is controlled with bone wax. The dura mater is lifted with the help of a needle having a fine hook at its tip and a Y shaped cut is made with fine scissors. The cut dura is folded back over the borders of the opening. The exposed cortex is covered with cotton wool or gauze soaked in warm Ringer solution until the experiment is commenced.

After the operation the cat is tied to the animal board and the head is fixed in a stereotaxic head holder by the nasal bones, palate and zygomata. The auditory meati remain open. If no stereotaxic head holder for the cat is available, good fixation of the animal suffices. Movement of the head against the electrodes is prevented by fixing the electrode carrier directly to the skull bones using two short screws. The electrodes must then be ball tipped spring mounted silver wires. If the electrodes are in special stands independent of the head holder Ag-AgCl or Hg-Hg_2Cl_2 electrodes with a 3—4 cm wick, following the movements of the cortex, are of adavantage. Inspite of the great advantages of a multi-channel ink-writing EEG apparatus for routine work, the recording of rapid components of primary responses is distorted mainly because of the considerable inertia of the mechanical recording system. If an exact picture of potential changes in the cerebral cortex is required a cathode-ray oscillograph must be used for recording. In that case either an independent pre-amplifier together with the amplifer of the cathode-ray oscillograph is used, or the output of the EEG amplifier is directly connected to the Y plates of the cathode-ray tube. In that case only the time base and cathode-ray tube of the cathode-ray oscillograph are used. When using a double beam C. R. tube or an electronic switch primary responses from two cortical areas may be recorded simultaneously with the ink-writer recording.

It is important to achieve exact synchronisation of the time base and the stimulus. Any of the methods described on p. 86 is therefore used in such a way as to ensure that the sweep starts 1—2 msec. before the stimulus. The stimulation artefact is important for calculating the latent period. The duration of the sweep is chosen from 500 to 50 msec. depending on which phase of the primary response is being studied. Pictures are taken with an open shutter during the single passage of the beam using an ordinary (p. 127) or special (p. 138) camera. If synchronisation of the time base and the stimulus is perfect it is possible to photograph several responses one over the other with the shutter permanently open. Thus one picture shows both variations in the individual responses and their average most typical shape. When photographing in the above way, the brightness of the spot is decreased so that only very weak curves are drawn on the photographic material. At the sites where the curves most frequently superimpose the tracing is then most distinct.

Oscillographic registration is carried out in similar leads as when using ink recording, but monopolar registration is preferable. When, e. g., determining the extent of the auditory projection area either the exposed skull bone, the metal electrode holder fixed to the skull, or the clamp on the cut skin, is used as a reference electrode. The active electrode is moved in a certain direction — e. g. in the parasagittal plane — from the border of the expected projection

area towards its centre. At 2 mm intervals several responses to an auditory stimulus (short clicks) are recorded. The sites from which recordings were taken are marked with a drop of india ink and drawn into the diagram of the exposed brain surface prepared beforehand. After having thus gone through the whole row a new parallel one is started 2 mm more laterally or medially. The whole lateral surface of the hemisphere is thus mapped out. Poor responses may be increased by local application of 0·1—1% strychnine nitrate (see page 356).

Evaluation of the recording further requires time and amplitude calibration. In a two channel apparatus one beam can be used for recording the sine wave signal of 50, 100 or 1000 cycle/sec. or a special time mark (cf. p. 131) together with the observed phenomenon. In a one channel apparatus the time signal is photographed now and again on the assumption that in the interval between these two recordings the function of the time base did not change.

Amplitude calibration is performed by introducing a known potential into the amplifier input. A square pulse is best, making it possible to characterise also the time constant of the apparatus.

3) Primary responses to repeated stimuli.

After ascertaining the localisation of the maximum response, e. g. to stimulation of the sciatic nerve in the rat or to sound stimuli in the cat, the ability of the cerebral cortex to react to repeated stimuli of the same intensity is tested in the lead giving the best response. If a stimulator equipped for measuring the refractory period is available, we use two slightly supramaximal stimuli and change the interval between them from 200 to 5 msec. The paired pulses are applied at 2—4 sec. intervals. After several responses the interval between both pulses is shortened. The relative refractory period is examined with particular thoroughness. If a stimulator giving repetitive stimuli in the range from 1 to 100 cycles/sec. is at hand, we use short-lasting (about 1 sec.) trains if stimuli of varying frequency adjusted beforehand. Here, of course, we determine not only the refractory period (the response to the first two stimuli) but also the ability of the cerebral cortex to respond to stimuli rapidly succeeding each other. It is therefore important, especially when using higher frequencies, to use longer intervals between short trains of stimuli (20—60 sec. or more) and thus prevent exhaustion of cortical neurones.

A certain variability of primary responses in spite of constant characteristics of the stimulus depends, among other things, on the phase of the spontaneous ECoG at which the afferent signal arrives. As spontaneous activity is controlled by the non-specific afferent system (Morison, Dempsey 1942, Li et al. 1956b) whose fibres terminate in all cortical layers, interaction between specific and non-specific impulses may occur in the cortex. In order to achieve synchronisation of specific impulses with normal or pathological cortical

activity an electronic trigger circuit is used (Shipton 1949, Hewlett 1951) with which some modern stimulators and stroboscopes are equipped. The stimulus is released when the output voltage of the EEG channel registering the activity of the corresponding projection area attains a certain value. Either increasing negativity or positivity may be used and also the interval between the releasing phase of EEG activity and the stimulus itself can be controlled.

Results:

1) The monopolar and bipolar recording of a typical experiment in a rat is shown in Fig. 163. It can be seen that responses to stimulation of the sciatic nerve by single electrical discharges have a characteristic distribution. The sites of maximum positive waves correspond to the maximum projection, provided, of course, that the indifferent electrode does not influence the shape of the recording. There is a certain overlap of the optic and somaesthetic projections on the periphery even though the areas of their maximum response are well defined and fundamentally correspond to the projection areas as determined in degeneration experiments of Krieg (1946a).

It is somewhat more difficult to determine the limits of a focus in bipolar recording. The latter's advantage, however, is a more acurate localisation of activity in smaller cortical areas. The principle of localisation is based mainly on the so called phase reversal. The characteristic focal activity will appear in tracing from a row of electrodes passing through the focus (Fig. 164A) as waves whose phase has been shifted by 180 °. Let it be assumed that the source of these characteristic waves — e. g. of primary response in the somaesthetic area — is situated directly below electrode 3, while electrodes 2 and 4 are almost outside the focus. Then, obviously, recorded activity will be highest in leads 2—3 and 3—4 while in leads 1—2 and 3—5 it will either be absent altogether or very low. As electrode 3 is connected once to the right and then to the left half of the EEG amplifier input, the same potential change at this electrode will cause the opposite movement of the pen in lead 2—3 and in lead 3—4. Synchronous potentials of the same shape but of opposite phase (mirror images) occuring in two tracings may thus be regarded under such conditions of recording as an expression of electrical activity below the electrode common to both leads.

Further diagrams illustrate other possibilities of localising foci with regard to a row of electrodes. In Fig. 164B the focus is between electrodes 3—4, so that the focal potentials reach both equally. For that reason signs of synchronous activity will be smaller in lead 3—4 than in leads 2—3 and 4—5, which record potential differences between inactive areas 2,5 and electrodes 3, 4 situated in the area of the focus. The phase reversal then appears between leads 2—3 and 4—5. Practically, however, this theoretical possibility occurs only rarely. Usually the focus is nearer to one of the electrodes, and the record-

ing shows a typical phase reversal. The amplitude of both waves shifted by 180° is not quite the same, however (see Fig. 163C, leads 2—3, 3—4).

Another frequently used method of bipolar localisation is the so called triangulation. In this method we connect any three electrodes to form a triangle in such a way that its sides correspond to the individual leads (Fig. 164C).

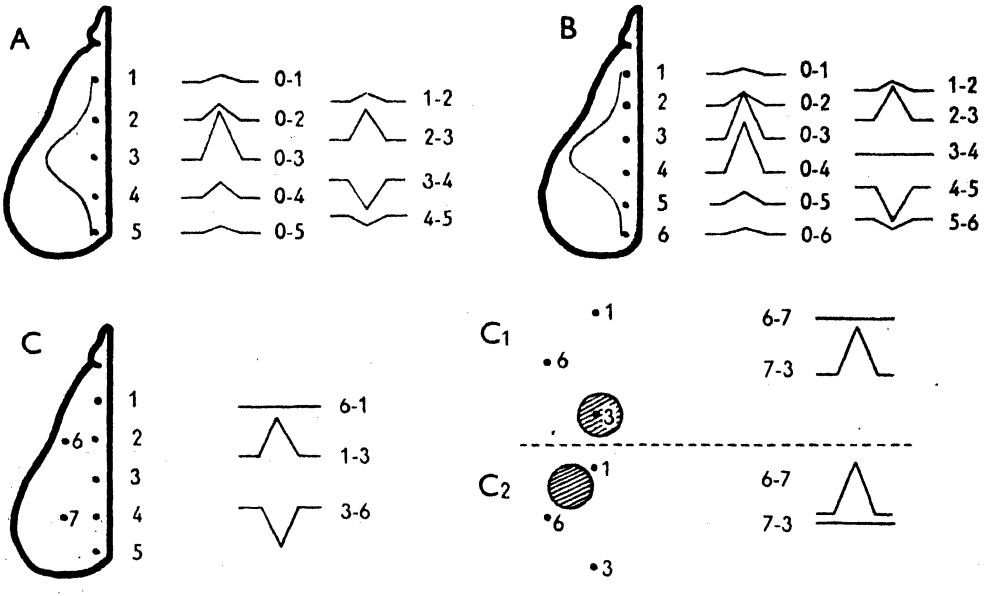


Fig. 164. Principles of localising focal activity. Above: localisation in monopolar (A) and bipolar (B) recordings. The curve in the diagram of the brain shows the spatial distribution of focal activity along the line joining the electrodes. Below: Triangulation. C — basic triangle, C_1 C_2 — two possibilities that correspond to the results obtained with the basic triangle leads. Shaded area indicates the focus.

When connecting we usually go in one direction (6—1, 1—3, 3—6). The leads in which the typical phase reversal appears contain the electrode nearest to the focus (Fig. 164C_1). Theoretically, of course, there is also the possibility (Fig. 164C_2), that a focus of opposite polarity is situated between the other two electrodes in such a way that its activity affects both symmetrically and thus is not apparent when recording from them. The use of a further electrode or electrodes (Fig. 164C) helps to distinguish between these two possibilities. A new triangle will then decide.

A bipolar recording from the same electrodes as in Fig. 163B is shown in Fig. 163C. If we apply the principles of localisation explained above we can easily find the focus of maximum responses to stimulation of the sciatic nerve.

All the above considerations are, of course, only valid on the assumption that the electrical phenomenon studied is limited to a certain exactly defined area of the hemisphere and that its time course is the same in all parts of that area. This theoretical assumption, of course, holds good only exceptionally as primary responses and other characteristic potentials spread immediately from the projection area into the surrounding regions (Adey, Carter and Porter 1954, Lilly and Cherry 1954). It is therefore necessary to define not a stationary but a moving electric field. This task can be fulfilled only with help of special techniques (toposcopy — Goldman et al. 1948, Walter and Shipton 1951, Lilly 1950, 1954, Petsche and Marko 1954, Livanov and Ananyev 1955, Ananyev 1956 for movement of electric waves along the cerebral surface, or by reconstruction of three dimensional movement of an electric field from individual tracings recorded from various depths — Howland et al. 1955). It must therefore be kept in mind that use of phase reversal for bipolar localisation of a focus of electrical activity is possible only if the following conditions are satisfied:

a) a characteristic, constant and well reproducible activity,
b) perfectly simultaneous activity in the compared leads,
c) the assumption of a stationary electric field.

Reproducibility is especially important as this makes it possible to localise phenomena whose time course is exactly determined even when using a single-channel apparatus. Thus e. g. the primary cortical response to stimulation of the sciatic nerve has an exactly defined latency that practically does not change during the experiment, provided depth of anaesthesia, blood flow and other physiological parameters remain unchanged. We can therefore compare electric responses in the same intervals from the stimulus in different regions and thus evaluate the phase reversal in recordings taken at different periods. This method is especially suitable when using a cathode-ray oscillograph. If there is a two-channel apparatus available it is even better to connect the lead giving the most characteristic response permanently to one channel and the other leads successively to the other channel, so that we can compare them consecutively with the first one.

2) Fig. 165 shows characteristic oscillographic recordings of primary responses from the auditory area of the cat's cerebral cortex to short acoustic stimuli. The following are easily distinguishable: the latent period, the positive phase of the primary response, the negative phase of the primary response. Since the sweep starts at the same moment when the stimulus is applied, no stimulus artefact is visible. The positive phase is constant and changes little. The negative phase, on the contrary, is variable. The latent period corresponds to the mechanical transfer of sound, stimulation of the organ of Corti and conduction of impulses along nerve fibres and nervous pathways across a number of synapses into the cerebral cortex. The positivity of the cortical

surface is an expression of the negativity arising at synapses of deeper cortical layers (4—5) where fibres of the specific afferent pathway terminate. The change of positivity to negativity is an expression of the fact that the area of negativity has spread to the cortical surface due to ortho- or antidromic activation of the superficially situated parts of the dendrites. Responses from

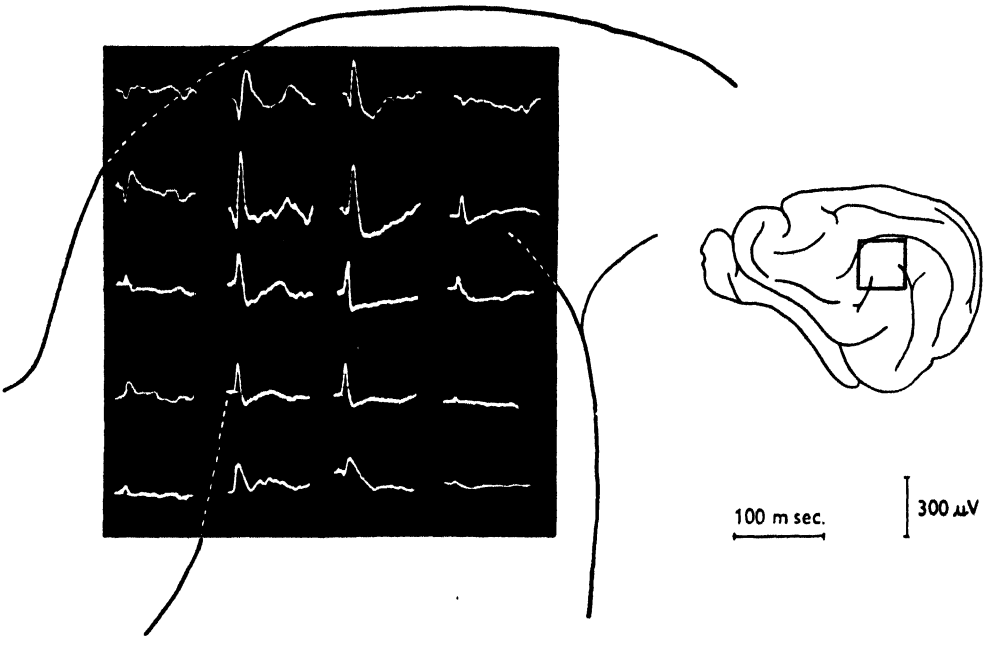
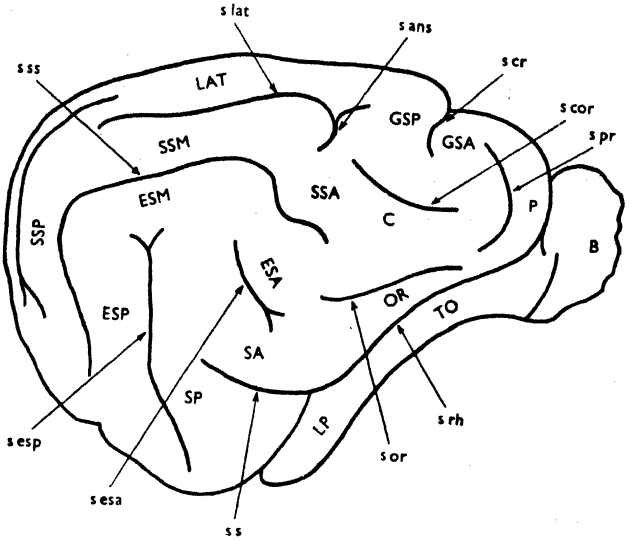


Fig. 165. Topography of primary responses to auditory stimuli in the cerebral cortex of the cat. Upward deflection indicates positivity of the active electrode.

all areas have a similar shape, as might be expected from the uniform structure of the cerebral cortex in those areas.

Oscillographic recording can be used for monopolar mapping of projection areas. Fig. 165 shows the result of such mapping of the auditory area in the cat. Fig. 166 shows the extent of the main analysers in the most important laboratory animals. Data from electrophysiological work from recent years and our own experimental material was used for its construction (Bremer and Dow 1939, Adrian 1940, Woolsey and Waltzl 1942, Marshall, Talbot and Ades 1943, Walzl and Mountcastle 1949, Patton and Amassian 1952, Kempinsky 1951, Artemyev 1951, Mickle and Ades 1952, Chernigovsky 1956 for the cat, O'Leary and Bishop 1938, Woolsey and Wang 1945, Woolsey 1947, Thompson, Woolsey and Talbot 1950, Adey, Carter and Porter 1954 for the rabbit, Woolsey and Le Messurier 1948, Le Messurier 1948, Benjamin and Pfaffman 1955, Libouban and Oswaldo-Cruz 1958 for the rat).

Detailed mapping of responses to certain stimuli in extensive areas of the cerebral cortex shows characteristic differences not only in amplitude but also in latent periods of primary responses in different parts of a cortical projection area.



a

Fig. 166a. The main anatomical structures in the cat's hemisphere.

B — bulbus olfactorius
C — g. coronarius
ESA — g. ectosylvius anterior
ESM — g. ectosylvius medius
ESP — g. ectosylvius posterior
GSA — g. sigmodeus anterior
GSP — g. sigmoideus posterior
LAT — g. lateralis
LP — lobus pyriformis
OR — g. orbitalis
P — g. proreus
SA — g. sylvius anterior
SP — g. sylvius posterior
SSA — g. suprasylvius anterior
SSM — g. suprasylvius medius
SSP — g. suprasylvius posterior
TO — tractus olfactorius
s ans — s. ansatus
s cor — s. coronarius
s cr — s. cruciatus
s esa — s. ectosylvius anterior
s esp — s. ectosylvius posterior
s lat — s. lateralis
s or — s. orbitalis
s pr — s. praesylvius
s rh — s. rhinalis
s s — s. sylvius
s ss — s. suprasylvius

It is possible to construct isolatent lines connecting sites at which the onset or peak of positive waves (or other characteristic parts of the response) appears at the same time after stimulation. The isolatent lines concentrate around one or more foci in which the positive wave appears first and then continues to spread irregularly into more distant areas (Mickle and Ades 1953, Lilly and Cherry 1954). According to the type of response we distinguish primary cortical projection areas (I) with the shortest latent period and maxi-

mum positivity and secondary projection areas (II or III). These are parts of the cortex immediately adjoining to area I through which they receive corresponding afferent impulses (e. g. auditory area II in the cat — Ades 1943, Bremer, Bonnet and Terzuolo 1954). The latent period in them is much

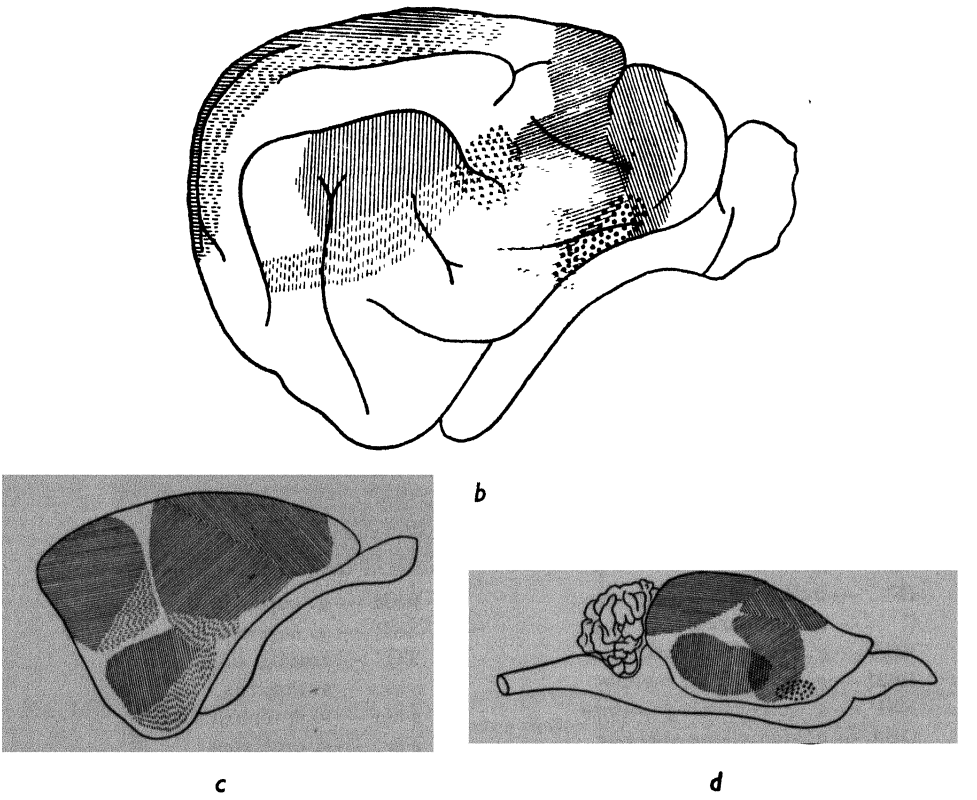


Fig. 166b,c,d. Map of the projection areas of the cerebral cortex of the cat (b), rabbit (c) and rat (d). Horizontal shading — optic area, vertical shading — acoustic area, oblique shading (from top right to left bottom) — somatic area, oblique shading (from top left to bottom right) — motor area, crosses — vestibular area, dotted — gustatory area. Interrupted shading indicates corresponding secondary and tertiary projection areas.

longer than in projection areas I (20 msec. instead of 6—10 in the cat) and their activity follows all changes occuring in area I. At other times we find responses with a short latent period even in those areas (e. g. auditory area III, the activity of which does not depend on the functional state of area I — Tunturi 1945, Mickle and Ades 1952). In such cases direct afferent connections with subcortical centres of the specific pathway evidently exist.

3) The absolute refractory period of the primary response to acoustic stimuli in the cat is 30—60 msec., the relative refractory period lasts 60—100

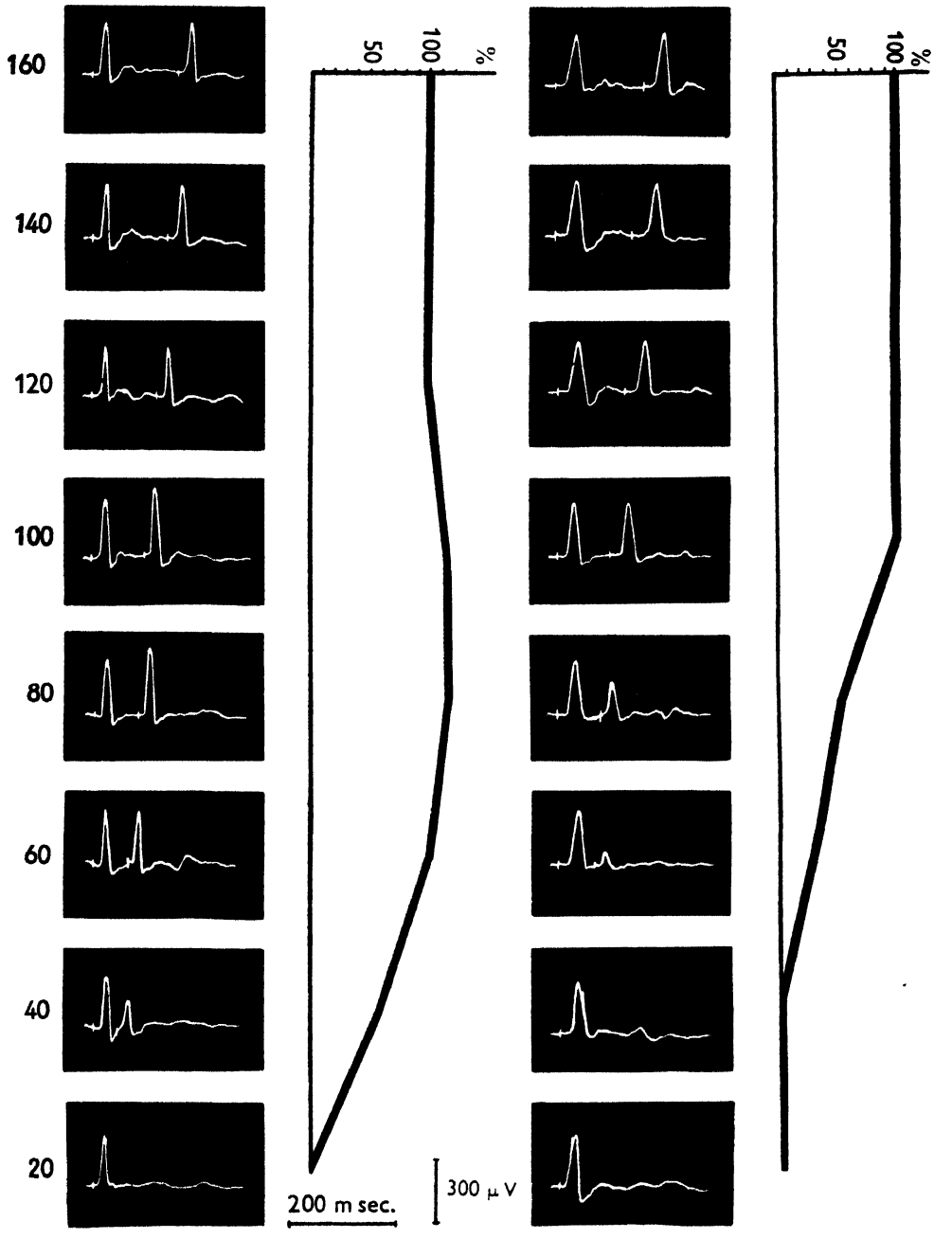


Fig. 167. The refractory period of the primary cortical response to acoustic clicks in the cat. Left: light anaesthesia. Right: deep barbiturate anaesthesia. Figures denote the delay of the second stimulus in msec. Upward deflection denotes positivity of the active electrode.

msec. according to the depth of anaesthesia. With longer intervals between stimuli it is possible in some experiments to register a transitory increase in the second response above the control level. Such excitability curves were described in the optic cortex by Marshall 1949, Gastaut et al. 1951, Chang 1952, in the somatosensory cortex by Heinbecker and Bartley 1940, Marshall 1941,

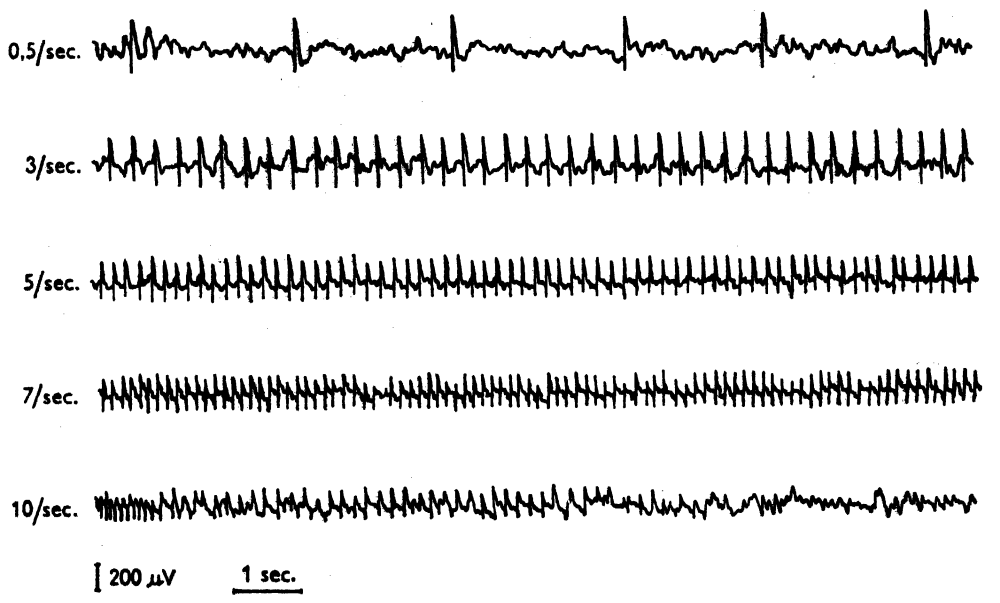


Fig. 168. Cortical responses to repetitive stimulation of the sciatic nerve in rat (dial anaesthesia). Monopolar recording. Downward deflection — positivity of the active electrode.

Jarcho 1949, in auditory cortex by Chang 1950, Rosenzweig and Rosenblith 1953. Fig. 167 shows a typical recording of the refractory period to paired acoustic stimuli in the cat. The amplitude of the response to the second stimulus as a percentage of that of the response to the first stimulus for different intervals between both stimuli, is illustrated by the curves in Fig. 167. They differ characteristically according to the depth of anaesthesia and the overall state of the animal.

A train of stimuli of a given frequency produces a series of cortical responses (Roitbak 1956, Smirnov 1953). If the frequency of stimuli is low, the cortex responds typically to every stimulus. With increasing frequency of stimulation the positive phase begins to predominate; the negative phase disappears occasionally at first and permanently later on. On further increasing the frequency of stimulation the response assumes a rudimentary character, the amplitude of the positive phase decreases and finally the projection area

begins to respond to every second or third stimulus only. In some cases it responds irregularly or ceases to respond altogether. The ability to follow the rhythm of stimulation (so called lability — Wedensky 1903) changes during stimulation. It can often be observed that the projection area responds to every one of the first few impulses, then only to alternate ones and finally without any regularity. The complete picture may develop during a train of stimuli with a frequency of 10/sec. lasting for several seconds (Fig. 168).

Secondary response

In deep barbiturate anaesthesia (dial 50 mg./kg.), when nearly complete suppression of spontaneous EEG activity has been achieved, sensory stimuli, particularly stimulation of the sciatic nerve, produce in addition to the primary response a further electrical response with an amplitude several times higher and with a latency of 50—100 msec. (Derbyshire, Rempel, Forbes, and Lambert 1936, Forbes, Battista, Chatfield and Garcia 1949, Forbes and Morrison 1939, Purpura 1955). In shape the secondary response is similar to the primary one but both its positive and negative components are prolonged to about 50 msec. (Fig. 169). Stronger stimuli are usually needed to produce the secondary response.

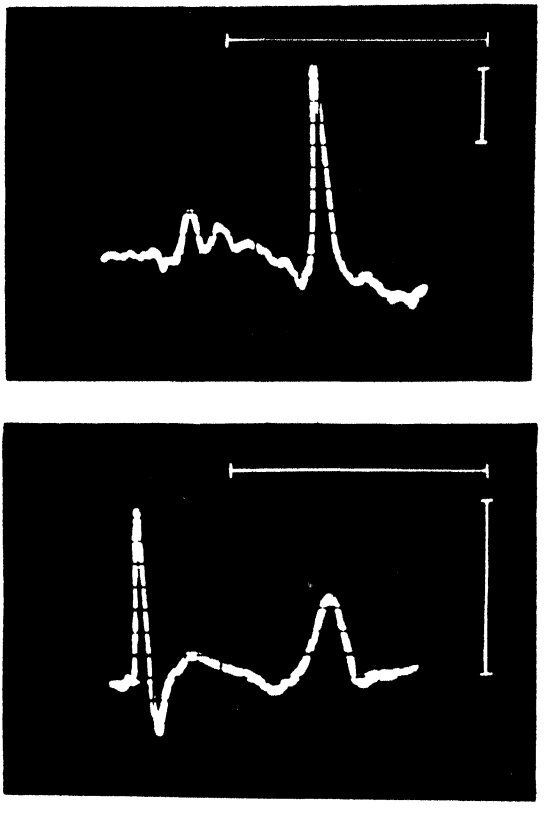


Fig. 169. Secondary response to acoustic stimulus in cat (dial anaesthesia). Top: recording from g. sigmoideus posterior. Bottom: recording from g. ectosylvius anterior. Monopolar recording. Upward deflection — positivity of the active electrode. Calibration: 100 msec, 200 μV.

In contrast to the primary response the secondary one is not limited to the specific projection area. In the cat it can be recorded from the whole surface of the exposed hemisphere even though its shape is not the same in the different areas. The secondary response is thus diffusely distributed regardless of the

stimulus producing it — be it an acoustic or optic stimulus or stimulation of the sciatic nerve. Simultaneous stimulation of 2 nerves (e. g. both sciatic nerves) results in considerable facilitation of the secondary response which is then often several times larger than the response produced by stimulation of each nerve separately.

The long latent period indicates the complex polysynaptic nature of the secondary response. This is also confirmed by experiments in which the refractory period of the secondary response is determined. The absolute refractory period lasts 0·2—0·5 sec., the relative 0·7—1·5 sec. The absolute refractory period of the primary response is usually less than 30—60 msec. With repeated stimuli the refractory period of the secondary response rapidly increases, individual responses fall out or disappear altogether by the time when a stimulus frequency of about 1 cycle/sec. is reached.

The mechanism of the secondary response has not as yet been elucidated completely. It would appear that this phenomenon is closely related to the synchronous barbiturate spindles, to the recruiting response and to the activity of the reticular system of the brain stem (cf. p. 409).

The term "secondary responses" is often also used for other extraprimary potentials appearing after stimulation of afferent systems outside the corresponding cortical projection areas in unanaesthetised animals or under chloralose anaesthesia — in the cat especially in associative cortical areas (G. suprasylvius, G. lat. ant.). Again these are mostly bilateral surface positive waves which do not change after extirpation of the corresponding primary projection area. Their latency is always longer than that of primary responses. It is, however, shorter than that of secondary responses in deep barbiturate anaesthesia (Buser and Borenstein 1956, Albe-Fessard and Rougeul 1955, 1956).

Conclusion: Monopolar or bipolar recordings of primary cortical responses permit mapping of projection areas of individual analysers or of their components and determination of their excitability.

E. Responses of the cerebral cortex to direct electrical stimulation

Problem: Record the direct cortical response to a short electrical stimulus and the spreading of this response over the cortex.

Principle: Stimulation of the exposed cortical surface with a short electric stimulus (1 msec. or less) produces a characteristic biphasic response spreading a certain distance from the stimulated point (Adrian 1936, Rosenblueth and Cannon 1942, Burns 1950, 1951, Chang 1951a, Bishop and Clare 1953, Clare and Bishop 1955, Roitbak 1955, Ochs 1956, Purpura and

Grundfest 1956, Vorontsov 1957, 1958). In unanaesthetised animals (encéphale isolé preparation) a weak stimulus produces a brief surface negative wave (Adrian's "surface response"), spreading at a rate of 2 m/sec. to a distance of 4—8 mm. A stronger stimulus evokes a surface positive wave of longer duration which spreads in all directions at a rate of 20 cm/sec. without any evident decrement (Adrian's "deep response"). In animals under barbiturate anaesthesia a surface negative wave predominates. The detailed nature of this response has not yet been elucidated. Participation of apical dendrites of pyramidal cells, Cajal cells of the molecular layer and short associative cortico-cortical connections have been postulated.

From the technical point of view the main problem is the reduction of the stimulus artefact to such an extent as to make possible recording of the response from close to the stimulating electrodes.

The following conditions must be achieved:

1) perfect isolation of the stimulating circuit from earth (isolating transformer, radiofrequency output — cf. p. 89) in order to minimise the capacitance of the stimulating electrodes to ground;
2) the use of differential amplifiers with a high discrimination of in-phase signals (cf. p. 118); omission of the grid resistors may sometimes improve the symmetry of the input circuit.
3) bipolar stimulation ensuring minimum current spread;
4) the use of non-polarisable stimulating and recording electrodes in order to limit poststimulation changes in the circuit;
5) the use of very short pulses (about 0·1 msec.) to prevent interference with the reaction;
6) geometry of the stimulating and recording electrodes. The monopolar electrode is placed roughly in the line midperpendicular to the stimulating electrodes. For the stimulus used this line (Fig. 170) corresponds to the zero potential level. The reference electrode is placed approximately on the same line but, of course, at a considerable distance from the site of stimulation.

In addition, various methods of electrical compensation of the stimulus artefact may be used (see e. g. Mozhayeva 1958). These will be mentioned later on.

Object: rat, 200 g. weight.

Apparatus: D. C. or A. C. (time constant at least 0·5 sec.) preamplifier with cathode-ray oscillograph, total amplification at least 50 μV/cm. A two channel apparatus is preferable. A stimulator giving single square wave pulses or condenser discharges synchronised with the time base of the oscillograph. An isolation unit (transformer, radiofrequency output). A time marker or a tone generator.

Other requirements: Surgical instruments, a 5 mm trephine, ball-tipped or wick electrodes for corticography, with electrode carriers. An animal board for the rat with a stereotaxic head holder.

Procedure: The rat is deeply anaesthetized with dial (40 mg./kg.). Curarisation is unsuitable since, according to Purpura and Grundfest (1956),

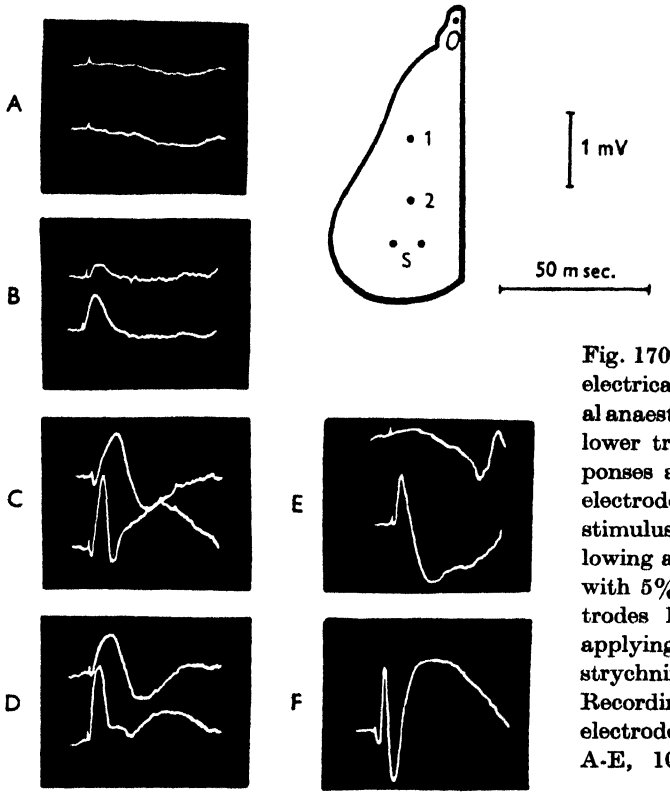


Fig. 170. Cortical response to direct electrical stimulation in rats (dial anaesthesia). Upper tracing 0—1, lower tracing 0—2. ABCD — responses at the proximal and distal electrodes, with gradually increasing stimulus strength. E — 5 min. following application of a filter paper with 5% novocaine between electrodes 1 and 2. F — 5 min. after applying filter paper with 1% strychnine between 1 and 2. Recording from the more distal electrode. Calibration: 50 msec. for A-E, 100 msec. for F. Negative deflection upward.

d-tubocurarine depresses direct cortical responses. If anaesthesia is to be avoided succinylcholine is used for immobilisation.

A trephine opening 5 mm in diameter is made over the parietooccipital region of one hemisphere. The cerebral cortex is widely exposed by rongeuring away the parietal bone. The dura is cautiously cut and the dural flaps are reflected.

The electrodes consist of plexiglass bars with thin steel springs (3 × 30 mm) on which flexible, ball-tipped (0·5 mm) silver wires (0·2 mm in diameter, 2·5 cm in length) are mounted. The spring ensures a constant pressure on the cortical surface, the thin flexible wire permits limited movement of the electrodes. Wick Ag-AgCl electrodes serve equally well for recording. Silver wire low-impedance electrodes are preferable for stimulation. As reference

electrode a needle in the frontal bones is used. The electrodes are situated as shown Fig. 170. The stimulating electrodes are 0·5—1 mm apart; the recording electrodes are placed 3—5 and 6—10 mm from the former.

After the electrodes are connected, the sweep is synchronized with the stimulus onset and regulation of the stimulus artefact is begun. With stimuli of low intensity and with a maximum brightness of the spot the amplitude and direction of the stimulus artefact are observed while moving the recording electrode along a line parallel to the stimulating electrodes. Usually a point is found, in the vicinity of which the stimulus artefact changes its polarity and thus reaches its minimum amplitude. Finer regulation of the stimulus artefact may be obtained by the use of one of the following methods.

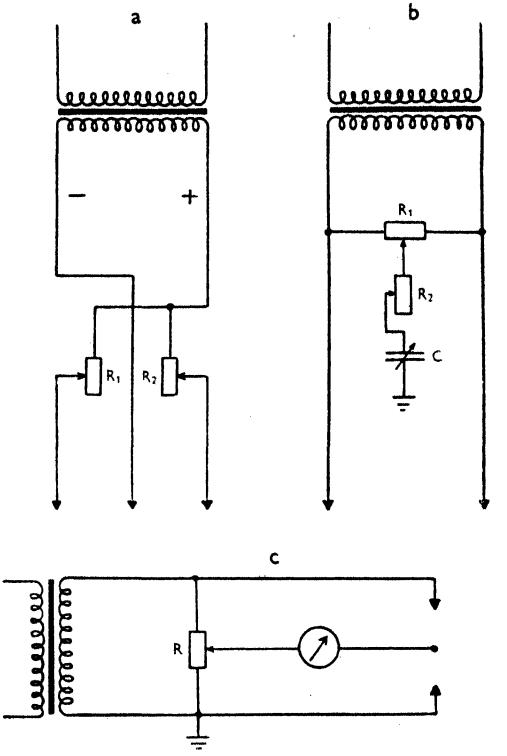


Fig. 171. Methods for electrical compensation of the stimulus artifact. For details see text.

a) The use of tripolar electrodes (Bishop and Clare 1953). The stimulating cathode is situated in the middle (cf. Fig. 171a). The positivity of both anodes is controlled by two variable resistors 1 MΩ permitting gradual changes in the shape of the electric field around the electrodes. This system may, of course, only be used if all three electrodes are firmly fixed in position. The most suitable position for the recording electrode is approximately found and the stimulus artefact is then adjusted with the help of the variable resistance in the anodes.

b) The use of balancing reactance. Part of the stimulating voltage from the potentiometer (10 kΩ), connected in parallel to the stimulator output is earthed through a variable RC network (0·0001—0·1 μF, 1—2 MΩ potentiometer — Fig. 171b). By suitable adjustment of the output potentiometer and the earthing reactance a signal is directed to earth the polarity, amplitude and duration of which compensate for the stimulus artefact. A simpler application of the same method of compensation is the use of so called Wagner's earth. The middle point of the output potentiometer is simply earthed. The use of this method of compensation excludes the use of a radiofrequency output.

c) Bridge arrangement of the stimulating and recording electrodes

(Philips 1956). One of the stimulating electrodes is earthed (the indifferent one in monopolar stimulation). The middle point of the potentiometer connecting both stimulating electrodes is used as reference (cf. Fig. 171c). A potential is found by trial and error corresponding to that of the recording electrode. The bridge is thus equilibrated. Between stimuli the stimulating electrodes are short-circuited by the low impedance output of the stimulator. The potential difference between these 2 electrodes and the active electrode is then registered. This method is especially suitable for recording high voltage phenomena, e. g. when working with microelectrodes. It can be used for registering processes occuring directly at the stimulated point.

After the stimulus artefact has been satisfactorily minimised, the intensity of the stimulus is gradually increased and the response is registered simultaneously from the proximal and distal electrode. Thus the shape of responses to threshold and to maximum stimuli is determined together with the extent of their spread in the cortex. The rate of spread of the response may be calculated from the distance of the recording electrodes and the relative delay of the different components of the response in the distal lead.

The physiological nature of the response may be tested by its sensitivity to various procedures. Asphyxia is most suitable. This is obtained easily by clamping the trachea for 1—3 minutes. The response can also be affected by topical application of drugs on pieces of filter paper 1—2 mm by 4—8 mm, applied between the stimulating and recording electrodes or between the proximal and distal recording electrodes. A 5% solution of Novocaine is used to suppress spreading of the response. A 1% strychnine nitrate solution increases the response.

For elucidation of the spreading mechanism subpial incisions are made, cutting the cortex between the stimulating and recording electrodes to a depth of 1—3 mm.

It is further possible to determine the refractory period of the response, its changes during frequent stimuli, interaction of two pairs of stimulating electrodes etc.

At the end of the experiment the animal is sacrificed and the response recorded from the dead brain with stimuli of different intensities. Such an experiment gives especially valuable information concerning the character of the electric field arising in the volume conductor of the brain around the stimulating electrodes.

Results: The direct cortical response is a biphasic positive-negative wave. Its positive component decreases with increasing anaesthesia. A weak stimulus produces a response only at the proximal electrode, a strong one also at the distal electrode. The spreading rate of the negative maximum is about 1—2 m/sec. (Fig. 170A–D). Anoxia suppresses first the positive and later also the negative component of the direct cortical response, so that after 1—1·5 min.

only the stimulation artifact can be obtained, as in the dead animal. After recovery of normal ventilation these parts of the response reappear in the opposite order.

A strip of filter paper saturated with 5% novocaine suppresses spreading of the response from the stimulating to the recording electrodes within a few minutes (Fig. 170E). Care must, however, be taken to prevent this change occuring as a result of short circuiting of the stimulating electrodes by excess fluid. Records obtained after removal of the filter paper and drying of the cortical surface are most reliable. The effect of novocaine persists for a short time following application and is rapidly reversible. A similar but irreversible effect is obtained with transcortical incisions. Depending on their depth they result either in a decrease or complete suppression of the direct response at the more distant electrode.

A strip of filter paper saturated with 1% strychnine nitrate produces a considerable increase of the direct response at both the proximal and distal electrodes. If the intensity of the stimulus is gradually decreased a value is found at which the negative potential is evidently divided into two components (Chang 1951b). The shape and latency of the first corresponds to the original negative wave of the direct response. The second wave, however, is higher, arises suddenly at a certain stimulus intensity and evidently corresponds to synaptic activation of deeper cortical layers (Fig. 170F).

Conclusion: A cortical response to direct electric stimulation represents a reaction in which probably various cortical elements participate and which thus permits a study of different aspects of cerebral cortical activity.

F. Mapping of nervous pathways on the cerebral nervous system using neuronography

Problem: Determine the projection of a cortical area of one hemisphere via neurones of the corpus callosum onto the contralateral hemisphere.

Principle: Electric stimuli of sufficient intensity cause a synchronous discharge of neurones situated in the area in which the electric field of the stimulus attains suprathreshold intensity. If these neurones give rise to a group of long fibres, it is possible to register along this path an electric wave corresponding to the synchronous volley. In particular activation of the terminal synaptic area of the pathway manifests itself by very conspicuous electric signs (e. g. cortical primary responses). Areas showing an electric response synchronous with the stimulus must therefore be connected via nervous pathways with the stimulated point.

• Local application of strychnine can be used instead of an electric stimulus. This is applied either using filter paper on the surface of nervous tissue or

in the form of a paste applied through a needle into deeper regions. Strychnine applied topically to the grey matter of the CNS produces spontaneous electric discharges so called "strychnine spikes" (Gozzano 1936, Dusser de Barenne and McCulloch 1936a, b, 1939, Dusser de Barenne, Garol and McCulloch 1941, Frankenhauser 1951), predominantly negative waves of high amplitude (1—3 mV) corresponding to synchronous activity of the poisoned neurones. A corresponding volley of impulses along axons of discharging neurones produces synchronous electric potentials even in remote areas. Strychnine spikes are limited to the distribution of neurone whose synaptic area is affected by strychnine. Only low voltage variations are registered from localities to which tracts from the strychninised area have to pass through one or more synapses. In distinction to electric stimulation

a) strychnine does not act on nerve fibres but exclusively on synapses (for that reason application to white matter remains without effect),
b) strychnine spikes spread only orthodromically. They can never be recorded from nerve fibres sending afferent impulses to the synapsis affected by strychnine.

These characteristics of strychnine spikes make it possible to use topical strychnine application for tracing centrifugal projection of a group of neurones. The disadvantage of this procedure lies on the fact that not all neuronal systems produce convulsive waves following local application of strychnine (Frankenhauser 1951), so that negative results obtained with strychnine neuronography do not exclude the possibility of nervous connection existing.

Local application of strychnine can also be used in another way. Low concentrations of strychnine, inadequate to cause spontaneous discharges, distinctly increase evoked potentials. If these are small, so that they are difficult to identify in the basic activity, local strychninisation of the point of recording results in their accentuation. At a site, on the other hand, that is not connected via nervous pathways with the area of stimulation local application of strychnine will be uneffective.

Object: Cat, 2—3 kg. weight.

Apparatus: A dual beam cathode-ray oscillograph with D. C. or A. C. amplifiers (total time constant of at least 0·5 sec. and a maximum amplification of at least 50 μV/cm); or an ink-writing multichannel electroencephalograph. A stimulator giving single square wave pulses or condenser discharges synchronised with the sweep of the oscillograph. An isolation unit to the stimulator output. A time marker.

Other requirements: Surgical instruments, a set of trephines, a dental electric drill. Wick Ag-AgCl recording electrodes and bipolar ball-tipped silver wire electrodes for stimulation, with electrode-carriers. An animal board with head holder for fixing the animal. 10% Dial, strychnine nitrate (crystalline and 1% solution).

Procedure: A cat in dial anaesthesia is operated as in experiment VIII D2. Wide craniotomy, however, is carried out over both hemispheres. Only a narrow trip of bone (3—5 mm) above the sagittal sinus is left in place to prevent accidental injury. The dura is cut and the dural flaps are reflected.

1) The stimulating electrodes are placed on the gyrus suprasylvius of one hemisphere and spaced 1 mm apart. Stability of contact is ensured with a fine spring pressing the electrodes to the cerebral surface. The recording wick Ag-AgCl electrode is placed on the corresponding area of the contralateral hemisphere, the reference electrode on the cut skin. The stimulus artifact is decreased by means described in experiments with direct cortical responses (cf. p. 351), its regulation, however, is not too difficult. Stimuli are applied at 1—2 sec. intervals, responses are observed or photographed. If only rough evaluation of polarity and amplitude is required, recording responses with a conventional ink-writing electroencephalograph suffices. First the site of the maximum response is approximately located. One recording electrode is permanently left at this point and the latter's response is recorded in one channel. The other recording electrode is moved systematically in the parasagittal and frontal planes in 2 mm steps and responses from various points of the grid are compared with the simultaneously recorded maximum response. Each position of the electrode is marked with a drop of india ink. After mapping one projection area the stimulating electrodes are moved 1·5—2 cm and a further projection area is mapped.

2) The extent of the projection of the stimulated site onto the contralateral hemisphere can also be tested in the following way. The stimulating and recording electrodes are placed on symmetrical sites in the anterior part of the gyrus suprasylvius. The maximal response to slightly supramaximal stimuli is found and then the stimulating electrodes are moved in 2 mm steps in the parasagittal plane occipitally or frontally until the response on the fixed recording electrode disappears. Then the stimulating electrodes are again placed on the original site and the area of the recording electrode is treated with a 1% solution of strychnine using a filterpaper 2 × 2 mm. The area surrounding the electrode is first carefully dried and excess fluid is removed from the paper in order to decrease strychnine diffusion as far as possible. Then the change in the callosal response in the strychninized area is studied. As soon as there is a clear-cut increase in the negative phase of the callosal potential the paper is removed. The potentials continue to increase under the influence of strychnine for several more minutes and then reach a constant level which is maintained for 20—40 minutes. When stabilisation of the increased response is attained the stimulating electrodes are again moved along the suprasylvian gyrus in the above manner and the responses are recorded. In a similar way the extent of various projections onto a certain point of the cerebral cortex can be determined after its treatment with strych-

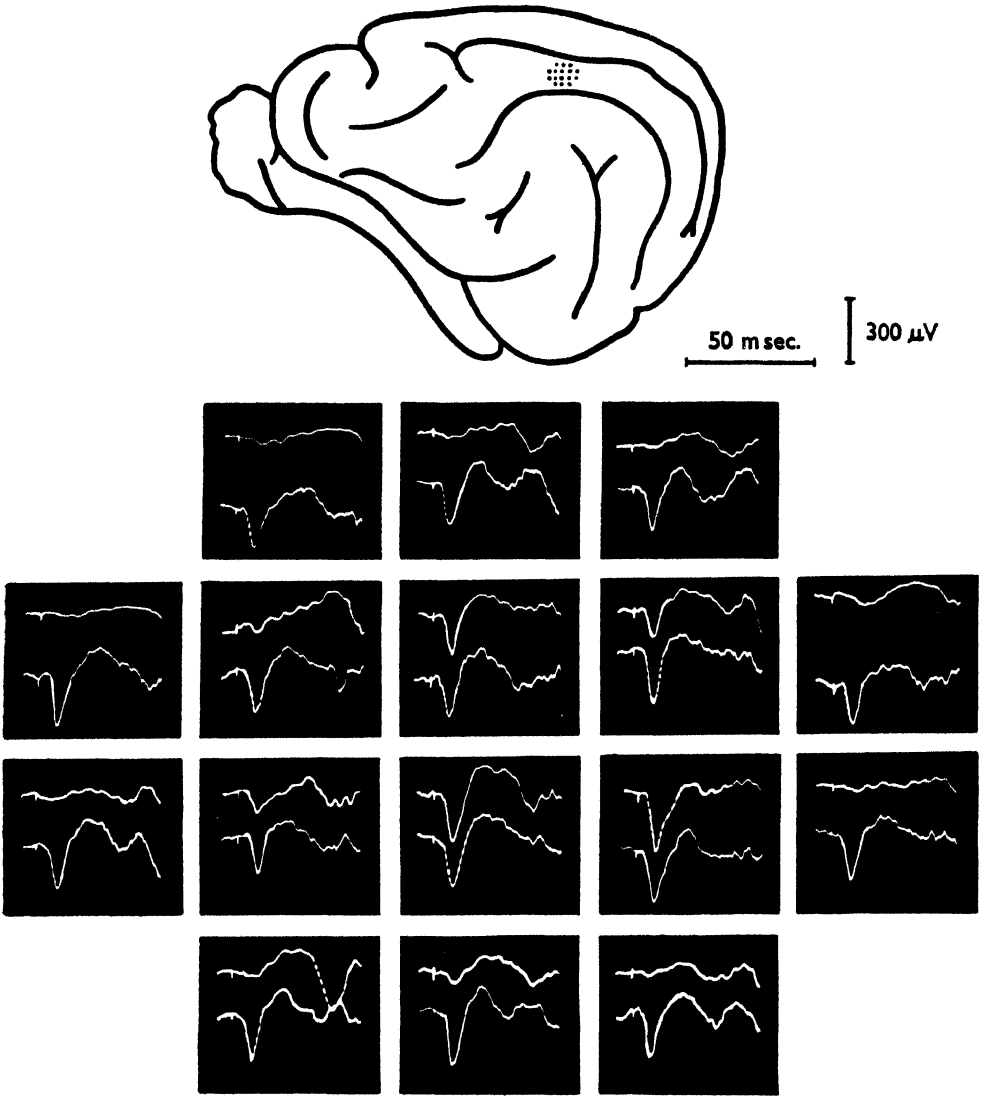


Fig. 172. Distribution of callosal responses to stimulation of a symmetrical point in the g. suprasylvius in the cat. Lower tracing — recording from the point of maximal response. Upper tracing — response from points indicated in the upper diagram. Positive deflection downward.

nine by stimulating other cortical or subcortical areas. It must be emphasised that the success of this method depends largely on the conditions for stimulation in different stimulated sites being completely identical. The distance between the stimulating electrodes must remain constant as must the moisture of the cortical surface and the contact pressure.

3) The experiment is continued by again applying strychnine onto the area treated. A filter paper soaked in saturated strychnine solution or a small crystal of strychnine may be used. Great care is taken to prevent diffusion of strychnine into the surroudinng areas. The paper (or crystal) is removed as soon as spontaneous strychnine spikes appear, usually within 2—5 min. Then one electrode is placed directly on the strychnine treated cortical point. The second electrode (or with multichannel EEG a row of electrodes) is placed symmetrically onto the opposite hemisphere (which had been stimulated earlier) and is gradually moved as in the previous experiments. Tracings are either photographed onto a continuously moving film with a stationary spot or on single frame pictures with sweep triggered by discharges in the strychnine treated area. Recording of the high and relatively slow strychnine spikes with an ink-writing multichannel EEG is especially suitable. After mapping the projections of strychnine spikes onto the opposite hemisphere the area treated with strychnine is rinsed with warm saline and may be covered with a bit of cotton wool soaked in Ringer solution. The slow disappearance of the strychnine spikes (30—60 min.) is observed in the EEG recording. The wick of the electrode in contact with the strychnine-treated area is exchanged since it might be soaked with concentrated strychnine solution and thus cause strychnine potentials in normal areas to which it is applied later. If strychnine is used repeatedly it is applied to a region of the hemisphere removed as far as possible from the previously treated area.

Results: 1) The electric response to stimulation of a symmetrical area of the contralateral hemisphere is similar to the primary response to sensory stimuli. First a positive wave appears lasting for 10—15 msec. and having a latency of 3—4 msec. This is replaced by a longer-lasting negative wave. The ratio of the amplitudes of the two phases changes according to the area stimulated and also depends on the depth of anaesthesia. The strongest response (up to several times higher than elsewhere) in the cat is obtained in the gyrus suprasylvius and the weakest in the sensorimotor cortex. These differences are probably due to the relative density of callosal neurones in different areas. The area from which maximum responses to stimulation of a certain fixed contralateral point may be obtained is very small — hardly more than 4 to 6 mm^2. For a further 2—3 mm it is surrounded by a zone in which the amplitude of the response rapidly decreases (Fig. 172). Potentials in this zone may, of course, be explained in part by physical spread of the current from the area of maximum projection.

2) Results with moving the stimulating electrodes are similar. The fixed recording electrode gives a maximum response on stimulation of a symmetrical site. If now the stimulating electrode is moved in the parasagittal or other direction the response rapidly diminishes until it disappears alto-

gether if the stimulating electrode is moved 4 mm from the site giving the maximum response.

The effect of local application of 1% strychnine is seen as a nearly instantaneous increase in electrical response in the intoxicated area (Fig. 173A). The positive component is hardly changed while the amplitude of the negative component increases up to 10 fold and is considerably prolonged. If during this initial stage of a strychnine effect the filter paper is removed, strychnine potentials will be produced only by a volley of impulses coming into the area treated with strychnine. Prolonged application of strychnine produces spontaneous strychnine spikes occuring without evident relationship to external stimuli. Electric stimuli, of course, then also cause strychnine spikes, provided they do not coincide with the refractory period of a spontaneous discharge.

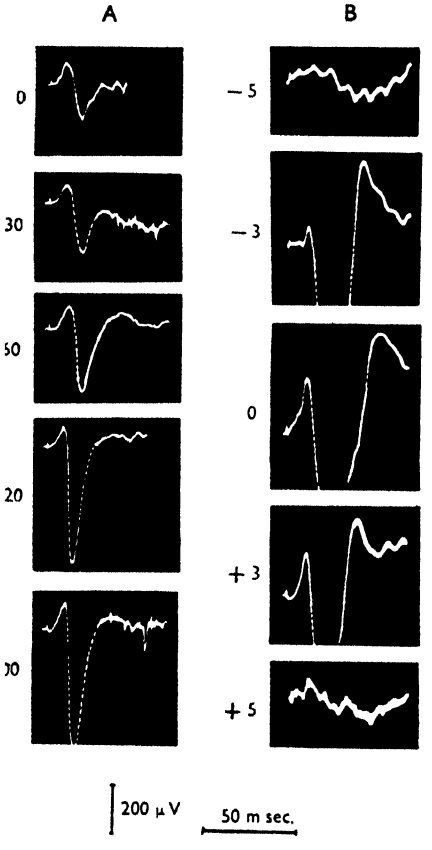


Fig. 173. The effect of strychnine on the shape and distribution of callosal potentials. A) Gradual increase in the negative component of the callosal response, after applying 1% strychnine. Figures indicate time in seconds following strychnine application. B) Maximal responses of the same point after strychninisation. The stimulating electrodes are moved along the contralateral g. suprasylvius. 0 — symmetrical point. Other figures denote distances in mm from 0. Upward deflection — positivity of the active electrode.

If now the stimulating electrodes are again moved the extent of the cortex activating the strychnine treated area on stimulation is larger (Fig. 173B). This is due to the fact that even a weak afferent influx, hardly discernable in recording from the normal cortex, produces a maximum all-or-none response in the intoxicated area. For this reason the transfer of the stimulating electrodes into an area not connected by nervous fibres with the strychnine treated region results in a nearly instantaneous disappearance of the response. If previously the spike occured after every stimulus, now it appears only occasionally and on further moving the electrodes it disappears altogether. The same technique may be used for mapping other projections and establish-

ing fine differences within them, as was shown for the auditory cortical area by Tunturi (1950), Hind (1953) etc.

In experiments a and b the extent of the projection depends, among other things, on the strength of the stimulus and the spacing of the stimulating electrodes. With increasing intensity of the stimulus and spacing of the stimulating electrodes the number of stimulated neurones increases and so does the size of the callosal projection area. It is therefore preferable, especially

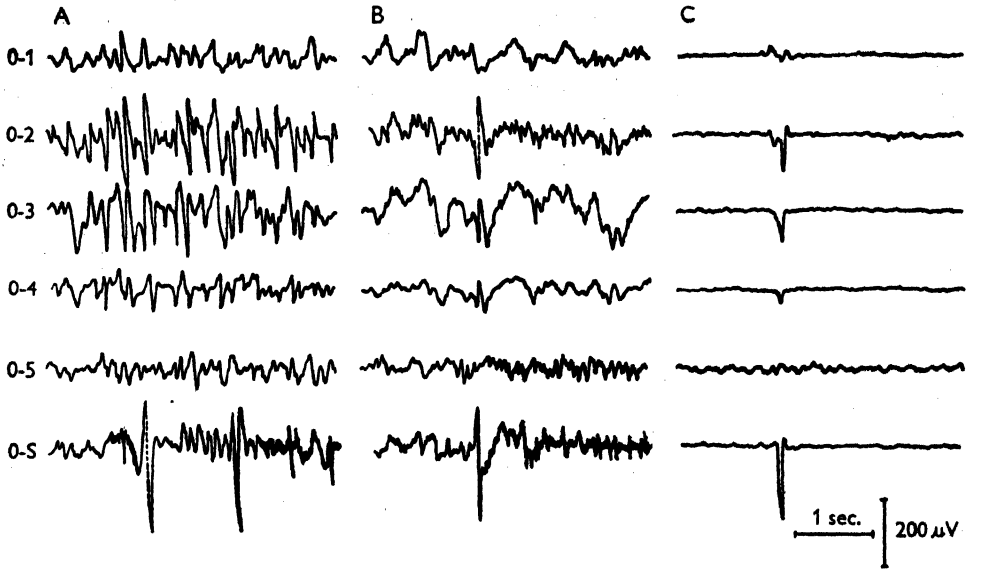


Fig. 174. EEG recording of projection into the contralateral g. suprasylvius of spontaneous strychnine spikes, evoked by application of strychnine crystal on the g. suprasylvius 1—5 — parasagittal row of electrodes in g. suprasylvius. Interelectrode distance 3 mm. ABC — deepening anaesthesia.

in experiments with strychnine, to use weaker (liminal) stimuli. Transsection of the corpus callosum is a reliable way of demonstrating that the recorded potentials are of callosal origin.

3) Spontaneous strychnine discharges are characterised by a negative wave having an amplitude of 1—2 mV and lasting for about 100 msec. followed by a less distinct positivity. They appear in irregular intervals (0·2—2/sec.), with higher concentrations of strychnine they may form trains with a frequency of 5/sec. Single spikes running at 1—2 sec. intervals are most suitable for neuronography. In addition synchronous spikes in the contralateral hemisphere are also registered. Their amplitude is highest in the symmetrical area (Fig. 174). The distribution of potentials is roughly the same as in experiments with electric stimulation — the area of the maximum response is

limited to several mm, then there is a rapid decline in amplitude. The shape of the response, however, is somewhat different. This, among other things, is due to the fact that callosal responses thus produced have no antidromic component. Callosal connections are always reciprocal. The electric current stimulates both ganglionic cells of homolateral callosal neurones (orthodromic conduction of the impulse) and the axon terminals of the contralateral callosal neurones (antidromic conduction of the impulse). A strychnine discharge in the cerebral cortex is only connected with orthodromic activity. Callosal fibres mainly terminate in superficial layers of the cortex, the activation of which appears as surface negativity. The antidromic discharge, on the other hand, terminates on cell bodies of the callosal neurones in deeper layers of the cortex, whose activity appears as surface positivity. The absence of antidromic impulses thus results in a relative weakening of the positive phase of the response.

Strychnine neuronography can be succesfully used for determining various cortico-subcortical or cortico-cortical connections. If the response is localised subcortically the stereotaxic method is used and the sites of maximum response are subsequently identified histologically (p. 396).

Conclusion: Electrical or strychnine neuronography is a valuable means for tracing direct fibre connections between various regions of the brain. The advantage of strychnine neuronography is that the direction of the tract is also determined, absence of the strychnine spikes in a given region, however, does not exclude direct pathways leading to it from strychninized focus. Increasing evoked potentials by local application of strychnine to the projection area permits detection of even very weak afferent activity.

G. Depth recording in the cerebral cortex

Problem: Record the response to stimulation of the sciatic nerve from different cortical layers.

Principle: In earlier experiments different methods of recording electric potentials from the cerebral cortical surface were desribed. Such a record, of course, is an expression not only of the activity of superficial cortical layers but also of the electric fields created by activity of deep cortical elements. Thus the positive phase of the primary response, signalising the arrival of impulses into a specific projection area corresponds in part to action potentials reaching the cortex via afferent pathways (in a similar way as positive action potentials from an electrode placed on the injured end of a nerve fibre are recorded — cf. p. 277), in part to synaptic potentials produced by these impulses on cell bodies and dendrites of pyramidal cells and interneurones (for review of the literature see Eccles 1951, Roitbak 1955, Albe-Fessard 1957).

According to others (Marshall, Talbot and Ades 1943) surface positivity corresponds to movement of impulses from deeper cortical layers towards the surface and the negative wave to spreading from the surface in the opposite direction. Precise data are obtained by experiments with microelectrodes introduced to different depths into the cortex. By comparing recordings from different depths with a simultaneous recording from the surface, the depth of primary negativity and its spread in the cortex can be determined relatively accurately. When interpreting electric recordings thus obtained it is assumed that the excited part of the neurones is depolarised and becomes negative with respect to other non-excited areas. This potential difference gives rise to a current flowing from the less negative (positive) pole of the neurone to the more negative one. The distant indifferent electrode applied to the point where current density is already insignificant will be negative relative to the source of this current and positive relative to the sink of the same current. Such positivity of one area produced by negativity of an adjacent area is due to differences between different parts of the same neurone (between dendrites and the cell body). The circuit is closed through the inside of the neurone. A brief account of the volume conductor theory is given in chapter VIII K.

Object: Rat 150—200 g weight.

Apparatus: Two channel cathode-ray oscillograph with a D. C. or A. C. amplifier (time constant at least 0·5 sec., input resistance 5—10 MΩ, total amplification at least 50 μV/cm. A stimulator giving single rectangular pulses or condenser discharges synchronized with the time base of the oscillograph. An isolation unit for the stimulator. A time marker.

Other requirements: Surgical instruments, trephine (diameter 5 mm), an animal board with a stereotaxic head holder for fixing the animal. Micromanipulation equipment with vertical microdrive of at most 0·5 mm. per revolution and an electrode holder. Insulated steel semimicroelectrodes with a tip diameter of 20—40 microns. Electrodes are best prepared from entomological needles or stainless steel wire (0·2 mm) by electrolytic treatment and consequent insulation with a varnish. For details see p. 160. Wick Ag-AgCl electrodes for corticography, bipolar platinum electrodes for stimulating the sciatic nerve. Electrode carriers. 10% dial. A binocular dissecting microscope.

Procedure: Under dial anaesthesia the skull of a rat is exposed and thoroughly cleaned. A trephine hole is made over each hemisphere (diameter 5 mm) situated so that one third is in front of the coronar suture. Trephining is carried out carefully in order to avoid injuring the dura mater as far as possible. The sciatic nerve is exposed in both thighs for a length of 2 cm and placed onto the stimulating electrodes. The nerves are prevented from drying by paraffin oil filled in a skin cup formed around the exposed nerve.

The experiment is commenced by determining the area of maximum primary response to stimulation of the sciatic nerve as in experiment VIII D 2 —

by gradual mapping of the exposed cortical surface moving the wick of the Ag-AgCl electrode. The reference electrode, a strong needle, is fixed in the frontal bone above the level of olfactory bulbs. At the point where the maximum surface positive response is obtained the dura is pierced with a sharp hook and the microelectrode is introduced. This is controlled visually under the dissecting microscope. The dura (and in the cat and rabitt also the arachnoidea and pia) must be cut in order to prevent excessive pressure on the brain surface during penetration through the strong connective tissue. Indentation of the cortex makes precise determination of depth impossible. The actual depth to which the electrode has been introduced can be estimated from the movement of the micrometer drive while watching for indentation of the cortex at the site of entry with the microscope. Since even after penetration of the

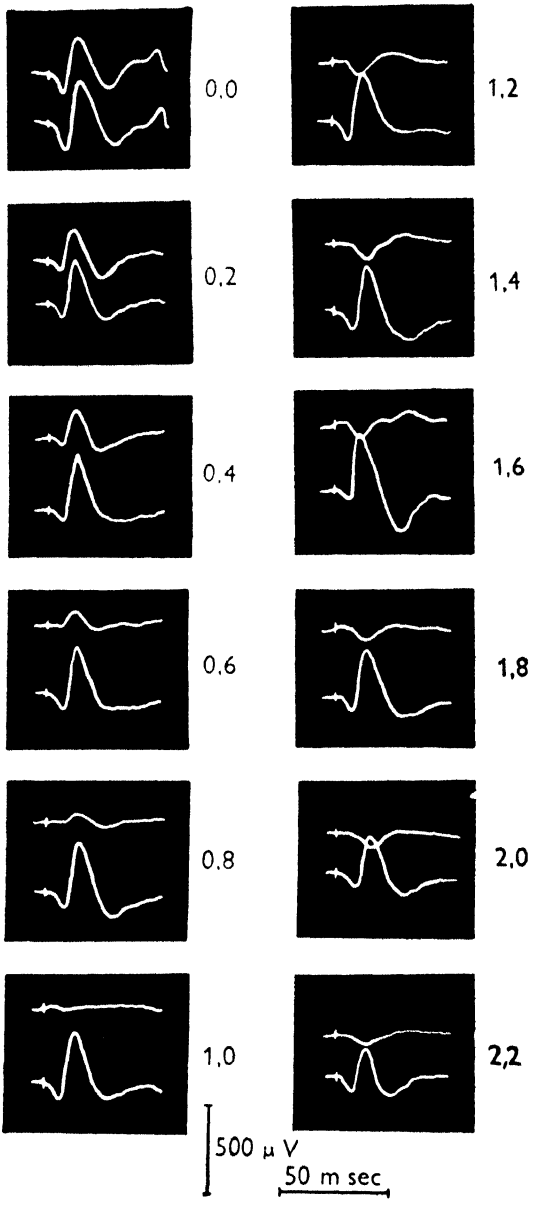


Fig. 175. Deep registration of primary responses on stimulating the sciatic nerve of the anaesthetised rat. Upper tracing — microelectrode recording. Lower tracing — surface recording. Figures indicate the depth of the tip below the cortical surface in mm. Positivity upward.

dura the surface is depressed to a certain extent it is best to introduce the electrode deeper by several tens of microns and then to raise it under view of the microscope until the cortical surface is again at the control level.

When the tip of the electrode just touches the cortical surface the primary response is registered both with the microelectrode and the wick electrode immediately adjacent to the point of insertion. The shape of the two responses is usually the same but the amplitude of the microelectrode record is sometimes lower particularly if the ratio of the electrode resistance to the input impedance of the amplifier is too high (cf. p. 92). In order to make comparison of deep recordings and surface response easier, amplification in the two channels is adjusted so as to obtain the same amplitude on both tracings. Then electrode is moved down 0·1 mm at a time until a depth of 2·5 mm is reached. Several responses are recorded simultaneously from both leads at every level. The same procedure may be applied when raising the electrode. If histological verification of the electrode position is required, a direct current is allowed to pass through the microelectrode (5—20 μA, 5—30 sec., microelectrode +) after the recording has been made. Iron atoms are thus deposited in the tissue and these can easily be detected histologically using potassium ferrocyanide, a 1% solution of which in 10% formalin is injected into the carotid artery after termination of the experiment. The localisation of the electrodes is shown by blue spots easily discernible in histological preparations stained according to Nissl or with haematoxylin-eosin.

Results: Fig. 175 shows responses from different depths of the rat's somatosensory cortex as compared with those from the surface. It can be seen that the amplitude and duration of the positive phase of the response decreases with depth while the negative phase is prolonged until it completely replaces the surface positivity. At a depth of 1 mm this negative wave becomes the direct mirror image (phase shift by 180°) of surface positivity, but its amplitude may be higher. The negative phase of the deep response is usually followed by a slight positivity. A more detailed analysis of changes in the primary response at different depths is attained by relating the polarity and amplitude of the deep response to the amplitude and polarity of the surface response at the moment when the latter attains its positive or negative peaks. If the amplitude of the deep response is expressed in % of the coincident positive or negative maximum of the surface response and if these values are plotted against the depth of registration, characteristic curves are obtained (Li et al. 1956a, b). It follows from these curves that the positive and negative components of the surface response have a different depth distribution. Change of positivity to negativity occuring at a depth of about 1 mm demonstrate where the primary active area of the surface positive wave commences. The maximum negativity in deep recordings indicates the centre of this area. In the time interval corresponding to maximum surface negativity, on the other hand, negativity in the deep recordings decreases more slowly and is replaced by positivity only at a depth of 1·2 mm. This indicates that the upper layers of the cortex are the primary active area for this component (Li, Cullen and Jasper 1956a).

On the basis of deep recordings an attempt may be made to reconstruct the process of spreading of the primary cortical response. When the surface positive wave commences, a focus of negativity is formed in deeper cortical layers which are depolarised by the arrival of the afferent volley from specific thalamic nuclei. The negativity immediately begins to spread towards the surface at a rate that can be estimated from the interval separating maximum negativity at different depths. This rate is usually less than 0·2—0·5 m/sec. The original focus of deep negativity disappears first, negativity of surface layers persists for another few tens of msec.

In a similar way the depth distribution of other cortical potentials — callosal responses (p. 355), secondary responses (p. 349), antidromic responses to stimulation of pyramidal pathways (p. 366), recruiting responses (p. 409), seizure potentials (p. 419), strychnine spikes (p. 356) etc. may be determined.

Conclusion: Simultaneous registration of electrical potentials from the surface and deep layers of the cerebral cortex permits precise characterisation of the distribution and movement of electric dipoles arising in the cortex during spontaneous or evoked activity and thus contributes to a better understanding of the functional anatomy of the cortex.

H. Antidromic and orthodromic stimulation of pyramidal paths

Problem: Determine the extent of the motor cortex in the rabbit by mapping responses of Betz pyramidal cells to antidromic stimulation of the medullary pyramids. Trace the pyramidal discharge produced by stimulating the motor cortex in fibers of the pyramidal tract at the level of the medulla.

Principle: Motor areas of the cortex may be mapped by stimulating the cerebral cortex and observing peripheral motor responses or electrical activity of nerve paths. The opposite approach, however, is also possible. Since the pyramidal tract is composed of axons arising directly from giant pyramidal cells of the fourth cortical layer (Betz cells) it is possible to produce by stimulation of these fibers an antidromic volley that reaches the cortex and causes depolarisation of the cell bodies and perhaps dendrites of the pyramidal neurones. The technique of stimulation and recording is similar to that in experiment VIII D. The situation, however, is complicated by the fact that the pure antidromic response is distorted by activity of afferent fibres terminating in the same cortical area which may be stimulated in the medullary region together with fibres of the pyramidal tract (particularly fibres of the lemniscus medialis — Landau 1956). Another factor making interpretation of results more difficult are recurrent collaterals of pyramidal cell axons which may excite further cortical cells orthodromically (Chang 1955b). A tho-

rough analysis of tracings, however, makes it possible to distinguish between true antidromic responses and potentials of other origin, and thus makes it possible to obtain information not only on the localisation and extent of cortical areas contributing to the pyramidal tract but also on antidromic activity in cortical neurones and especially in the dendrites.

With the opposite arrangement of stimulating and recording electrodes — stimulation of the cortex and recording in the medulla — characteristic responses can be recorded in the pyramidal tract. These consist of a sharp wave with a latency of 1—2 msec., followed by one or more successively smaller waves at about 2 msec. intervals. It has been shown by Patton and Amassian (1954) that the first wave (D) corresponds to direct stimulation of pyramidal cells or their axons in the cortex while the other waves (I) are an expression of re-excitation of pyramidal cells via cortical interneurones. The intensity of cortical stimulation remaining constant, the amplitude and area of the D and I deflections of the evoked pyramidal response may further be analysed from the aspect of excitability changes in pyramidal cells (D waves) and cortical interneurones (I waves).

Object: rabbit, 2—3 kg.

Apparatus: A cathode-ray oscillograph with an A. C. amplifier (time constant at least 0·3 sec., total amplification at least 50 μV/cm). A stimulator giving either single or paired rectangular pulses or condenser discharges, synchronised with the time base of the oscillograph. An isolation unit (transformer, radiofrequency output). Time marker.

Other requirements: Surgical instruments, dental drill, animal board and head holder or a stereotaxic instrument for rabbit, permitting ventral and dorsal approach to the head. Bipolar needle electrodes for stimulating the pyramids (spacing of the tips 0·5 mm.). Spring-mounted, ball-tipped silver or platinum electrodes (diameter 0·25 mm) for corticography. Electrode carriers. 1% nembutal or pentothal. 1% chloralose in 10% urethane.

Procedure:

1) The rabbit, anaesthetised with i. v. pentothal, is fixed to the animal board on its back. After low tracheotomy and insertion of a tracheal cannula the pharynx is exposed and pulled sidewards. Deep muscles are carefully removed and the base of the occiput is exposed over about 10 × 10 mm. An opening of 4—6 mm diameter is made with the dental drill between the foramen magnum and the caudal border of the pons. The dura is not cut for the time being and the borders of the cut skin are sutured. Then the animal is released and again fixed prone on the board. The skin of the head is cut sagittally and trephine holes are made above the fronto-parietal area of the hemisphere (roughly above coronal suture). After an interval of 30—60 min. the animal is fixed in the head holder of the stereotaxic apparatus in such a way that the skull base is easily approachable from below. Light anaesthesia

is maintained by repeated injections of pentothal or by gradually replacing pentothal by a solution of 1% chloralose in 10% urethane. The latter in amounts of 4—5 ml/hr. is recommended by Chang (1955b) as especially suitable in this experiment.

The trephine opening at the base of the occipital bone is again exposed and the dura cut in the mid-line. The bipolar stimulating needle electrodes are placed onto the medullary pyramid 3—5 mm below the border of the pons. Electrodes are applied with the help of the vertical microdrive of the electrode carrier under visual control (dentist's mirror). Recordings are taken directly from cerebral surface after cutting the dura, the arrangement being similar to that in experiments with primary cortical responses. In order to ensure a good functional state of the cortex the trephine opening may be covered with warm paraffine oil, previously shaken with Ringer solution. The recording electrodes are placed onto the cortical surface at 2 mm intervals. At each point several responses to stimulation of the pyramids are recorded (stimuli in at least 1 sec. intervals). The reference electrode is a metal clamp fixed to the cut skin above frontal bones or a needle fixed into those bones.

After finding the area of maximum response the following procedures may be used for analysing its individual components:

a) determination of its refractory period. This is done as in experiments with primary responses (VIII D 3),
b) three-minute anoxia (introduction of N_2 into the tracheal cannula),
c) local application of 1% strychnine to the area of the recording electrode (as described in experiment VIII F 2),
d) micro-electrode recording of cortical responses from different depths (as in experiment VIII G).

2) The second part of the experiment is done on the other hemisphere if possible. Two silver ball-tipped stimulating electrodes are placed at a distance of 2—3 mm from each other onto the surface of the motor cortical area. Registration is made in the homolateral medulla with the electrodes used for stimulation in the first part of the experiment. Stimuli are applied at minimum intervals of 2 sec. The values for threshold and maximal stimuli are determined in the cortex and by moving both the cortical and medullary electrodes the optimum responses are found. For more detailed analysis of their components anoxia is applied for one minute by introducing N_2 into the tracheal cannula while the cortex is being stimulated rhythmically with submaximal stimuli (submaximal, in order to make it possible to observe not only a decrease but also an increase in excitability as judged from changes in the response). The development of the medullary response is studied during anoxia together with its subsequent restitution.

Results: 1) The extent of antidromic projection in the rabbit and cat according to the work of Porter (1955), Woolsey and Chang (1948), Landau

(1956), Lance and Manning (1954) roughly corresponds to the motor regions, shown in Fig. 166b. Responses, however, are recorded not only from the cortical motor area but also from somaesthetic area I. The typical responses in the centre of the projection area are nearly monophasic surface positive waves with an amplitude of about 200 μV and a duration of about 5 msec. (Fig. 176A). These are an expression of the arrival of antidromic volleys in

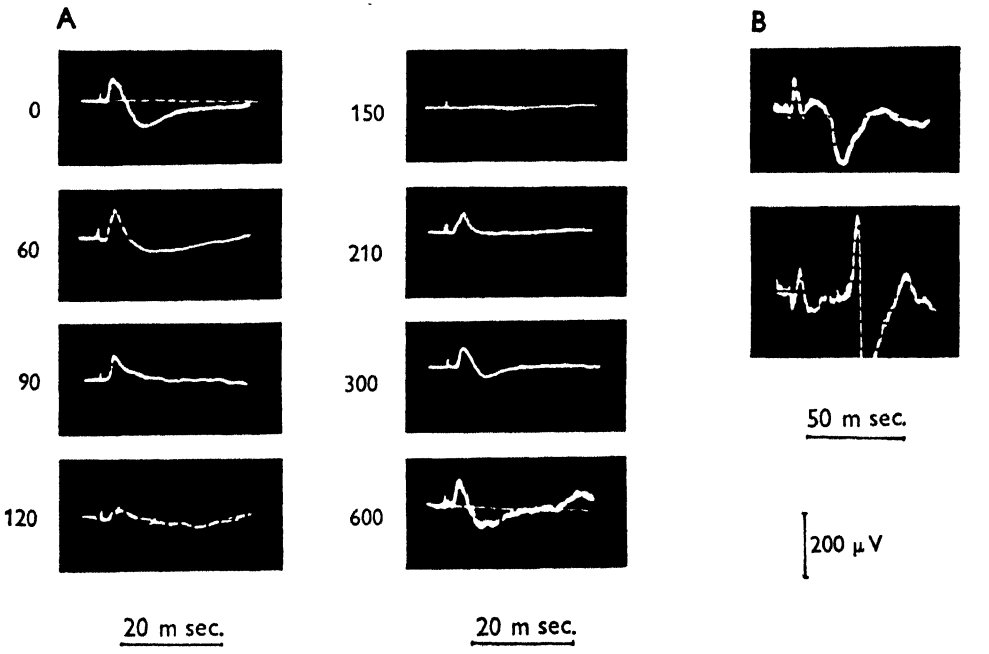


Fig. 176. Cortical response to antidromic stimulation of the pyramids in the rabbit. A) Effect of 3 min. anoxia. Figures denote time in sec. from start of anoxia. B) Late components of the antidromic response (above) and the effect of strychnine (below). Positivity upward.

the cortex and of depolarisation of cell bodies of pyramidal neurones. They are characterised by a very short latency, not exceeding 2 msec., thus excluding the possibility of considering them as orthodromic stimulation of ascending fibres of the medial lemniscus. The positive wave changes smoothly into a low negative potential lasting for about 10 msec. and corresponding to antidromic propagation of depolarisation from the cell body to the dendrites (Chang 1955a). In the rabbit under chloralose-urethane anaesthesia it is further possible to record a surface negative wave of a high amplitude and changing shape, with a latency of 15—20 msec. This, according to Chang (1955b) is an expression of activation of cortical neurones via collaterals of pyramidal axons at the cortical level. According to Landau (1956) and Porter (1955) it is the result of orthodromic stimulation of fibres of the medial lemniscus.

The initial positive wave has a very short refractory period as compared with the primary responses (2 msec. instead of 6—20 msec.). During anoxia the positive wave is preserved for a longer period than the negative one and also reappears sooner (Fig. 176A). The antidromic responses are more resistant to anoxia than the primary responses. The local application of strychnine in no way changes the antidromic response or its early phase but usually increases late waves if they are present (Fig. 176B.) This indicates that they are postsynaptic in nature. The microelectrode recordings from different depths of the cortex show a phase reversal of the antidromic response at a depth of 1—1·5 mm. The latency of the positive component is the same in all cortical layers, that of the peak of the negative component is the longer the closer it is to the surface. This may indicate conduction of depolarisation in the dendrites from the cell body towards their apical branching at a rate of about 0·5 m/sec. (Chang 1955a).

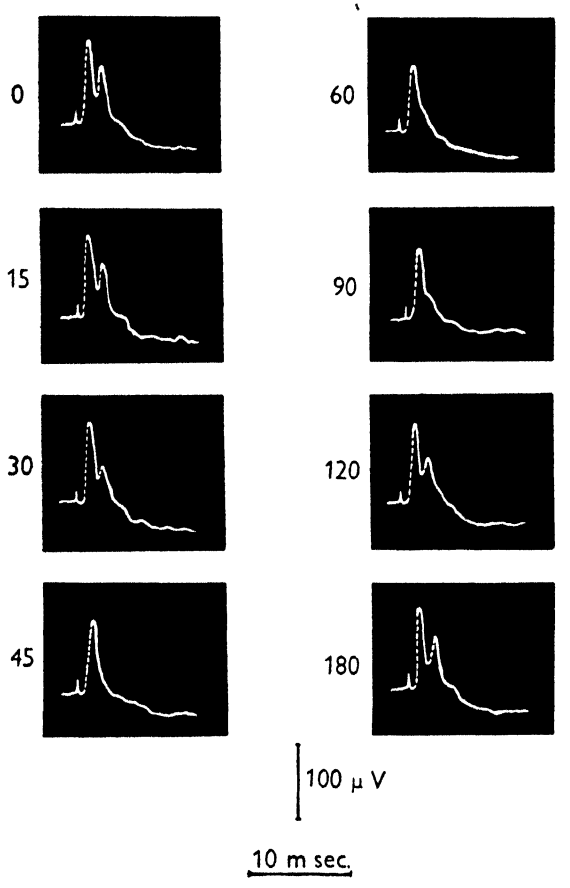


Fig. 177. Responses to stimulation of the motor cortex in the rabbit, recorded from the region of the bulbar pyramids, and the effect of 1 min. anoxia. Figures denote time in sec. from the start of anoxia.

2) Fig. 177 shows the characteristic response registered from the medullary pyramids when stimulating the motor cortex. The first wave (D) is well in evidence as is also subsequent, usually less distinct wave or waves (I). The threshold for D waves is lower than for I waves. During anoxia the amplitude of both D and I waves at first increases for a short period (if a submaximal stimulus is used) and then first the I, and later also the D waves decrease considerably or disappear altogether. Restitution occurs in the opposite order. This result is in agreement with the assumption that I waves corres-

pond to the activity of cortical interneurones, which are much more sensitive to anoxia then pyramidal cells. The greater resistance of D waves might, to a certain extent, be due to the direct action of the stimulating current on axons of pyramidal cells in the cortex.

Even without stimulation of the cerebral cortex, spontaneous potentials occasionaly appear at the medullary electrodes. These indicate synchronous discharges of pyramidal cells. Simultaneous recordings from the cerebral cortex and the pyramids show that these discharges coincide with the appearance of sleep spindles (p. 328) in the motor area (Whitlock, Arduini and Moruzzi 1953, Brookhart and Zanchetti 1956).

Conclusion: Antidromic stimulation of the pyramidal tract makes it possible to define the extent of motor areas and to demonstrate some aspects of antidromic conduction in the cortex. Pyramidal tract responses to stimulation of the cortical motor area, on the other hand, make it possible to study changes in excitability of pyramidal cells and cortical interneurones.

I. Steady potentials and impedance of the cerebral cortex

Problem: Determine the polarity and impedance of the cerebral cortex in rats and register its changes during temporary cortical ischaemia.

Principle: The cortical surface of mammals is 15—25 mV positive to other electrically indifferent areas. This steady potential may be considered as a baseline on which phasic changes of EEG activity occur. Cortical polarity is the result of the regular structure of the cerebral cortex (Aladzhalova, Koshtoyants 1957) and probably depends, in the first place, upon the potential difference between dedrites and bodies of pyramidal cells. It is very stable and changes significantly only following severe interference with the metabolism of nervous tissue (anoxia, asphyxia, some intoxications — Leão 1947, Van Harreveld and Stamm 1953b, Goldring et al. 1953, Bureš 1957a, b, Bureš and Burešová 1957) or in the course of some processes simultaneously affecting extensive groups of cortical neurones (epileptic seizure, spreading depression — cf. p. 376). Even considerable changes of short duration are reversible (depolarisation lasting for tens of minutes).

From the technical point of view it is of advantage to use simultaneously several methods for determining cortical polarity. The choice of the reference point is particularly important. It is best to determine cortical polarity as the demarcation potential between a normal and damaged area of the cortex. Recording is carried out according to directions described in the previous experiments concerned with registration of steady potentials (Chapter III.).

Another parameter characterising elementary functions of the cortex is the cortical impedance, measured between two cortical areas (Leão and

Ferreira 1953, Freygang and Landau 1955, Van Harreveld and Ochs 1956, 1957, Aladzhalova 1954, 1955). The real component of cortical impedance (resistance) is especially important. This can easily be determined using a low frequency A. C. bridge and non-polarisable silver disc electrodes coated with silver chloride. Of course the current must be such as not to stimulate the cerebral tissue itself, and the resistance of the electrodes must be considerably less than that of the cerebral tissue between them. Finally electrodes must be placed in such a way that the majority of the current flows through the cortex, for otherwise actual cortical impedance would not be measured. Determination of resistance of cerebral tissue corresponds to measuring the conductivity of the extracellular space of the cerebral cortex.

Object: Rat 150—200 g.

Apparatus: Double beam cathode-ray oscilloscope with D.C. amplification of at least 1 mV/cm or a cathode-ray oscillograph with an A.C. amplifier and a special rotating switch permitting registration of steady potentials after their transformation into short pulses (Zachar 1955; see also page 121). An A.C. bridge for determining resistance and capacitance with a measuring frequency of 500—1000 cycles/sec. Calibrator. Balancing potentiometers.

Other requirements: Surgical instruments, trephine 2·8 mm, non-polarisable Ag-AgCl screw electrodes (diameter 3 mm), $Hg\text{-}Hg_2Cl_2$ wick electrodes with stands, an animal board for the rat with a stereotaxic head holder. A rubber band (10 × 200 × 0·5 mm) with a clamp, a respiration pump with a rubber tube fitting over the rat's snout, dial 10%.

Procedure: A rat under dial anaesthesia (40 mg./kg.) is tied to the animal board on its back and the trachea is exposed in such a way that between it and the other tissues of the neck there is a space of about 1 cm. Through the latter a rubber band is passed (1 cm in width). The animal is then placed on its abdomen, the scalp is cut and the skull bones are cleaned.

When registering cortical polarity two threphine holes 5 mm in diameter are made above the frontal and occipital regions of one hemisphere and one hole of the same diameter above the occipital region of the other hemisphere. The rat's head is then fixed in the stereotaxic holder and a rubber tube 1 cm in diameter connected to the respiration pump is slipped onto the snout (Fig. 178). Both ends of the rubber band running below the trachea are passed round the neck and through a special bracket (Fig. 178).

An earthed calomel cell electrode is placed into the anterior trephine opening (R) and further calomel cell electrodes connected through compensators to the input of the D. C. amplifiers or to the mechanical switch are placed into the openings A, B. Before placing them onto the cerebral surface the electrodes are shortcircuited by connecting their wicks, and their potential differences to the reference electrode are adjusted to zero with compensators. After applying the electrodes to the brain calibration is carried out by introduc-

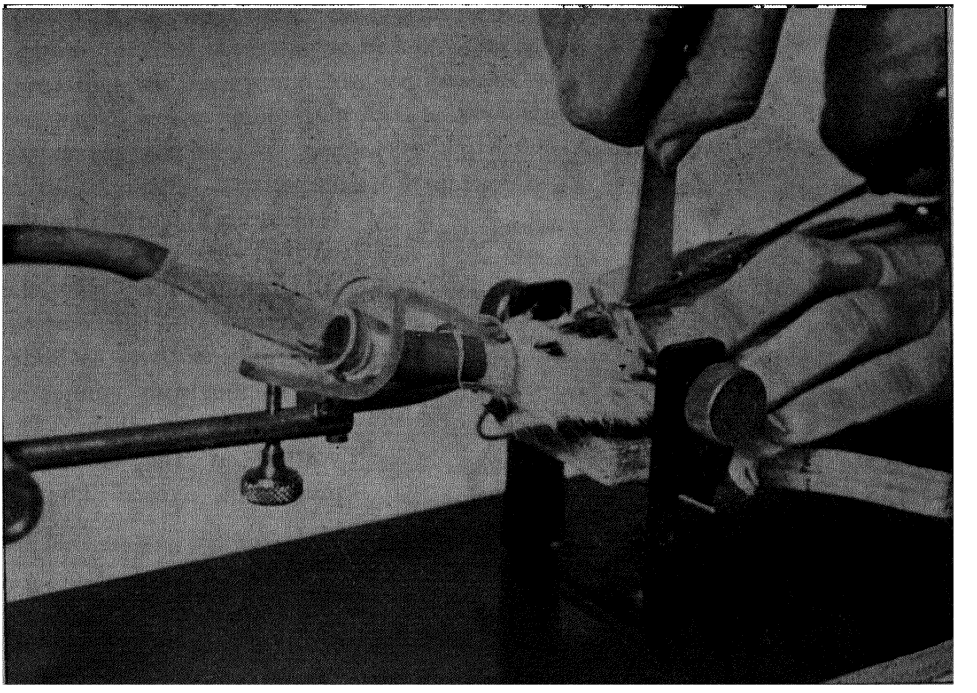


Fig. 178. Arrangement for experiments with transient ischemia of the brain. The snout of the rat is fixed to the tube leading from the respiratory pump. Rubber band passes through a bracket on the neck of the animal.

ing a known D. C. potential (5—10 mV) in series between the reference electrode and earth.

After briefly recording potential differences AR and BR, the brain tissue in the anterior trephine opening R is thermocoagulated by applying a heated metal rod (diameter 4 mm) for several seconds to the cortical surface and the cortical demarcation potential produced between the injured area R and areas A and B is recorded. Sometimes a slow negative potential appears in area B. This is due to a wave of spreading depression (cf. VIII J) produced by thermocoagulation. Otherwise, however, the potential differences AR and BR remain unchanged. After ten minutes of recording the rubber band is tightened by a pull of about 5 kg. and fixed with a hemostat. Simultaneously artificial respiration is started (30—60/min.). An open system is sufficient. The changes of cortical polarity are recorded in leads AR and BR. The rubber band is released after 10—20 minutes of ischaemia and recording is continued for another 15—30 minutes.

When measuring impedance only two trephine openings (diameter 2·8 mm) are made over one hemisphere in another rat. The distance between

the centres of the openings should be 6 mm. Silver screws coated with AgCl on their lower surfaces with a diameter of 3 mm are screwed into those openings. They are connected through flexible insulated wires with the measuring bridge. In order to avoid polarisation of the electrodes a low frequency alternating current (preferably 500—1000 cycles/sec.) is used for measuring impedance. Stimulation of the cerebral cortex by the current is prevented by controlling the potential difference on the cortical electrodes with a cathode-ray oscillograph connected in parallel to them. The voltage at the electrodes must not exceed 20 mV.

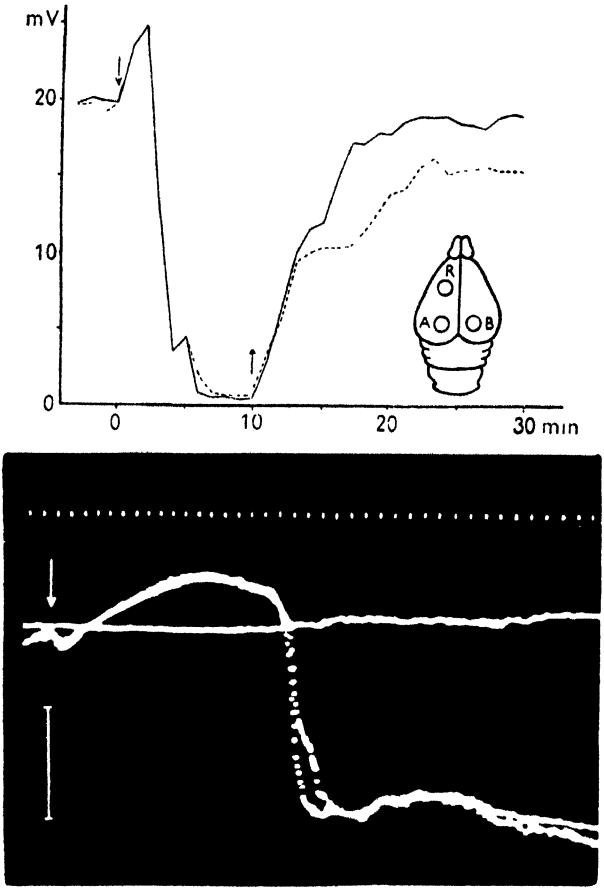


Fig. 179. Terminal anoxic depolarisation. Top: redrawn course of depolarisation and repolarisation. Full line — BR, dotted line — AR. The arrow indicates the beginning and end of 10 minute ischaemia. Diagram: distribution of electrodes. Bottom: oscillogram of rapid depolarisation. Arrow — beginning of ischaemia. The horizontal trace shows the potential level from which deflections AR and BR must be measured. Calibration: 10 mV, time mark 10 sec.

After recording the original resistance of cerebral tissue for several minutes the experiment is continued in the same way as when determining terminal anoxic depolarisation. Individual determinations of resistance are made at 15—20 sec. intervals. Later, particularly during resuscitation, intervals of 1 minute suffice.

Results: The interruption of blood supply to the head, as applied in the above experiment does not result in complete circulatory isolation of the head but does produce complete arrest of blood flow in the carotids and considerably decreases the supply of blood from the spinal and vertebral arteries. The effectiveness of circulatory arrest may be demonstrated by comparing ischae-

mic changes after strangling and decapitation. No significant differences between the effects of these two procedures can be observed as far as cortical polarity and impedance are concerned. The chief advantage of the method used here is the likelihood of recovery, with normal cardiac function during the period of brain ischaemia (Grenell 1953). A typical experiment with terminal ischaemic depolarisation is shown in Fig. 179.

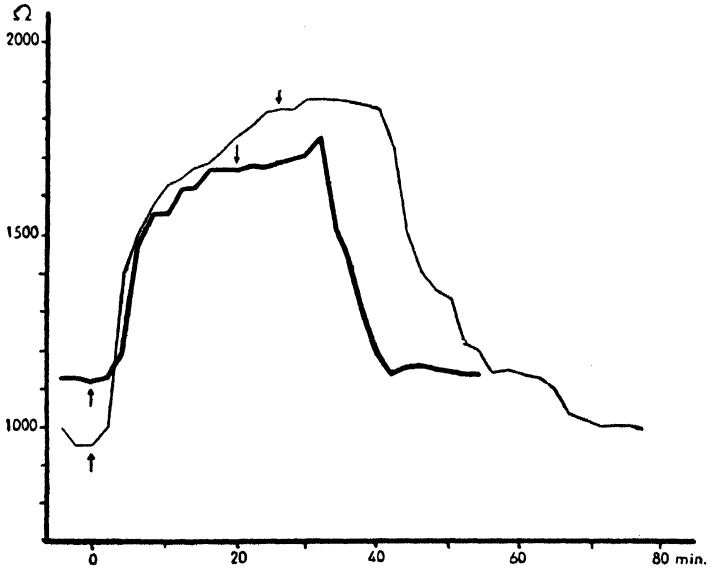


Fig. 180. Changes in resistance of the cerebral cortex during and after transient brain ischemia (20 min. — heavy line, 25 min. — thin line). Arrows indicate the beginning and end of ischemia.

The cortical steady potential which initially has a value of about 20 mV (Bureš 1957b) is preserved for 1—3 min. after interruption of the blood supply but then it begins to fall to zero, at first rapidly and then more slowly. Depolarisation appears earlier in the hemisphere in which an area has been coagulated. If normal cardiac activity is maintained during brain ischaemia, resuscitation may be successfull even after complete disappearence of cortical polarity. Revival is indicated by the recovery of cortical polarity. The rate of repolarisation is inversely related to the duration of ischaemia.

The cortical impedance is measured by adjusting the resistance and capacitance boxes so as to balance the bridge. From the values of resistance (R) and capacitance (C) the impedance (Z) can be computed using equation:

$$Z = \frac{R}{\sqrt{1 + \omega^2 R^2 C^2}}$$

As the balancing capacitance (C) does not usually exceed 0·02 μF, Z differs only slightly from R. For R values of the order of 1000 Ω and measuring frequency 500—1000 c/sec., resistance values read directly from the bridge at balance may be used as measure of tissue impedance.

The resistance of the cerebral cortex begins to rise from the moment blood flow is interrupted. This rise is most rapid when the slope of the depolarisation curve is steepest (Fig. 180). Within 10—20 minutes, depending apparently on the completeness of the circulatory isolation of the head, the resistance of the cerebral cortex increases from the original 800—900 Ω to 1400—1600 Ω. Then it continues to rise slowly until, after 3—5 hours a maximum of 3000 to 4000 Ω is reached. These changes are reversible up to a certain increase in resistance (and thus a certain duration of ischaemia). If, however, the resistance increases by more than 100%, there is little hope of complete recovery of normal nervous functions.

Intracellular electrolytes, enclosed within a membrane of high resistance, do not significantly contribute to the conductivity measured with a low frequency current applied via extracellular electrodes. The increase in resistance is, therefore, produced primarily by changes in conductivity of extracellular fluid. According to Van Harreveld and Ochs (1956) it is possible to calculate directly how much the relative volume of the extracellular space must decrease in order to achieve a corresponding decrease in conductivity, assuming a stable composition of the extracellular fluid. It has been shown histologically that the initial sudden increase in cortical resistance is accompanied by swelling of nerve cells and dendrites (Van Harreveld 1957). This is evidence for extensive movement of water and electrolytes between intra- and extracellular spaces during ischaemia. A corresponding increase in permeability of cellular membranes helps to explain the depolarisation of the inherent potential difference between the surface and deep layers of the cerebral cortex.

Conclusions: Reflex activity as well as phasic EEG potentials disappear within several tens of seconds following brain ischaemia. In the period following, during which succesful resuscitation is still possible, cortical steady potentials and resistance of cerebral tissue are the main indicators of changes occurring in the brain.

J. Spreading depression

Problem: Produce spreading depression in the cerebral cortex of the rat with different stimuli, and record its manifestations. Show the antagonistic actions of K^+ and Ca^{++} ions in producing spreading depression.

Principle: Electrical, thermal, mechanical or chemical stimuli, acting on the surface of the cerebral cortex, produce after a short latent period a local

decrease in amplitude of the ECoG. This does not remain confined to the area of the stimulus but spreads over the cortical surface in all directions at a rate of about 2—5 mm/min. (Leão 1944). The advancing front of the wave of depression is accompanied by a slow negative potential of about 8 mV in amplitude followed, after 1—2 min., by a less pronounced but longer lasting positive wave. The wave of steady potential can also be recorded from deeper cortical layers but disappears completely in the white matter. The depression of spontaneous EEG activity lasts 5—6 min. at a given cortical locus. Coincident with the reduction in EEG amplitude there is a decrease of primary responses in cortical projection areas and in excitability of the motor areas. The wave of depression is also accompanied by increased cortical blood flow, decreased cortical pO_2 and an increase in cortical impedance. For review of literature see Marshall (1959).

Recording of electrical manifestations of spreading depression is achieved with the usual techniques (cf. VIII B, VIII I) which need not be further explained. The characteristic wave of increased blood flow accompanying depression can be demonstrated by thermoelectric registration of blood flow changes in the cerebral cortex. Using a conventional EEG apparatus with a switch in the input D.C. potentials are recorded from cooled thermocouples applied to the cortical surface (Burešová 1957). The temperature of the thermocouple depends upon the equilibrium between the constant withdrawal of heat from the metal of the thermocouples by a constant temperature cooling bath (0°C) and the changing supply of heat to the thermocouples from blood circulating in the surrounding tissues. The difference in temperature between two thermocouples of exactly similar construction placed on symmetrical areas of the brain reflects differences in blood flow through these areas.

Spreading depression can be used as a means of studying some problems of cortical metabolism, in particular electrolyte metabolism. The antagonism between K^+ and bivalent cations, well known in other excitable tissues, can also be demonstrated in the cerebral cortex. The effect of K^+ is suppressed by adding Ca^{++} or Mg^{++} to KCl solutions of a concentration producing depression. Local application of Ca^{++} or Mg^{++} to certain cortical areas prevents the invasion of spreading depression into the treated areas.

Object: Rats weighing 150—200 g.

Apparatus: An ink-writing EEG apparatus with at least 4 channels and slow rate of paper movement (1—2 mm/sec.). A simple mechanical switch driven by a shielded electric motor for the channels in which steady potentials are to be recorded.

Further requirements: Needle electrodes for electrocorticography (cf. p. 321). Nonpolarisable wick calomel electrodes filled with Ringer solution. Glass semimicroelectrodes (50—100 μ) filled with saline and with an Ag-AgCl core. An animal board with a stereotaxic head holder for the rat. A stereo-

taxic apparatus for the rat with vertical microdrive. Surgical instruments, dental drill. Cooled thermocouples (Ag-constantan) for recording changes in blood flow in symmetrical areas of the brain (Fig. 181). An anode battery with a 1 MΩ potentiometer and nonpolarisable wick Ag-AgCl electrodes for polarising the brain. Different solutions of KCl and KCl + $CaCl_2$ or KCl + + $MgCl_2$, see below.

Procedure: The skull bone of a rat under dial anaesthesia is exposed in the usual way. The animal is tied to the board and fixed to the stereotaxic head holder. Trepanation and fixation of the needle electrodes is carried out according to the kind of experiment.

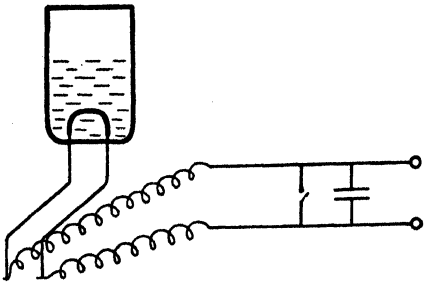


Fig. 181. Cooled thermocouples: a vessel with crushed ice and a silver wire (1 mm in diameter). Constantan leads to EEG amplifier. Mechanical switch in the input.

1) Initiation of spreading depression with different stimuli.

Trephine holes are distributed according to Fig. 182: a large opening (diameter 4 mm) above the occipital pole of the hemisphere, two small openings (2·5 mm) rostrally from the first. Along these openings a row of 4—5 needle electrodes is placed. Above the other hemisphere openings and electrodes may be placed symmetrically. The needle electrodes are connected in a bipolar fashion (Fig. 182). Steady potentials are recorded from points A″, A′ (or B″, B′) against the reference electrode situated above the non-stimulated hemisphere. The reference electrode may be earthed. Care must, however, be taken not to earth the animal at any other point (head holder, board etc.) If the needle electrodes are earthed through the input resistance of the EEG amplifier a condenser must be introduced into every lead interrupting the galvanic connection of the electrode with earth. Steady potentials would otherwise be measured against all other earthed points and would thus be uncontrollably distorted.

The EEG is recorded in 3—4 leads, the steady potential in another two. The steady potential is first adjusted to zero by compensators in series with the active electrodes (cf. p. 182). Since it is the amplitude that changes most in the EEG recording, the speed of paper movement may be limited to 1—2 mm/sec. After 10—15 min. of control recording, during which we make sure that the level of EEG activity is stable and that the steady potential difference between the hemispheres does not vary by more than 1 mV, spreading depression is produced.

a) Faradic stimulation with bipolar Ag-AgCl electrodes (distance between the tips 1—2 mm) may be used to produce spreading depression.

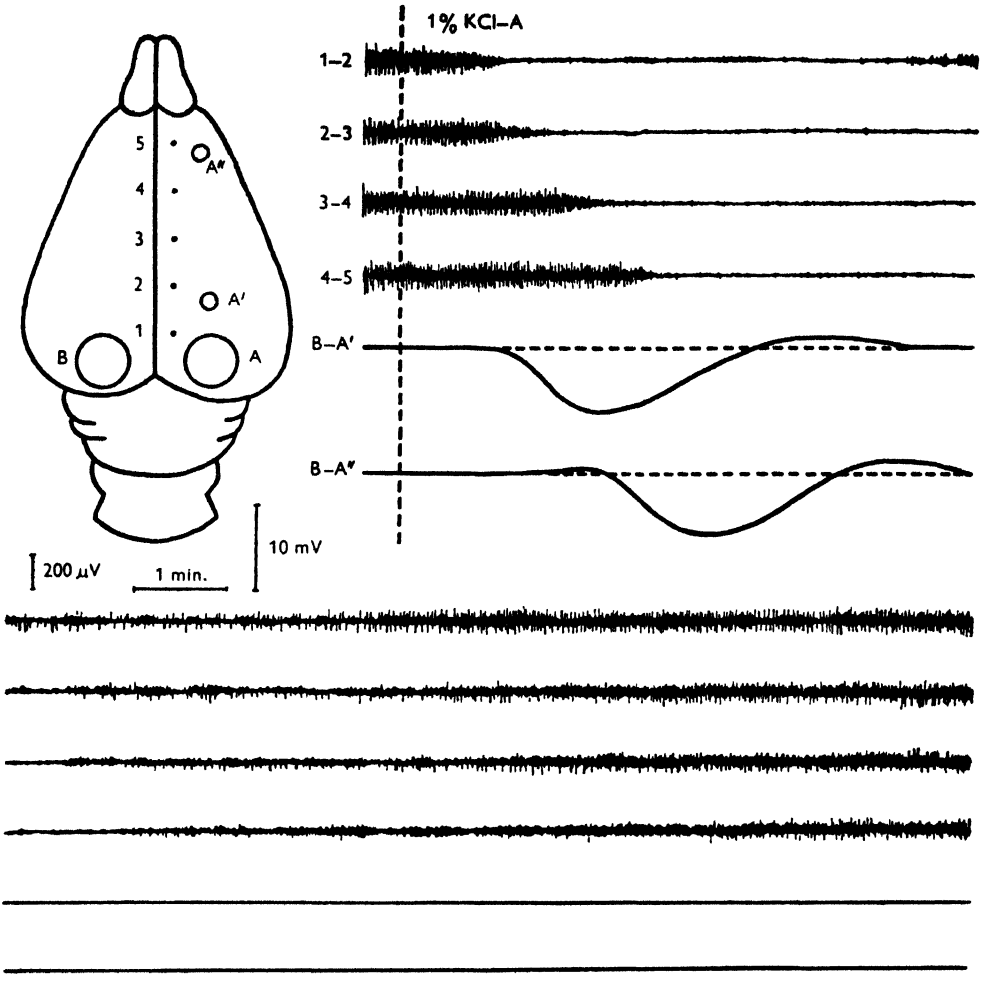


Fig. 182. Tracing of spreading EEG depression in an anaesthetised rat. The interrupted vertical line indicates application of the stimulus. Negativity of A downward. For details see text.

A direct current, however, gives more constant results (Leão 1944, Leão and Morrison 1945, Whieldon and Van Harreveld 1950, Van Harreveld and Stamm 1953a, Marshall 1959). The current from an anode battery is regulated by a potentiometer and measured with an ammeter in series. The intensities used vary from 0·2—3·0 mA, the duration of the stimulus is limited to 5—10 sec. A wick Ag-AgCl electrode is applied to the large trephine opening, the other is fixed to the cut skin in the neck. During polarisation recording of the steady potential with the calomel electrodes is interrupted.

b) Spreading depression can be produced easily by mechanical stimuli,

e. g. tapping the cortical surface with blunt pincers (Leão 1944). A more exact determination of threshold, however, is made by measuring the energy of a body falling onto an area of the hemisphere from a known height. By gradually increasing the path of free fall and thus the kinetic energy of the falling body, the threshold for producing spreading depression can be determined exactly (Zacharová and Zachar 1958).

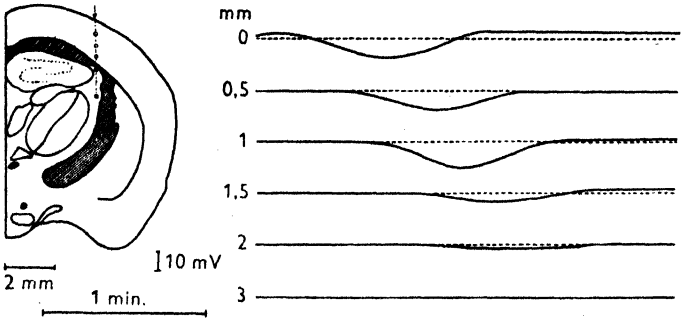


Fig. 183. Deep registration of slow potentials accompanying spreading depression in the rat. Figures denote depth of the microelectrode in mm. Negativity downward.

c) Spreading depression may be produced by topical application of a number of substances onto the cortical surface — KCl, glutamine, asparagine, NH_4Cl, veratrine, NaCN, 2,4—dinitrophenol, NaF, strophanthine etc. They are applied using squares of filter paper 2 × 2 to 3 × 3 mm in size soaked in the solution desired. The concentration just producing depression is determined. The papers are applied to an area exposed by a large trephine opening. If spreading depression develops the filter paper is removed and the cortical surface washed with Ringer solution.

2) Recording of a wave of steady potential from different cortical layers.

The procedure is the same as in paragraph 1), but only two large trephine openings (5 mm diameter) are made over each hemisphere (cf. Fig. 183). Into the anterior opening solutions producing spreading depression are applied. The capillary electrode fixed to the electrode carrier of the stereotaxic apparatus is introduced into the posterior one. Steady surface potentials are registered from the area of the posterior opening with wick calomel electrodes. The reference calomel electrode is placed on a symmetrical point and earthed. If the resistance of the capillary electrode is not too high, it may be directly connected to the input of the EEG apparatus, which is periodically short-circuited by an electrically driven switch (1—2/sec.). At first the cortical surface is only just touched by the microelectrode and the wave of steady

potential accompanying spreading depression due to 1% KCl is recorded with the macro- and microelectrode applied to the same site. Then the electrode is pushed further down in steps of 0·5 mm and at each level a new wave of steady potential is recorded. The amplitudes of slow potential waves in simultaneous deep and surface recordings are compared. Calibration of the macro-

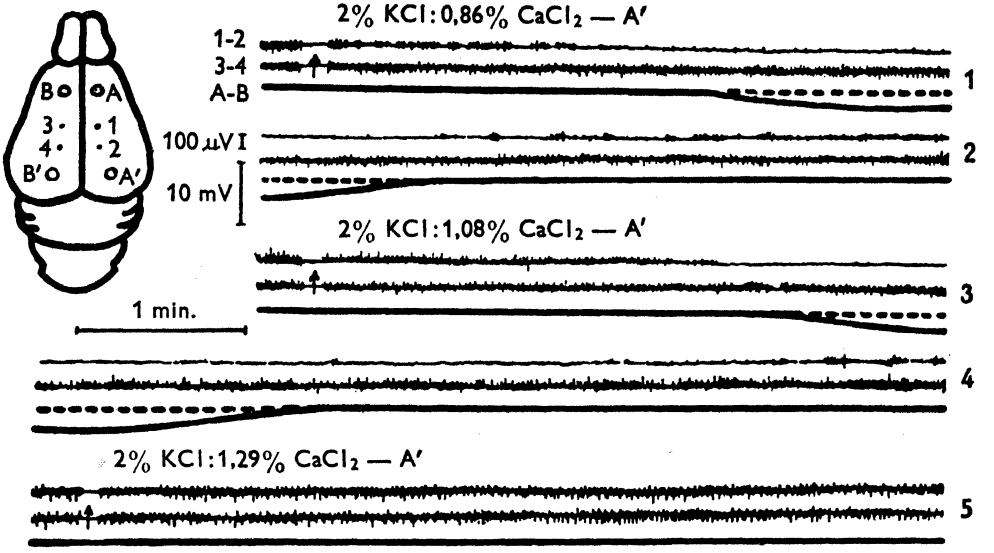


Fig. 184. Blockade of spreading depression by adding $CaCl_2$ to an effective concentration of KCl. Negativity of A downward. For details see text.

and microelectrode leads is carried out by introducing a known D. C. potential between the reference electrode and earth. Intervals of 30—40 minutes should be left between individual waves of depression.

3) The effect of bivalent cations on the initiation and spread of spreading depression.

Trephine openings and electrodes are arranged as shown in Fig. 184. A filter paper soaked in an effective KCl solution (e. g. 2% KCl) or in a mixture of 2% KCl with $MgCl_2$ or $CaCl_2$ is applied to the posterior trephine opening. After depression has been produced in one hemisphere with KCl solution, a KCl solution containing a certain concentration of the bivalent ion is applied to the other hemisphere. If depression occurs KCl solution of the same concentration but containing more bivalent cations is again applied to the first hemisphere, which has meanwhile recovered from the previous application. Intervals of at least 40—60 minutes are left between experiments on the same hemisphere. The experiment is continued until a certain ratio R^{++}: K^+ is

no longer capable of producing spreading depression. This ratio is determined for both hemispheres.

In another experiment (see Fig. 185) it can be demonstrated that waves of spreading depression do not enter a cortical area locally treated for 10 minutes with sufficiently strong solutions of $CaCl_2$ or $MgCl_2$. First a wave of spreading

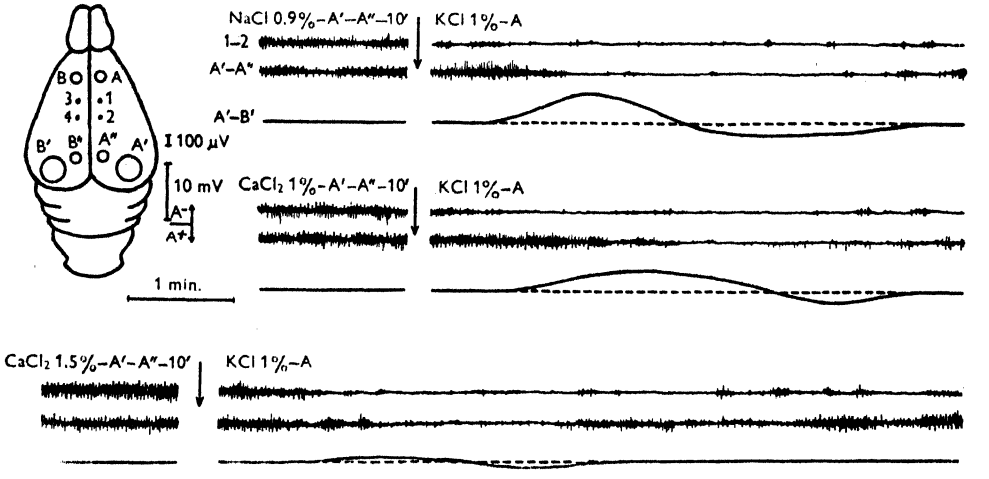


Fig. 185. Blockade of spreading depression in cortical areas treated with $CaCl_2$. For details see text.

depression produced from the region A by 1% KCl is recorded in the area A'A". Then a solution of $CaCl_2$ ($MgCl_2$) is applied to these areas. 10 minutes later the protecting solution is removed and a new wave of depression is produced from the distant trepanation A. The change in amplitude of EEG and of the slow potential is observed.

4) Changes in blood flow during spreading depression.

Spreading depression is produced, as shown in Fig. 186, from the small opening A (B), steady potential waves and changes in blood flow are recorded from the large openings A'B'. In addition EEG activity is registered with needle electrodes. Accurate recording of blood flow largely depends upon the correct placement of thermocouples. Their round silver facets (2 mm in diameter) must be previously adapted to the cerebral surface concerned by adjusting slope and distance between them. Only then are the suspended thermocouples, together with the cooling vessel, carefully lowered until they lightly touch the surface of the hemispheres without exerting pressure. Contact is ensured by a thin film of fluid formed between the thermocouples and the cerebral surface. Measurement will be successful only if the surrounding temperature is perfectly stable and if there are a minimum of air currents.

Very careful exclusion of factors setting up temperature gradients (light bulbs, opening and closing of doors etc.) is therefore recommended. The direction of the steady potential recording while increasing the temperature of one thermocouple is checked by approaching it with a warm object. After control recording lasting for 10—15 minutes, during which time the difference

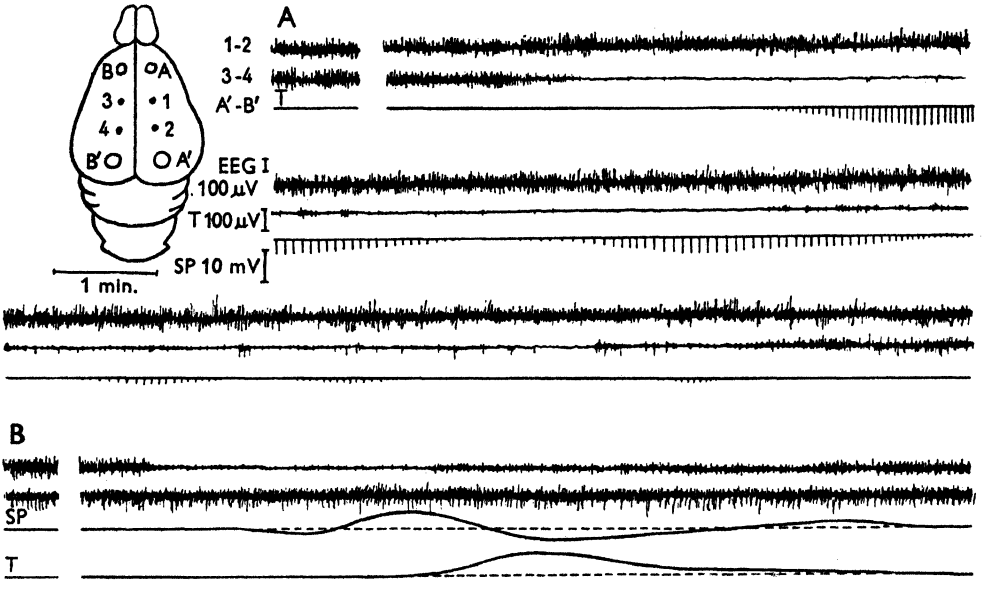


Fig. 186. Recording of vasomotor changes accompanying spreading depression. Top: potential difference between cooled thermocouples, registered by transforming D. C. potential to rectangular pulses. Bottom: comparison of the tracing of a slow potential wave with vasomotor changes, observed in the same part of the cerebral cortex. Both curves redrawn. Negativity (SP) or temperature increase (T) of A′ upward.

between the cooled thermocouples does not change by more than 0·2—0·5°C (10—20 μV), spreading depression is produced by applying 2% KCl to the trephine opening A (or B).

When depression has passed, the cooled thermocouples are removed and replaced by calomel electrodes. A new wave of spreading depression, produced in the same way is recorded. The two recordings are compared, beginning with depression in the ECoG, and thus the time relations between changes in blood flow and steady potentials are determined.

Results:

1) Fig. 182 shows spreading EEG depression and the wave of negative potential after stimulating area A with a direct current (either cathode or anode may be used but cathodic stimulation is more effective) or with a mechanical

stimulus (e. g. a metal rod (4 g weight) falling onto an area of about 10 mm^2 from a height of 5—10 mm).

Chemical threshold stimuli usually produce spreading depression after a much longer latent period (up to 10 min.) during which time a local reaction develops at the site of application of the solution.

Spreading depression is a reaction of the "all or none" type. Once it leaves the limits of the focus it continues to spread regardless of the former's continuing existence until it extends over the whole cerebral cortex. The rate of spread depends, among other things, upon the temperature of the brain. It can be calculated from the time lag between different phases of EEG depression or of the slow potential wave in two distant leads. The steepest part of the negative wave, corresponding to the moment when negativity has attained 50% of the maximum, is most suitable for calculation. The velocity of spreading EEG depression is calculated from the time interval between those points in the slow potential recordings from the two cortical areas and from the spatial distance between them.

The refractory period, during which a new wave of spreading depression cannot be produced from the same site and by the same stimulus, lasts for about 4 min. Since EEG activity does not recover within such a short period, only slow potentials indicate the onset of a new wave of depression. Strongly supraliminal chemical stimuli applied to the cerebral surface (e. g. 25% KCl) produce a whole series of slow potential waves recurring at intervals of about 6 min. The amplitude of the slow potential waves following rapidly one after the other gradually decreases, the EEG remains permanently suppressed. Following a series of slow potential waves, convulsive potentials may appear on the recording instead of decreased EEG activity.

2) The steady potential recorded with a capillary electrode from different depths of the cerebral cortex has the same shape and duration as in the surface recording (Fig. 183). Its amplitude remains unchanged or even increases down to a depth of 1 mm. In deeper layers, however, it decreases and when the white matter or subcortex is entered by the electrode the slow potential disappears altogether. By comparing recordings from different depths with simultaneously made surface recordings it can be ascertained that depression spreads also vertically in the cortex. The negative maximum occurs later in deeper layers in proportion to depth. The rate of radial and tangential spread is roughly the same.

3) It is possible in all experiments to find a certain minimum ratio $[Ca^{++}]$: $[K^+]$ which prevents an otherwise effective KCl concentration from producing spreading EEG depression (Fig. 184). The mean values of this ratio are best calculated using probit analysis of the relation between the value $[Ca^{++}] : [K^+]$ and the percentage of experiments in which spreading depression was produced with this ratio.

The concentration ratio $[R^{++}]:[K^+]$ varies for different bivalent cations. Mg^{++} is most effective, Ba^{++} least so. The blocking ratio also depends on the blocked concentration of K^+.

Finally the spread of a wave of depression into a cortical area protected by Mg^{++} or Ca^{++} can be prevented (Fig. 185). Application of $CaCl_2$ or $MgCl_2$ decreases EEG activity and primary responses at the site of application. This decrease, however, is of a different character from that evoked by spreading depression. Protection manifests itself by a considerable decrease of the steady potential wave entering the treated area and by maintenance of spontaneous and evoked activity in that area although other cortical regions are depressed. The protective effect is most evident during the later phases of the action of $MgCl_2$, when its inhibitory effect on the EEG and primary responses has already disappeared but when the protective effect against spreading depression is still fully preserved. The relationship between the concentration of Ca (Mg) and the amplitude of the slow potential wave entering the treated area 10 minutes after application of the bivalent cation is shown in Fig. 187.

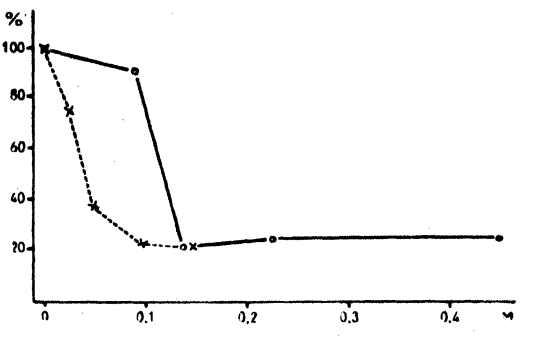


Fig. 187. Decrease in the amplitude of negative potential of spreading depression in cortical areas after 10 min. application of $MgCl_2$ (dotted line) or $CaCl_2$ (full line) in different concentrations. Ordinate: amplitude of negative potential in % of normal. Abscissa: molar concentration of the applied solution of the $CaCl_2$ or $MgCl_2$.

4) Temperature changes recorded with cooled thermocouples during spreading depression are shown in Fig. 186. An evident increase in the temperature of the hemisphere affected with depression can be seen on a relatively stable base line. A comparison of the temporal course of the steady potential wave and thermoelectric potential shows that the temperature increase sets in later than cortical negativity and attains a maximum when the cerebral cortex is already completely repolarised. Often several gradually decreasing waves can be seen in the temperature recording. These follow the first wave of increased temperature and indicate an undulating return of blood flow to normal. On the basis of relations between the steady potential and the change of blood flow it may be concluded that increased blood flow is not a cause but rather an after-effect of spreading depression. The vasomotor change described here is, however, characterised only qualitatively and without further elaboration of technique more precise quantitative evaluation cannot be attempted.

Conclusion: Spreading EEG depression is a special reaction of the cerebral

cortex to abnormal stimuli probably not occurring under normal physiological conditions. A study of this phenomenon, however, permits an analysis of the relation between electrical phenomena and metabolism in the cerebral cortex (Bureš 1956). Spreading depression may further serve as a method enabling the temporary and reversible suppression of normal cortical function.

K. The theoretical basis of recording electric potentials in a volume conductor

The complex structure of the central nervous system with millions of active elements makes it exceptionally difficult to interpret electrical records of brain activity. Potentials recorded with macroelectrodes are the vector sum of elementary electrical fields of a large statistical assembly of neurones, the individual elements of which may show contrary changes. For that reason the relation between macro- and microelectrode recording will be solved only by detailed analysis of the behaviour of a sufficiently large and representative statistical sample of neurones from a given area. The problem cannot be discussed in detail in this short chapter. Only some fundamental working principles will be presented in order to characterise more exactly the source of electric activity by macroelectrodes.

1) Membrane potentials of neurones are the chief source of electric potentials recorded from structures of the brain. Since in different parts of an inactive neurone the membrane potential is evidently not the same (the membrane of the cell body, dendrites, axons) a potential difference can arise between its parts. This is perhaps the basis of the resting electrical polarity of nervous structures (cf. steady potential of cerebral cortex, p. 371, Sutin and Campbell 1955). During synaptic or antidromic stimulation there is always an asymmetrical change in membrane potential in different parts of the neurone and this again results in external potential differences.

It must be emphasised, that potential differences always arise only within one single neurone. The electric current flows in one direction through the extracellular space and in the opposite direction through the interior of the cell. The assumption that a potential difference is produced between active and inactive cells has no physical justification whatsoever. If an inactive and an excited cell with a homogenously distributed membrane potential are placed in a conducting medium, no potential difference can be recorded between them. Such cells would be isopotential on their external surface and thus no current or corresponding external field would be produced. This is evident from the diagram in Fig. 188.

2) The asymmetric distribution of the membrane potential along the

neurone (permanent or temporary) results in external electrical asymmetry of the nerve cell, which in a simplified form appears as an electric dipole. The external current passes from the site with the larger membrane potential to sites of lower potential. The external positivity of a certain part of the neurone does not necessarily indicate hyperpolarisation of the corresponding part of the membrane. The membrane potential at the source may be normal or even decreased, but of course is less than at the sink.

The source will be positive when recording against a distant reference electrode, the sink negative. If the reference electrode approaches the source it will gradually acquire the latter's potential. This results in a decrease in positivity recorded directly from the area of the source. Placing the reference electrode near the sink, on the contrary, decreases the negativity recorded directly from the site of depolarisation. The voltage is the product of the integrated value of the current density component and specific resistance of the medium. From the integrated components of the current density along the line joining two electrodes the magnitude of the potential measured can be calculated by applying Ohm's law. The specific resistance of the medium is assumed to be constant. If the sink is spatially smaller than the source, the density of the current in the area of the sink is greater than in the area of the source. This manifests itself by more pronounced but circumscribed negativity.

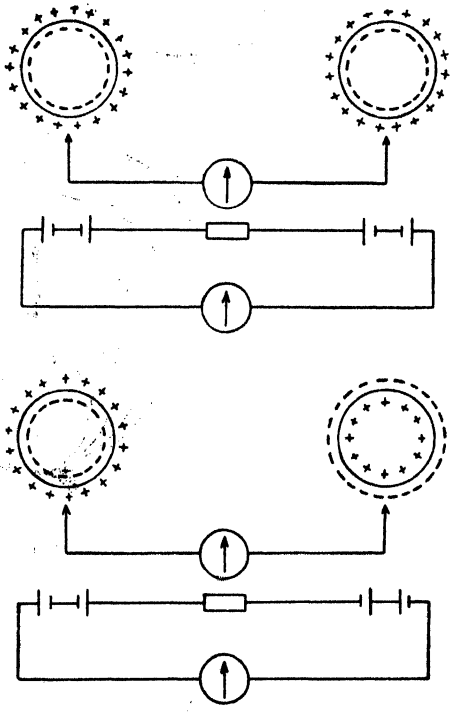


Fig. 188. Potential difference between two cells with symmetrically distributed membrane potential. Top: both cells in resting conditions. Bottom: one cell excited. In both cases the electrical diagram of the circuit is shown.

3) In a volume conductor the current arising between two areas of a neurone with different membrane potentials spreads in far reaching lines of current. In the distant parts of the field the direction of the lines of current flow is nearly reversed in relation to the intrapolar sector. Model experiments studying configuration of the electrical field of the nerve action potential in a flat conductive medium (Lorente de Nó 1947, Kostyuk 1960) and theoretical treatment of this problem (Offner 1954) show that under such conditions

the monophasic action potentials changes to a triphasic one (cf. experiment p. 279). This model experiment is of fundamental significance also for the interpretation of electrical manifestations of the movement of excitation along neurones in the brain. Assuming that the fundamental manifestation of

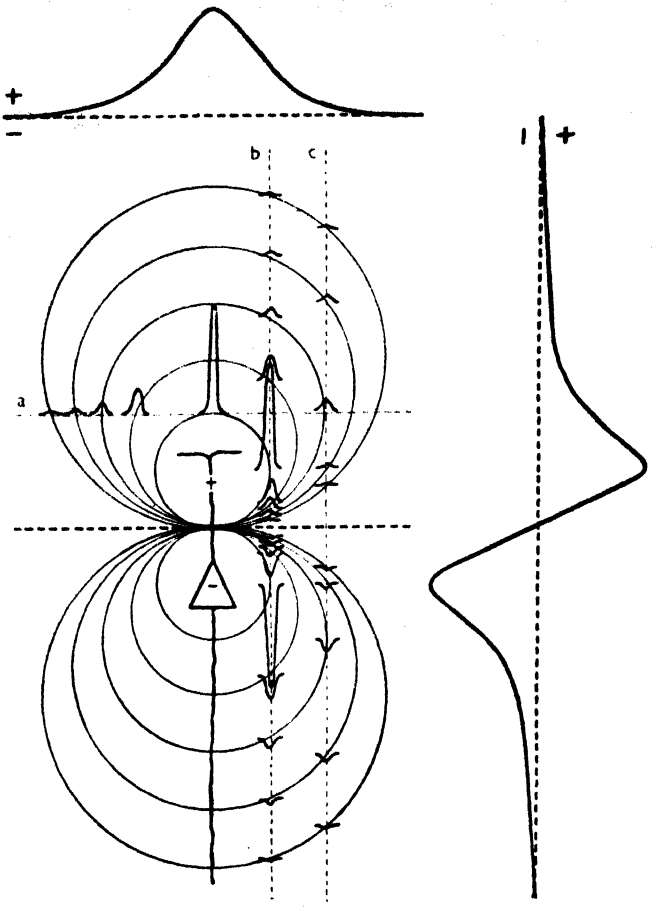


Fig. 189. The field arising around an electric dipole, with characteristic equipotential lines. The thin, interrupted lines represent sections through this field perpendicular to the axis of the dipole (a) or parallel to it (b, c). The amplitude and polarity of deflections at the corresponding points of the field are illustrated. Top: spatial distribution of the dipole potential in plane a). On the side: spatial distribution of the dipole potentials in plane b).

excitation is negativity, the positive deviation may be explained by the fact that the electrode is localised in the area of the current source or by an *IR* drop, produced by backcurving of the lines of current flow. The change of positive into negative potential indicates that excitation has spread to the area of the electrode. A positive wave may thus be an expression of excitation

approaching the electrode, a negative wave an expression of excitation reaching the area of the electrode.

4) The shape of the physiological dipole and its position in the volume conductor of the brain may be determined either directly, by exploring the electrical field with microelectrodes, or indirectly, by projecting the dipole onto the surface of the volume conductor.

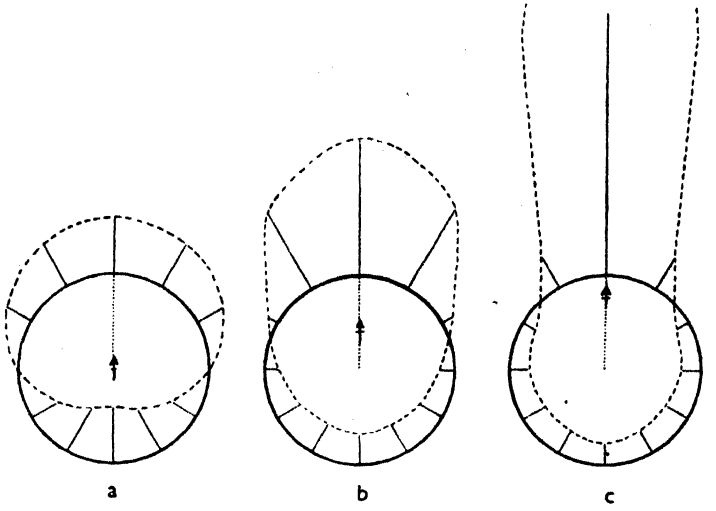


Fig. 190. The surface potentials of a sphere containing a radial dipole at a depth lr (a), 0·6r (b) and 0·2r (c) below the surface. The potentials are drawn in radial coordinates. Zero corresponds to the surface of the sphere, plus values are plotted outside and minus value inside. The angle denotes the angular distance of a recording point from the radius containing the dipole.

The first method was described in detail in the experiment with depth recording of primary responses (VIII G). Introducing a microelectrode vertically into deep layers of the cortex at the site of maximum surface response makes it possible to compare activity in different cortical layers with the simultaneously recorded surface potential. Initial surface positivity of the primary response is replaced by an initial negativity at a certain depth. This indicates that the microelectrode has reached the other side of the corresponding dipole. On further advancing the electrode down the amplitude of negativity gradually decreases. Fig. 189 shows the characteristic shape of the electrical field through which the microelectrode passes when introduced parallel to the axis of the dipole.

Characteristic voltage distribution may also be found on the surface of a spherical homogenous conductor containing a radial dipole. It is evident that its projection will be the more pronounced the greater is the dipole moment and the closer it is to the surface of the conductor. It may thus be

stated that the site of maximum response corresponds to the area of localisation of the dipole. Using more exact analysis the depth of the dipole can be calculated from surface distribution of the potential. According to Shaw and Roth (1955) the potential (V_p) on the surface of a homogenous spherical conductor with radius R which contains a dipole whose axis lies on a radius is defined as:

$$V_p = \frac{M^2}{R^2}\left(\frac{1-f^2}{f(1-f^2-2f\cos\varphi)}\right) - \frac{1}{f}$$

where M is the dipole moment, f the distance of the centre of the dipole from that of the sphere, φ the angle between the radius for point P and the radius of the dipole. Fig. 190 shows the theoretical distribution of the potential for different depths of the dipole. The depth of the dipole can be determined from the angular distance between the site of the maximum response and the site of phase reversal.

5) In a volume conductor the potential recorded at a given point is the resultant of activity of close and distant elements, the electric fields of which often reach to a considerable distance. It is difficult to distinguish between such distant and close activity using conventional technique. Methods based on recording of current instead of potential make it possible to obtain more exact information concerning the distribution of sources and sinks in a small volume of tissue (Howland et al. 1955).

Consider a square area A, B, C, D (Fig. 191) in a section plane or on the surface of nerve tissue. The parts of neurons contained in it are either isopotential in relation to those lying outside or sources or sinks of current. If the currents passing in or out through the individual sides of the square are added algebraically, the total contribution of the given area in an outward direction is given. Currents arising outside this square area are excluded automatically since if they enter through a side of the given square they

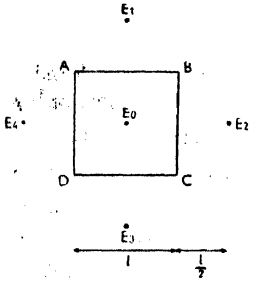


Fig. 191. Determination of sinks and sources, on the basis of the average current flowing across the sides of hypothetical square ABCD. For details see text.

must leave it by another one (Kirchhoff's law). Thus they cancel each other.

Currents flowing through individual sides of the square are determined from potential differences between monopolar leads from electrodes localised in the centre of the square and against the mid-point of its sides at distance $l/2$, according to the equation:

$$i_1 = \frac{l}{\varrho}\,\frac{E_0 - E_1}{l} = \frac{E_0 - E_1}{\varrho}$$

where ϱ is the specific resistance of the medium.

The current out of the whole square is

$$i_0 = \frac{1}{\varrho}(4E_0 - E_1 - E_2 - E_3 - E_4)$$

The so-called Laplacian is a practical realisation of this principle (Perl and Casby 1954). Five electrodes arranged as shown in the diagram in Fig. 191 are placed on the cortical surface. The potential recorded between E_0 and the average from electrodes E_1, E_2, E_3, E_4 is proportional to the current i_0 and according to Perl and Casby permits a more exact localisation of primary projections than the usual recording of primary responses.

If the distribution of electrical sources in a cross section of the volume conductor is to be analysed in detail another procedure must be adopted (Howland et al. 1955). The whole plane of the section is mapped out by inserting a microelectrode into different depths at regular steps. A series of recordings is made from each position of the electrode. This method can only be used if variability in the responses is minimal. The exact position of the electrodes is checked histologically and marked on a large photograph of the section. Using cross interpolation of the potential values the irregular net of the actual electrode positions is transformed to a regular square lattice which serves for calculating the elementary currents. In such a way the planar distribution of sources and sinks of currents at different phases of the realisation of a certain reflex act may be determined. If a three-dimensional current distribution is required an elementary cube would have to be used. This could be determined from potentials from further points distributed in such a way that they would form a regularly spaced cubic lattice.

IX

Electrophysiology of subcortical structures

A. The stereotaxic method

The use of the stereotaxic method described by Clarke and Horsley in 1906 is a necessary prerequisite for electrophysiological and anatomical research into subcortical centers. The principle is simple: an electrode (metal needle, glass capillary) or other instrument is introduced mechanically without direct visual control into a certain nerve structure the position of which is given with reference to a three-dimensional system of coordinates determined by external landmarkers on the skull or by other reference points. Stereotaxic apparatuses of various constructions for different animals have been described by Horsley and Clarke (1908), Clarke and Hendersen (1920), Ranson (1934), Harrison (1938), Clark (1939), Krieg (1946b), Stellar and Krause (1954), Carpenter and Whittier (1952), Hume and Ganong (1956), Cort (1957). A number of modified stereotaxic apparatuses for man has also been described (e. g. Hayne and Meyers 1950, Jasper and Hunter 1949, Bailey and Stein 1951, Spiegel, Wycis, Marks and Lee 1947 and others). For review of literature see Pavlov (1958) and Szentágothai (1957).

The usual type of stereotaxic apparatus consists of two fundamental parts: a head-holder for fixing the head of the animal in an exactly defined position and an arrangement permitting measurable movements of the electrode carriers in rectilinear or radial coordinates (Fig. 192).

The first part usually consists of two adjustable screws or plugs that fit into the external auditory meati and a bar (or bars) holding the upper jaw. Fixation determines the basal reference plane permitting orientation of the system carrying the electrodes with respect to the skull and brain.

In principle this is a system of three planes perpendicular to one another. The basal plane is chosen so as to be roughly parallel with the base of the brain. It passes through both external acoustic meati and the anterior reference point differing according to the animal and apparatus used. In the majority of laboratory animals the lower surface of the upper jaw just behind the canine or incisor teeth (Cort 1957) or the lowest point on the orbital margin (Jasper,

Ajmone-Marsan 1952b) is chosen as the anterior point determining the basal plane. Some authors, however, also use other basal planes. Thus, e. g. Whittier and Mettler (1949) in the macacus use a plane passing through the external auditory meati and the upper orbital margin. In stereotaxic atlases for the rat (Krieg 1946b) and rabbit (Sawyer, Everett and Green 1954) the basal plane is defined as perpendicular to the sagittal plane and passing through the bregma and a point 1 mm (rat) or 1·5 mm (rabbit) above lambda.

The sagittal plane passes through the head medially in the region of the sagittal suture, the frontal plane is given by the line joining both auditory meati, through which it passes perpendicularly to the basal plane. Since in the majority of apparatuses the basal plane is identical with the horizontal one, the other two planes take a vertical course. In a number of stereotaxic apparatus there is arrangement permitting inclination of the fixed head in a rostro-caudal direction and thus also inclination of the basal plane to the horizontal.

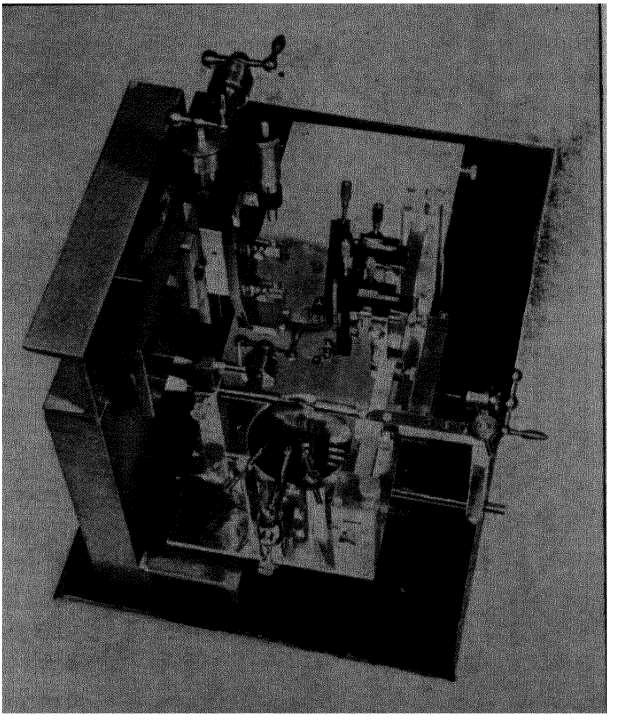


Fig. 192. Universal stereotaxic apparatus permitting simultaneous introduction of a number of electrodes at different angles and basal approach to the brain. Adaptable for work with the cat, rabbit and rat.

The electrode carrier assembly makes it possible to achieve precisely measurable movement of the electrodes in rectilinear (most frequently used today) or radial coordinates. The vertical movement is sometimes equipped with a micrometric drive or an hydraulic system (Li, Cullen, Jasper 1956a). This permits exact introduction of the electrode into the depth of the brain. Movement in the horizontal plane is achieved either by moving the electrode carrier mounted on a special frame permitting antero-posterior and medio-lateral movements and their measurement on a scale, or, if the electrode carrier is fixed, by moving the head holder fitted to a lathe slide (Fig. 192).

Both these methods may be combined in various ways. It is also possible exactly and measurably to incline the electrode carrier in the sagittal or frontal plane by at least 45°. This permits approach to those parts of the brain that are not directly accessible from the dorsal surface of the skull (e. g. mesencephalon in the cat).

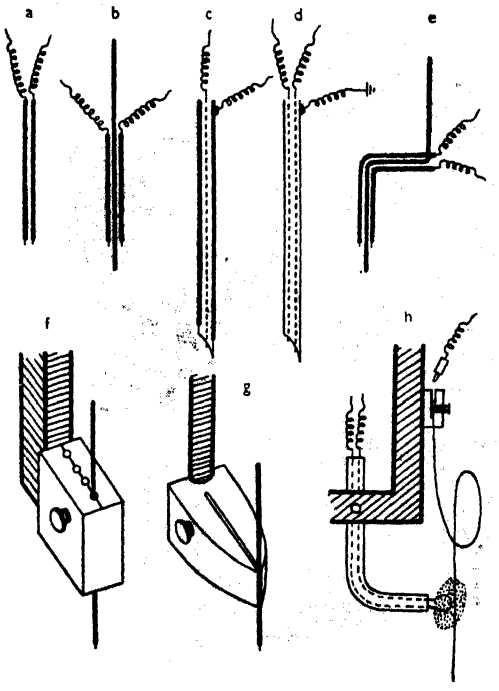


Fig. 193. Different types of electrodes and electrode carriers used with the stereotaxic apparatus. a) bipolar electrodes, b) bipolar electrodes with a fixing wire. c) concentric electrodes. d) bipolar electrodes with a needle sheath. e) electrodes for implantation (the bend permits firm fixation to the bone), with an auxiliary fixing wire for the electrode holder. f) g) different types of electrode carriers. h) arrangement for rapid release of the stereotaxically-introduced electrode, according to Baumgarten (1957).

The electrode itself is either firmly connected to the carrier or, if it is to be implanted, in such a way that it can easily be released. Baumgarten (1957) for instance, recommends fixing the electrode to a platinum wire with a drop of paraffin. Electric heating of the platinum loop at a suitable moment melts the paraffin if rapid release of the stereotaxically introduced electrode is required (Fig. 193h).

Electrodes applied stereotaxically are used for three fundamental purposes: for recording activity of deep structures, for stimulating them and for producing deep lesions.

In all cases the use of the stereotaxic instrument may be acute — i. e. the anaesthestised or curarised animal remains in the head holder of the stereotaxic instrument throughout the experiment — or it may serve as a preparation for a chronic experiment — i. e. after implanting the electrodes or producing the lesion in an anaesthetised animal there follows a long term period of observation of behaviour or recording electric activity from chronically implanted electrodes. In chronic experiments the whole procedure must be aseptic. The fur is removed from the head of the animal by first cutting and then applying a depilatory (barium sulphide). The head is fixed in the stereotaxic head holder by screws introduced into the external meati and by bars or clamps for the upper jaw. Care is taken to place the head symmetrically and sufficiently firmly in a position corresponding to the basal plane. Then the

scalp is painted with iodine washed with 70% alcohol. Surgical instruments are sterilised in the usual manner. Trepanation and the introduction of the electrodes are usually carried out in two stages.

In the first stage, after cutting the skin and cleaning the skull bones, the inclination of the basal plane is adjusted to bregma and lambda if necessary. Coordinates of the zero point are then established using a heavy marking needle. The sites of trepanations are marked on the skull surface with reference to the zero point. If the point of intersection of the 3 reference planes is used as zero its coordinates must be determined before fixing the skull in the head holder, for the marking needle and each of the electrodes. The use of an auxiliary zero point on a horizontal bar exactly 70 or 100 mm above the interaural line makes it possible to obtain zero coordinates without removing the head from the head holder. Without removing the animal from the stereotaxic apparatus trephine openings are drilled with a dental drill or a hand trephine at the sites marked. The openings must be of adequate size, small if the electrode is to be introduced chronically, larger if the localisation of the electrode is going to be monitored during the experiment by physiological responses to stimulations.

In the second phase the actual stimulating, recording or coagulating electrodes (coaxial, concentric, bipolar etc. — Fig. 193a–d) washed with ajatin, zephiran or another disinfectant are fitted into the carrier (Fig. 193fg). The horizontal coordinates of the trepanation are again tested (to make sure that the head was not moved during trepanation), the dura mater is pierced with a sharp needle and the electrode is introduced. In chronic experiments the upper bone layer surrounding the trephine opening is previously roughened with a dental drill or a scalpel. After thoroughly drying the bone the electrode fixed to an auxillary needle is introduced (Fig. 193e) and fixed to the roughened bone with phosphate cement or acrylate. After this has dried perfectly the fixing needle is released from the electrode carrier.

Determination of stereotaxic coordinates

The position of a nervous structure is determined using a system of three coordinates indicating its distance from the point of intersection of the three zero planes or from a certain point on the skull (bregma, lambda). Stereotaxic maps showing the position of brain structures in such systems of coordinates have been worked out for the monkey (Olszewski 1952, Clarke and Henderson 1920, Atlas and Ingram 1937), the cat (Clarke and Henderson 1911, Winkler and Potter 1914, Ingram, Hannett and Ranson 1932, Gerard, Marshall and Saul 1936, Hess 1932, Jimenez-Castellanos 1949, Jasper and Ajmone-Marsan 1952b), the rabbit (Sawyer, Everett and Green 1954) and the

rat (Krieg 1946b). There are, however, sometimes considerable discrepancies between the data from such atlases and actual findings. These are due to different shapes of the skull in the used representatives of a certain species. If we intend to study a certain structure it is of advantage to use the atlas only as a first approximation and to determine its more precise localisation using animals of the laboratory breed and the stereotaxic apparatus actually to be employed. The procedure is as follows: the anaesthetised animal is killed by perfusing the carotid arteries with saline followed by 10% formaline. The whole head is then placed into 4% formalin for several days or weeks. Then it is fixed in the head holder of the stereotaxic apparatus in the usual position. The soft parts are removed and the bone is carefully cleaned. Using a strong marking needle fixed in the electrode carrier the coordinates of the zero reference point are obtained. Then an extensive craniotomy is made uncovering the whole of the dorsal cerebral surface down to the first spinal segment. This is done very carefully since the head must not be moved. The dura is carefully removed. A piece of razor blade is then fitted into the electrode carrier in such a way that it is parallel to the frontal plane and frontal sections are made at 0·25—0·5 mm intervals at the sites of the expected localisation of the required center, using the mediolateral movement of the electrode carrier for cutting. This is done in rostrocaudal sequence and the cut nervous tissue is removed. Sections are made until the required structure appears. The marking needle is then again fixed to the electrode carrier and the coordinates of the required point are determined.

If the structure cannot be identified macroscopically serial sections must be examined histologically. This method is only succesful if the inclination of the coronary sections corresponds to the frontal plane of the brain in the animal fixed in the stereotaxic apparatus. This is made easier if a block of cerebral tissue is cut out of the skull fixed in formalin in the way described above.

Fixation in formalin results in a certain decrease in cerebral volume. This may change the spatial relations between different cerebral structures and the skull. Still greater deformations occur during embedding in paraffin (the freezing technique is preferable), cutting and preparation of serial sections. It is therefore of advantage to verify the coordinates obtained from heads fixed in formaline by lesions or iron deposits (see page 365) placed in normal brains. Small electrolytic lesions (cf. below) are produced in several points within and around the structure looked for. Then the animal is sacrificed, the brain fixed and prepared for histological examination. The position of the required structure is determined in serial sections with respect to the known stereotaxic coordinates of the lesions. The stereotaxic coordinates from the atlas or the formalin treated brain are corrected according to the results obtained.

Completely homogenous material (animals of the same breed, age, weight, proportions of the skull etc.) is necessary if the stereotaxic technique is to be used succesfully. Even so it is necessary to treat the data obtained from a fairly large number of animals statistically and to determine average values and their variation.

Stereotaxic lesions

Many methods are used to produce nervous lesions stereotaxically: mechanical destruction of tissues, injections of poisons or chemical substances, cauterisation, heating of implanted metal wires within an electromagnetic field of shortwave diathermy, implantation of beta or gamma emmitors (gold or radon seeds) etc. The method of electrolytic lesions, however, is the most valuable and most frequently used.

Either single or concentric bipolar electrodes are introduced into the structure to be destroyed. The electrodes are well insulated with glass or varnish and have a smooth, conical, carefully cleaned tip. Platinum, silver, steel or constantan are used as material. Before use the insulation of the electrodes is tested. The electrode is connected to the — pole of a 1·5 V battery and immersed in saline. The + pole is connected to a plate electrode placed in the solution and the formation of bubbles is observed. When using the monopolar technique the needle electrode has a positive potential against the large indifferent electrode placed on the cut skin, on the back, in the rectum or on the exposed muscles of the nape of the neck. Anodal lesions are preferable to cathodal since during electrolysis less gas is formed at the anode. Thus mechanical tissue injury is less and the shape of the lesion is more regular. An anode battery or rectifier is used as current source (voltage about 500 V). The intensity of the current is regulated by a 1 MΩ potentiometer connected in series and continuously measured with a miliammeter. The size of the lesion is roughly proportional to the quantity of electricity that has passed through the electrode. Usually a current of 2—10 mA is used for 15—30 seconds. The resulting lesions are spherical or oval in shape with a diameter of 2—6 mm. depending on the intensity and duration of the current. When using bipolar concentric electrodes the outer part is used as the anode and the core as the cathode. With the same intensity and duration of current lesions are slightly larger and more irregular than with the monopolar technique. High frequency current (0·5—1·0 megacycles per sec.) may be used instead of D. C. current but the insulation of the electrodes is then critical. Imperfect insulation results in irregular lesions along the electrode track due to current leaks.

The location of a lesion can usually be controlled only anatomically after the termination of the experiment. In some cases in which a successful lesion causes the disappearance of a characteristic function, the effectiveness of the stereotaxic intervention can be monitored during the experiment by observing changes in that function. Fig. 194 shows primary cortical response

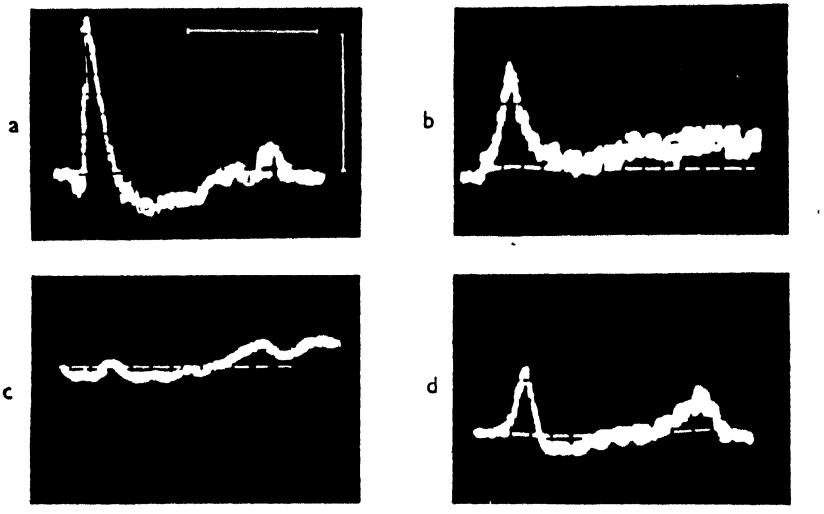


Fig. 194. Cortical response of the anaesthetised rat to acoustic stimuli before and after bilateral lesion in the region of the inferior colliculi. a) Control tracing. b) After bilateral lesion. c) After enlarging the lesion laterally. d) Two hours after placing lesions. Calibration: 200 μV. Time mark: 50 msec.

to acoustic stimuli during electrolytic destruction of the lemniscus medialis at the level of the inferior colliculi in the rat. Only after the second lesion has been placed does the response disappear. It must, of course, be remembered that the disappearance of a response immediately after electrolysis does not necessarily imply complete destruction of the corresponding nervous structure. In a number of neurones at the periphery of the lesion injury is reversible and their function may be recovered within a variable period.

In addition to electric current, nervous tissue can be stimulated or destroyed by implanting some slowly diffusing substances (e. g. powdered NaCN, alumina cream, a suspension of penicillin, strychnine paste etc.).

The use of the stereotaxic apparatus for introducing recording or stimulating electrodes in acute and chronic experiments will be described in detail in further experiments.

B. Primary responses in the subcortical centres of afferent systems

Problem: Record in the rat responses to stimulation of the sciatic nerve from centres of the somatosensory pathway (posteroventral thalamic nucleus, cortical samaesthetic area), to acoustic stimuli from auditory centres (corpus geniculatum mediale, colliculus inferior, cortical auditory area) and to visual stimuli from centres of the optic pathway (corpus geniculatum laterale, colliculus superior, visual cortex).

Principle: A synchronous volley of impulses produced by a short stimulus spreads along nervous paths into the cerebral cortex with a slightly increasing temporal dispersion. It elicits characteristic potentials in individual synaptic relays of the specific path. These usually are diphasic waves, an initial positivity followed by a negative phase. The response lasts for 10—30 msec. and reaches its maximum amplitude in the corresponding centre. If microelectrodes with a tip diameter of less than 30—40 μ are used, unit activity can usually also be recorded together with the slow synaptic potentials.

When simultaneous recordings are made from the subcortex and the corresponding cortical projection area, the interval necessary for conduction of the impulse from the subcortical relay into the cortex can be well determined. The following means can be used to check the location of the recording electrode in the nuclei of the path studied:

1) The difference in latency between the subcortical and cortical response is small (it corresponds roughly to the time of conduction and the expected number of synapses),
2) stimulation with the recording electrodes elicits a similar cortical response with an appropriate latency,
3) application of strychnine to the area of the subcortical electrode results in strychnine spikes spreading to the next higher centre.

Technically the most difficult task is to find the corresponding subcortical centres. Usually it is necessary to use the stereotaxic apparatus and to refer to stereotaxic atlases for coordinates of the centre. It is therefore of advantage to use animals for whom such atlases are available — especially the cat, rabbit or rat. Yet even if such a map is strictly followed the optimum localisation of the electrode must always be found experimentally by probing the expected area until the maximum response is obtained. In any case, it is of course necessary to verify the localisation of the electrode histologically at the end of the experiment.

Object: Rats, weighing about 200 g.

Apparatus: A two channel A. C. or D. C. amplifier (time constant at least 0·5 sec., input resistance 5—10 MΩ) and a cathode-ray oscillograph for recording. Total sensitivity at least 50 μV/cm. A stimulator giving single square

wave pulses or condenser discharges synchronised with the time base of the cathode-ray oscillograph. An isolation unit (isolating transformer, RF output). Time marker.

Other requirements: Surgical instruments, a set of trephines, a dental drill. Stereotaxic apparatus for the rat. Steel needle electrodes (0·2—0·3 mm wire gradually tapering to a tip diameter of 30—40 μ well insulated except for 50—100 μ of the tip). Wick Ag-AgCl electrodes and steel needle electrodes fitted for corticography (cf. p. 321). Bipolar hooked platinum electrodes for stimulating the sciatic nerve. A stroboscopic apparatus giving light flashes of high intensity. 10% dial, 10% formalin with a 1% ferrocyanide solution.

Procedure: The dorsal surface of the skull of a rat under dial anaesthesia is exposed by cutting the skin in the midline. The parietal, occipital and temporal skull bones are scraped clear and dried, after dissecting the temporal muscle. The animal is fixed in the stereotaxic apparatus in such a way that bregma is 1 mm higher than lambda. The external auditory meati are left free and fixing screws are applied to the rostral part of the temporal bone since sound stimuli are to be applied. Then a sharp strong needle with which the coordinates of the zero reference point (bregma) have first been determined is used to mark the centres of the trephine openings on the skull from which it will be attempted to reach the individual subcortical structures. Table 4 gives their coordinates in mm from the bregma assuming that the bregma-lambda distance is 8 mm.

TABLE 4

Structure	coordinates			diameter of trepanation
	antero-post.	lateral	depth	
coll. inf.	8	2	4—5	4
c. g. med.	5	2·5	6	3
coll. sup.	5·5	1·5	4	4
c. g. lat.	4	3·5	5—6	4
posterovent. nucl. thal.	2·5	2·5	5	4

Only one trephine hole is made over each hemisphere. The surface of the bone surrounding the trephine opening is roughened with the dental drill to ensure a good contact for the phosphate cement applied in further stages of the experiment. After trephining the stereotaxic coordinates of the zero point are again checked.

The area of maximum cortical response to the corresponding stimulus is found first (cf. p. 335). Wick Ag-AgCl electrodes are used for mapping in areas exposed by trepanation, needle electrodes in those covered by bone. The indifferent electrode is fixed in the frontal bone above the level of the olfactory

bulbs. The cortical response is recorded during the further course of the experiment in one of the two channels. The subcortical needle electrode is fixed to the electrode carrier and the corresponding stereotaxic coordinates for the zero point are again determined.

The electrode is then inserted into the previously exposed cortical area to a point 1·5 mm above the expected centre. Then the electrode is further advanced gradually at 0·25 to 0·5 mm intervals and one minute after each step several (10—20) responses to stimuli, the effect of which is checked simultaneously by the cortical primary responses, are recorded with this

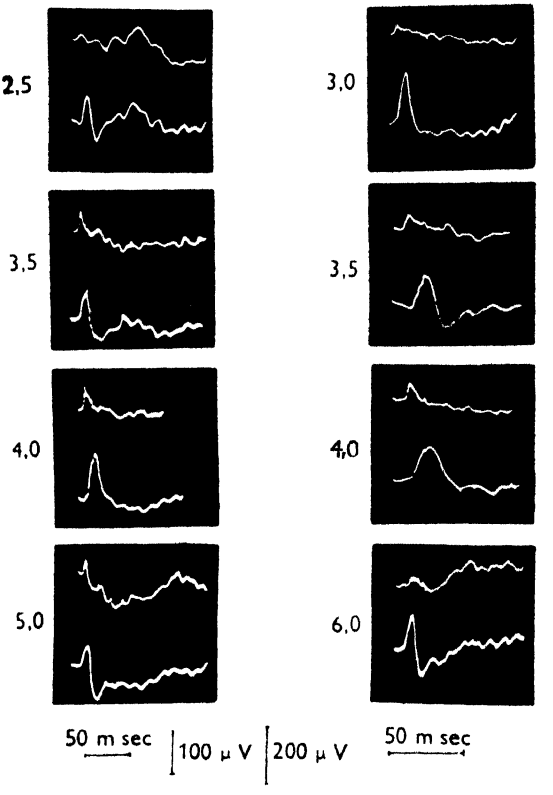


Fig. 195. Recording of primary responses to acoustic stimuli from the inferior colliculi (upper tracing) and the cerebral cortex (lower tracing) during progressive introduction of the deep electrode (AP9, L2). Figures denote the depth coordinate of the tip, according to the stereotaxic atlas. At a depth of 3·5 and 4·0 mm, tracing with a more rapid time base were made. Positivity of the active electrode upward. Calibration: 100 μV for the deep and 200 μV for the surface electrode.

electrode (Fig. 195). In such a way the electrode is advanced 1·5—2·0 mm deeper than would correspond to the depth of the centre according to the stereotaxic atlas. If no potential is found throughout the path which should be related to the cortical primary response, the electrode is pulled out and shifted 1 mm rostrally (or caudally, medially, laterally) and again introduced. Large pial vessels are of course avoided (special care to avoid injuring the sinus is necessary when trephining above the collic. inf.).

If a response is obtained in a certain position or positions of the electrode, we attempt to define the extent of the responsive area and to find the maximum response by systematically exploring the surrounding area. It must be borne in mind, however, that the introduction of several relatively large electrodes into a small volume of brain tissue may in itself adversely affect its normal

function. It is therefore necessary to limit the number of exploratory insertions to a minimum.

As a test for the specifity of the potential produced by a certain stimulus other receptors or nerves are stimulated. The electrode is fixed at the site of the maximum response if further subcortical electrodes are to be introduced into the same animal stereotaxically. A drop of phosphate cement is placed on the bone at a point nearest to the electrode and the electrode is fixed with another drop. This is then released from the stereotaxic electrode carrier after the cement has hardened. Thus several electrodes may be introduced into different cerebral regions in the same rat. The localisation of the electrodes may be verified further by using the deep electrode for stimulation and observing the cortical response. For monopolar stimulation a large indifferent electrode (an Ag-AgCl plate wrapped in cotton wool soaked in saline) is applied

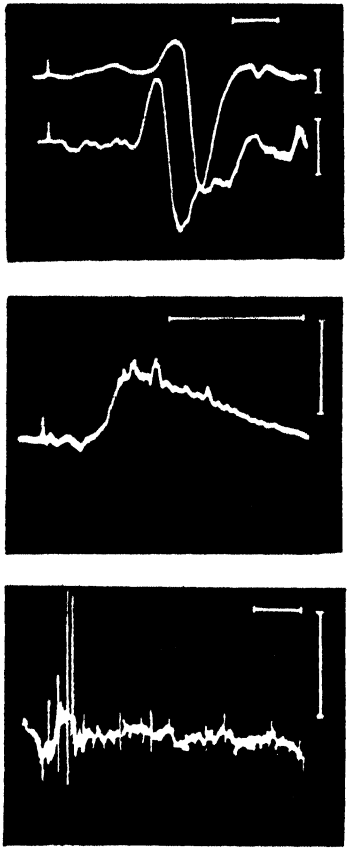


Fig. 196. Examples of specific responses in subcortical centres. a) Colliculus superior, optic cortex — response to an optic stimulus. b) Thalamus — slow potential with superimposed unit activity in a bipolar recording. Stimulation of the sciatic nerve. c) Colliculus inferior — acoustic stimulus. Microelectrode recording. Calibration: 100 μV. Time mark: 20 msec.

to the cut skin and muscles of the nape of the neck. Bipolar stimulation is, of course, better since it permits more exact localisation of the focus.

Results: A number of papers, mainly using the cat, have been devoted to a detailed study of subcortical responses in specific relays of sensory pathways. The somatosensory pathway has been studied by Therman (1941), Morin (1953), Cress and Harwood (1953), Berry, Karl, Hinsey (1950), Harwood and Cress (1954), Hunt and O'Leary (1952), Marshall (1941), Morin (1952), Mountcastle and Henneman (1949), Schricker and O'Leary (1953), Cohen and Grundfest (1954) and others. Auditory projection has been studied by Kemp, Coppé and Robinson (1937), Kemp and Robinson (1937), Ades (1944), Ades and Brookhart (1950), Thurlow, Gross, Kemp and Lowy (1951), Campbell

and Matzke (1950), Tunturi (1946), Rose and Galambos (1953), Galambos, Rose, Bromiley and Hughes (1952) and others. Many papers are also concerned with optic projection: Bishop and O'Leary (1942a, b), Bishop and Clare (1951), Bishop (1953), Bishop and Mc Leod (1954), Lennox (1956), Cohn (1956), Vastola (1955) and others.

In spite of the large variability in responses due to different orientation of the active elements with regard to the electrode and the small dimensions of the centres studied the majority of monopolar responses obtained with electrodes of larger diameter has the character of a positive—negative sequence. The initial positivity recorded in the area of maximum response corresponds to the afferent activity, the negativity to synaptic potential and to the postsynaptic volley. The response usually lasts about 15—30 msec. The latent period is always shorter by 2—3 msec. than that of the primary cortical response for a response at the thalamic level (c. g. lat., c. g. med., posteroventr. nucleus) and even shorter for lower centres (Fig. 196a, b). If electrodes with a smaller tip diameter are used (less than 30 μ) unit spikes can be recorded in addition to the slow potentials. They are usually observed during the negative phase of the synaptic potential, as an expression of postsynaptic neuronal discharge (Fig. 196c).

The shape of the response depends also on the synchronisation of the afferent signal. Direct stimulation of the optic nerve after enucleation elicits in the corpus geniculatum laterale of the cat a short (4 msec.) response with clear cut presynaptic and postsynaptic spikes (Bishop and McLeod 1954). The corp. gen. lat. with its „one to one" synapses is particularly suitable for responses of this type. When using an intensive flash in rats, a prolonged potential is obtained from the corp. gen. lat. This is similar to potentials from other subcortical structures and is due to increased temporal dispersion of impulses arriving from the retina.

As far as lateralisation of subcortical responses is concerned there is a pronounced contralateral response on stimulation of a sciatic nerve or one eye. This is not the case for acoustic stimuli which elicit nearly equal responses in both the homo- and contralateral collic. inf. and corp. gen. med. after unilateral stimulation.

At the end of the experiment the position of the electrodes is marked either by an electrolytic lesion or by depositing a small amount of iron from the steel electrode into the surrounding tissue (30—50 μA, 10 sec., electrode positive). The iron ions are then detected by fixing the tissue in 10% formalin with 1% ferrocyanide. The iron appears as small blue spots that are clearly visible in serial sections stained with conventional technique.

Conclusion: A study of electrical responses at various levels of a specific pathway makes it possible to follow the spread of a synchronous afferent volley in the brain.

C. Electric activity of the cerebellum

Problem: Record the spontaneous electrocerebellogram and responses of the cerebellar cortex to afferent stimulation.

Principle: Spontaneous electrical activity of the cerebellum of unanaesthetised mammals recorded with surface macroelectrodes manifests itself as very fast low voltage potentials (Adrian 1935, Dow 1938: 150—300/sec., 10—50 μV). Recording of this type of bioelectric potentials makes great demands on the amplifier which must not only have low noise (less than 5 μV) but also high amplification, linear up to 2—5 kilocycles/sec. A cathode-ray oscillograph must be used as the recording instrument.

An electrocerebellogram registered with surface macroelectrodes by the usual ink-writing EEG apparatus does not, of course, show any of this activity because of the inertia of the pen. The recording does not differ fundamentally from the ECoG but is usually of lower amplitude (Swank and Brendler 1951, Cooke and Snider 1954).

The cerebellum also reacts characteristically to stimulation of peripheral receptors and nerves or of the cerebral cortex and other central structures (Dow 1939, Dow and Anderson 1942, Adrian 1943, Grundfest and Campbell 1942, Snider and Stowell 1944, Snider and Eldred 1951, Combs 1954, Bremer 1952, Bremer and Bonnet 1951, Szabo and Albe-Fessard 1954, Bremer and Gernandt 1954, Albe-Fessard and Szabo 1955, Jansen 1957, Morin, Catalano and Lamarche 1957). Depending upon the type of anaesthesia used, the shape of cerebellar responses is fairly irregular and their projection unstable. They always show an initial positive wave if monopolar recording is used.

Object: Cat weighing 3—4 kg.

Apparatus: A cathode-ray oscillograph with an A. C. amplifier (time constant from 0·01 to 0·5 sec., overall amplification 20—50 μV/cm. linear to 5000 c/sec., peak to peak noise maximally 5 μV). A multichannel ink-writing EEG apparatus of conventional design. A stimulator giving single square wave pulses or condenser discharges synchronised with the time base of the oscillograph. An output isolation unit for the stimulator. A time marker.

Other requirements: Surgical instruments, trephines. Dental drill. Stereotaxic apparatus for the cat. Bipolar steel or silver stimulating electrodes. Ball-tipped platinum or silver recording electrodes mounted on springs or wick Ag-AgCl electrodes. Electrode carriers. A loudspeaker. Photostimulator. 10% pentothal. D-tubocurarine or other relaxant. Ether. 1% novocaine.

Procedure: The trachea of the cat under ether anaesthesia is exposed low in the neck, tracheotomy is performed and a tracheal canulla inserted. D-tubocurarine is then given i. p. and as soon as it starts to take effect, artificial respiration is begun (30 breaths/min.). The scalp is incised in the midline after local 1% novocaine infiltration. Using a dental drill openings are trep-

hined above the right hemisphere and in the occipital bone in the midline (diameter 8—10 mm). The openings are enlarged by rongeuring away the bone until nearly the whole area of the right hemisphere and the major part of the dorsal and dorsocaudal surface of the cerebellum in the region of the vermis and the hemispheres is exposed. Bleeding is continuously stopped with bone wax. Then, also under local novocaine anaesthesia, the sciatic and saphenous nerves in left thigh and the radial nerve in the right forearm are exposed. After this we wait for 30—60 minutes. Small additional doses of curare are given if necessary.

Electrical potentials are recorded with electrodes fixed in electrode carriers of the stereotaxic apparatus. A bipolar recording of spontaneous activity is made with a distance of 0·5 to 1·5 cm between the electrodes. For monopolar recordings of evoked potentials a metal clamp fixed to the cut skin is used as the reference electrode.

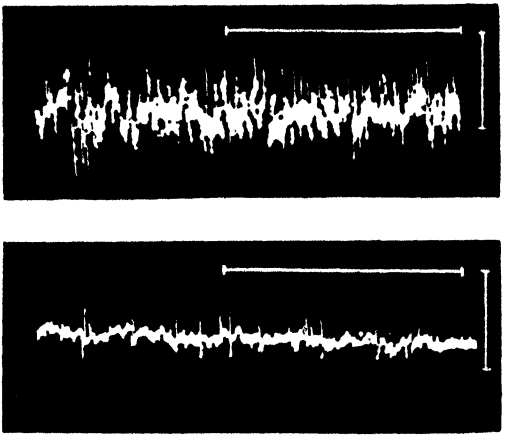


Fig. 197. Surface electrocerebellogram in the curarised cat before (top) and 10 min. after (bottom) an i. p. injection of 50 mg./kg. pentothal. Calibration: 200 μV, 100 msec.

Fast spontaneous activity is recorded with the shortest time constant available (0·01 sec.) and with the H. F. filter completely switched off so that the upper part of the frequency range of the amplifier can be fully utilised. The sweep velocity or the rate of film movement must be adjusted to the frequencies recorded. For control purposes the recording from the cerebellum is compared with tracing from the surface of the cerebral cortex made under identical conditions with the same electrodes and same setting of the amplifier. The noise level is either controlled at the end of the experiment by recording with the same electrodes, amplification and filters several minutes after the death of the animal or during the experiment by substituting an equivalent resistance for the electrodes and the animal.

In addition an ink recording may be made with an amplifier of the EEG apparatus connected in parallel to the same electrodes. Here another time constant (0·3—1 sec.) is used and if necessary also muscle filters. If possible the ECoG is recorded simultaneously and the two records are compared.

The character of the cerebellar responses to electric stimulation of the sciatic and radial nerves is then determined, as well as that to acoustic and

optic stimuli and to electrical stimulation of the somatosensory, auditory and optic projection areas of the cortex. Short clicks are used as acoustic stimuli. These are produced be feeding amplified impulses from the stimulator into a loudspeaker. Light flashes from a stroboscope serve as visual signals. By systematically moving the recording electrodes at 2 mm steps the extent

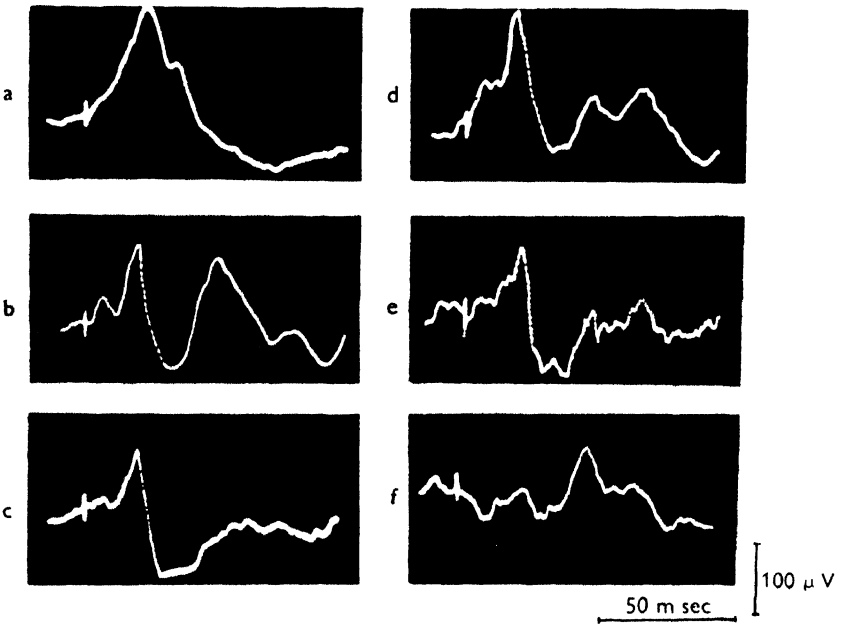


Fig. 198. Examples of typical evoked potentials in the cerebellar cortex. a) tibial nerve — anterior lobe. b) acoustic stimulus — vermis. c) radial nerve — paramedian lobe. d) stimulation of acoustic cortex — anterior lobe. e) stimulation of somatic cortex — vermis. f) optical stimulus — vermis. Positivity of active electrode upward.

of the electrically responding cerebellar cortex is determined for each stimulus. When recording evoked potentials a longer time constant is used (0·3—1·0 sec.) and no filtration of fast components is applied. Responses are recorded with single sweeps synchronised to the stimulus and with camera shutter opened.

In the second phase of the experiment, spontaneous and evoked electric activity of the cerebellum are followed during barbiturate anaesthesia. Fifty mg./kg. penthotal are given to an animal which is beginning to recover from the effect of curare and changes in the electrocerebellogram and electrocorticogram are compared. When anaesthesia has attained a constant level as shown by characteristic spindles in the cerebral cortex (cf. experiment VIII C) mapping of cerebellar projection areas is repeated and again the shape of the responses and the extent of their projection are noted.

Means described in other experiments may also be used to analyse cerebellar activity, e. g. microelectrodes (cf. p. 362), anoxia (cf. p. 368) and application of strychnine (cf. p. 359).

Results: The electrocerebellogram of the curarised unanaesthetised cat is characterised by fast low-voltage activity (Fig. 197), which can sometimes

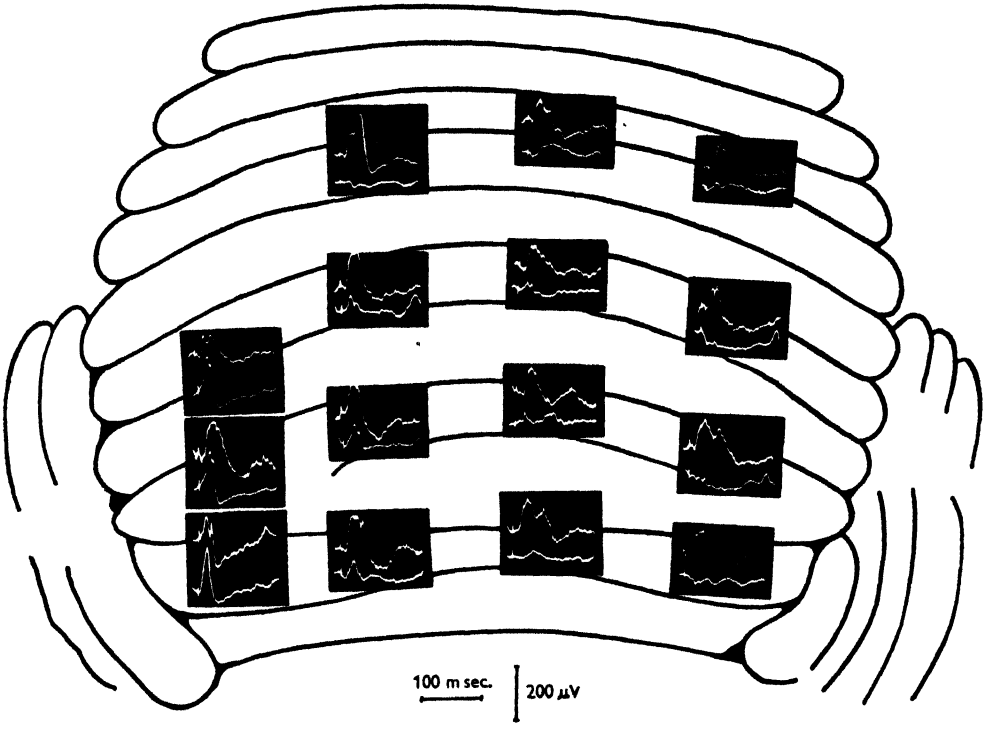


Fig. 199. Projection of cerebellar responses to stimulation of the left superficial radial nerve. Recording from anterior lobe of the cerebellum in curarised cat, before (above) and after (below) administering Pentothal. Positivity of active electrode upward.

be distinguished only with difficulty from the noise of the amplifier. Such small waves lasting 2—4 msec. and having an amplitude of 20—50 µV cannot be found in the ECoG registered in the same way. Under the influence of general anaesthesia this fast activity rapidly disappears. It is closely related to unit discharges of cerebellar cortical neurones (Purkinje cells and cells of the granular layer).

The ink recording of the electrocerebellogram with a longer time constant shows that this fast activity is superimposed on much slower waves that do not differ substantially from ECoG waves. The characteristic effect of barbiturates appears in the electrocerebellogram as well as in the ECoG, in the form of increased amplitude and appearance of slower frequencies.

Cerebellar and cortical potentials are, however, only rarely synchronous. This indicates that the subcortical structures controlling synchronisation of electrical activity of cortical structures of the telencephalon and rhombencephalon are not identical.

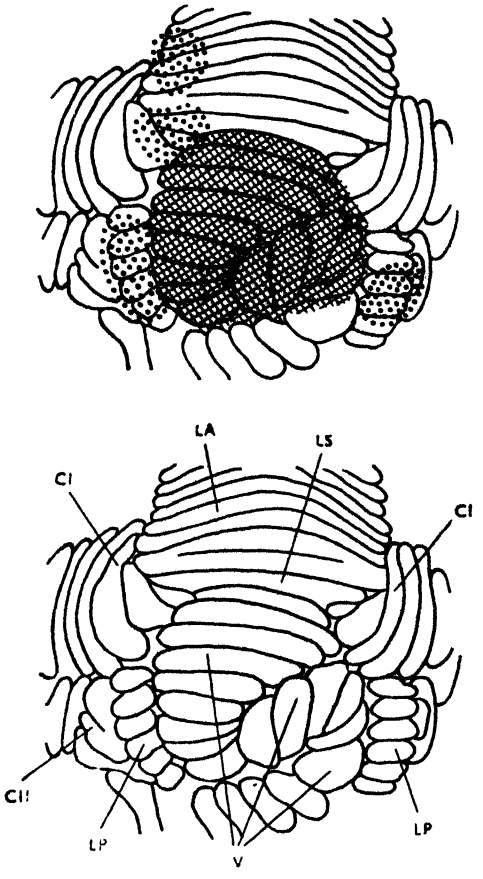


Fig. 200. Localisation of responses to different stimuli in the cerebellum. Cross-hatched area – auditory and visual projections. Dotted area — somatic projection.

CI — crus I
CII — crus II
LA — lobus anterior
LP — lobus paramedianus
LS — lobus simplex
V — vermis

Evoked potentials, although showing considerable variability, are mainly surface positive waves lasting 10 to 20 msec., with an amplitude of 50 to 100 μV (Fig. 198). This positive wave is very similar to primary responses in the cerebral cortex and is usually followed by a slower and less pronounced negativity. The latent period depends on the structure stimulated. Stimulation of peripheral nerves evokes a cerebellar response with a latency of 10—20 msec., for acoustic stimuli the latency is 10 to 14 msec., for optic stimuli 40—50 msec. and after stimulation of the cerebral cortex the response appears after 4 to 10 msec. The main positive deflection is sometimes preceded by 4—6 msec. by a smaller (20—50 μV) positive wave or waves. The interpretation of the cerebellar potentials is not easy—mainly because of the complex convolution of the cerebellar surface into deep gyri and sulci. It appears that the small positive waves preceding the main component of the response are due to activation of the granular layer by arriving afferent impulses (Morin, Catalano and Lamarche 1957). The positive phase of the positive-negative response is an expression of arrival of afferent impulses into the cerebellar cortex and of postsynaptic activation of the granular layer. The negative phase, on the other hand, is due to activity of superficial elements of the cerebellar cortex (dendrites of Purkinje cells).

The projection of different afferent systems into the cerebellum is not strictly specific. Particularly in the unanaesthetised animal, the projection

areas overlap considerably. Barbiturates, on the other hand, narrow the projection areas. Fig. 199 shows examples of the distribution of potentials evoked by stimulation of the left superficial radial nerve before and after pentothal administration. The extent of the projection of further afferent systems according to Snider and Stowell (1944), Combs (1954), Hampson (1949), Morin et al. (1957) and our own results is shown diagramatically in Fig. 200.

Conclusion: Despite a certain structural similarity between the cerebral and cerebellar cortex, the latter is characterised by very intense fast spontaneous activity, the nature of which is not yet understood (Bremer 1958). The extensive overlapping of the primary responses in the cerebellar cortex indicates a diffuse distribution of the afferent inflow permitting convergence of impulses of heterogenous modalities.

D. Nonspecific subcortical influences in the cerebral cortex

Problem: Record the electrical phenomena evoked in the cerebral cortex by stimulation of nonspecific systems in the medulla oblongata, mesencephalon and thalamus.

Principle: Electric stimulation (100—300/sec.) of some subcortical structures changes the sleeping type of ECoG (slow waves, spindles — cf. VIII B, C) into the waking type simultaneously throughout the cortex in an animal under light chloralose anaesthesia or in the unanaesthetised "encéphale isolé" preparation. Electrocorticography and the behaviour of the animal show that it is aroused as after stimulation of peripheral sense organs. Moruzzi and Magoun (1949), Lindsley et al. (1949, 1950), French and Magoun (1952), French (1952), French et al. (1953), Narikashvili (1953), Segundo et al. (1955) and others using stimulation and extirpation, determined the extent of the corresponding areas of the brain mainly in the ventromedial reticular formation of the medulla oblongata, the reticular formation of the mesencephalon, subthalamus (zona incerta, corpus Luysi, Forel's field) and in nonspecific nuclei of the thalamus.

Slow stimulation (6—10/sec.) of nonspecific thalamic nuclei in the cat under barbiturate anaesthesia produces a special type of generalised ECoG reaction (Dempsey and Morison 1942a,b, 1943, Morison and Dempsey 1942). The response is found over the whole cortex especially in the associative areas, its latency is 20—35 msec. and it is predominantly surface negative. If stimulation of the same intensity lasts for several seconds, the responses show a gradual increase and then decrease in amplitude — "waxing-waning". Hence the term "recruiting response". The work of Jasper (1949, 1954), Jasper et al. (1946, 1952a, 1955), Starzl et al. (1951) and Verzeano et al. (1953)

exactly defined those areas in the thalamus that produce this type of reaction when stimulated: nucleus centralis lateralis, nucleus paracentralis, nucleus parafascicularis, nucleus supragenicularis, centrum medianum, nucleus centralis medialis, nucleus submedius, nucleus rhomboideus, nucleus reticularis thalami, nucleus ventralis anterior.

The recruiting response is similar in many respects to spontaneous barbiturate or sleep spindles (Morison, Finley, Lothrop 1943) characteristic for the EEG of anaesthetised or drowsy animals. This indicates its thalamic origin.

The relationship between the bulbo-ponto-mesencephalic activating system and the diffuse thalamic system is not quite clear. According to some (Starzl, Taylor and Magoun 1951) these are two components of the same complex. Others (Gellhorn 1952, 1953, Gellhorn et al. 1954) maintain that two opposing systems exists, an activating (reticulohypothalamic) and an inhibiting (purely diencephalic). For review of literature see Zimkina 1958, Buser 1957.

Object: Cat weighing 3—4 kg.

Apparatus: Ink writing EEG apparatus. A cathode-ray oscillograph that can be connected to the output of the EEG channels. A stimulator giving rectangular pulses with a frequency of 0·5 to 500 cycles/sec. An isolation unit for the stimulator. A time marker.

Further requirements: Surgical instruments, trephines, dental drill. Stereotaxic apparatus for the cat. Screw electrodes for corticography (diameter 2 mm). Bipolar needle electrodes for subcortical stimulation (steel needles, diameter 0·1—0·2 mm, length 5 cm, distance between the tips 1 mm).

Procedure: 1) Encéphale isolé preparation (Bremer 1935, 1936). The cat is anaesthetised with ether. First the subcutaneous and later also deep tissues of the neck are infiltrated with 1—2% novocaine. After introducing a tracheal cannula, the cat is fixed in the stereotaxic head holder with the head elevated and a long sagittal skin incision is made from the frontal bones to about 3—4 cm behind the occipital protuberance. The neck muscles are dissected away from the occipital region of the skull and the atlas. The occipital joint is exposed and the atlanto-occipital membrane is cut between the occipital bone and the atlas. Through the opening thus formed a blunt leucotomy knife is inserted and the spinal cord is severed. Spinal shock may be prevented by chilling the cord or local application of anaesthetics at the level of transsection. Care is taken not to injure the spinal arteries so as to preserve normal blood supply to the brain base. Immediately after the spinal cord is transsected artificial respiration is applied (17—20/min). It is suitable to use a thermostatic animal board and to introduce a cannula connected to a mercury manometer into the femoral artery for measuring the blood pressure. If the last falls below 80 mm Hg, continuous i. v. infusion of adrenaline is given.

The cortical screw electrodes are fixed to the exposed skull bones in the usual way (experiment VIIIB). The bipolar needle electrodes are inserted according to coordinates of the atlas into the bulbopontine reticular substance and fixed to the bone with phosphate cement.

Following all surgical procedures, administration of ether is interrupted. Only an hour later, when the last effect of ether has disappeared is the actual experiment commenced. The type of EEG activity is decisive as only in a drowsy state can a clear cut arousal be obtained. If sleep pattern is pronounced, the reticular formation is stimulated (100—300 cycles/sec., 0·5—1·0 msec., 2—5 sec.). A voltage of 0·5 V is used first, and the stimulus intensity is increased to 5 V gradually, with successive stimuli separated by about 1 minute intervals. That threshold stimulus voltage is sought which will produce a pronounced arousal reaction in the cortex. With suprathreshold stimuli, the duration of EEG desynchronisation after cessation of stimulation is observed. We attempt to determine the extent of the reticular area from which the ECoG can be activated by moving the electrodes vertically or inserting them in another frontal or parasagittal planes.

In experiments with recruiting responses, the same preparation is used. The cortical electrodes remain in place without change but monopolar recording is used. The metal frame of the stereotaxic apparatus or a skin flap serves as reference electrode. The paired stimulating electrodes (of the same shape as when stimulating the reticular substance) are inserted according to stereotaxic coordinates of the atlas into the region of the nucleus ventralis anterior or nucleus centralis medialis. Stimulation is commenced several minutes after introducing the subcortical electrodes. Trains of stimuli of 2 sec. duration are used (frequency of 6—9/sec., pulse duration 0·5—1·0 msec., voltage 2—10 V). As far as possible the response is recorded simultaneously from several cortical electrodes. By changing the localisation of the stimulating electrodes, the depth and, if possible, other coordinates of the thalamic structures giving the recruiting response, are determined.

If two stimulators are available, one may attempt to stimulate simultaneously thalamic structures producing a recruiting response and the reticular activating system. When the recruiting response is pronounced the reticular activating system is stimulated for 2—3 sec. with a stimulus producing a good arousal response. Changes in cortical potentials are observed.

The following experiments are further suggested:

a) Registration of cortical response to stimulation of the reticular substance by single stimuli or stimuli of low frequency (up to 10/sec.).
b) The effect of high frequency stimulation (100—300/sec.) of the non-specific thalamus on the sleep EEG pattern.
c) The effect of stimulating the reticular substance on primary cortical responses produced by stimulating peripheral nerves or receptors.

Results: Periods of sleep activity alternating with periods of waking activity can be seen in the spontaneous ECoG of the "encéphale isolé" preparation. To a certain extent the sleeping or waking state of the animal can be judged from movement of the eyes, the pupils and the ears (Bremer 1936). During sleep the eyes are turned upwards and the pupils are fixed and constricted. In the waking preparation, the eyes and ears move and the animal reacts to light. Since the preparation receives afferent impulses from cerebral nerves it can be aroused by sensory stimulation, particularly by acoustic

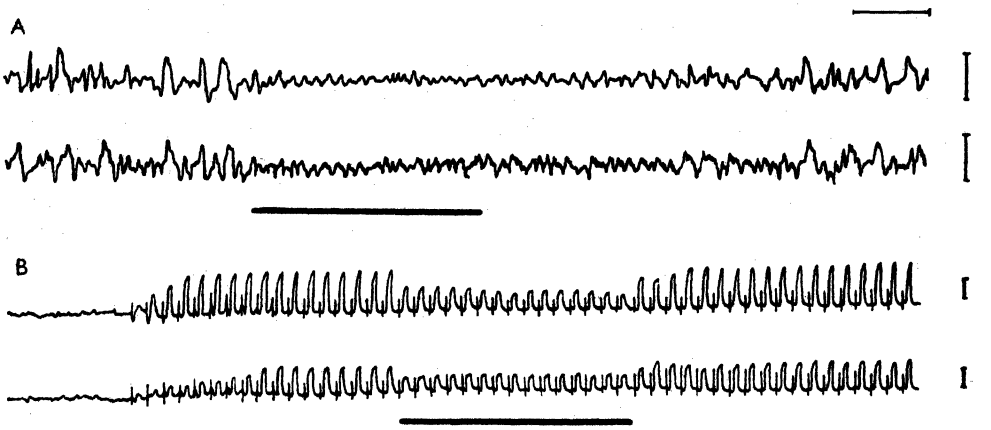


Fig. 201. The effect of reticular stimulation (300/sec., 1 msec., 3 V) on sleep activity in the cerebral cortex (A) and on the recruiting response (B). Cat, encéphale isolé preparation. Calibration: 1 sec., 200 μV.

and trigeminal stimuli. It, therefore, happens sometimes that nociceptive stimuli due to the surgery and fixation of the head in the stereotaxic head holder maintain the preparation in a state of wakefulness. Usually local anaesthesia of all sites that might be a source of pain and a small dose of chloralose (5—10 mg/kg i. p.) produce the required level of sleep activity.

Fig. 201A shows the effect of stimulating the reticular substance in the left half of the midbrain on the ECoG of both hemispheres in a drowsy animal. Both hemispheres are activated equally. With threshold stimuli (decrease in stimulus voltage or frequency), however, activation may be homolateral. More intense stimuli produce fast low activity persisting for a longer time after termination of stimulation. Different threshold values correspond to different localisations of the stimulating reticular electrodes so that it is possible to map the extent of the areas giving optimum results.

The effect of slow stimulation of the nonspecific thalamic nuclei is shown in Fig. 202. A gradual increase in duration of responses and their diffuse projection are characteristic signs. The extent of cortical projection as well

as the shape and latency of the response is, of course, considerably dependent upon the localisation of the thalamic electrodes. Hence, in addition to well defined generalisation, we find cases with predominating frontal or occipital distribution of the responses. The response is usually bilateral but more pronounced on the side of the stimulus. In monopolar recordings, increasing cortical responses are surface negative and attain their maximum amplitude in associative areas of the cortex, in the cat, particularly in the suprasylvian

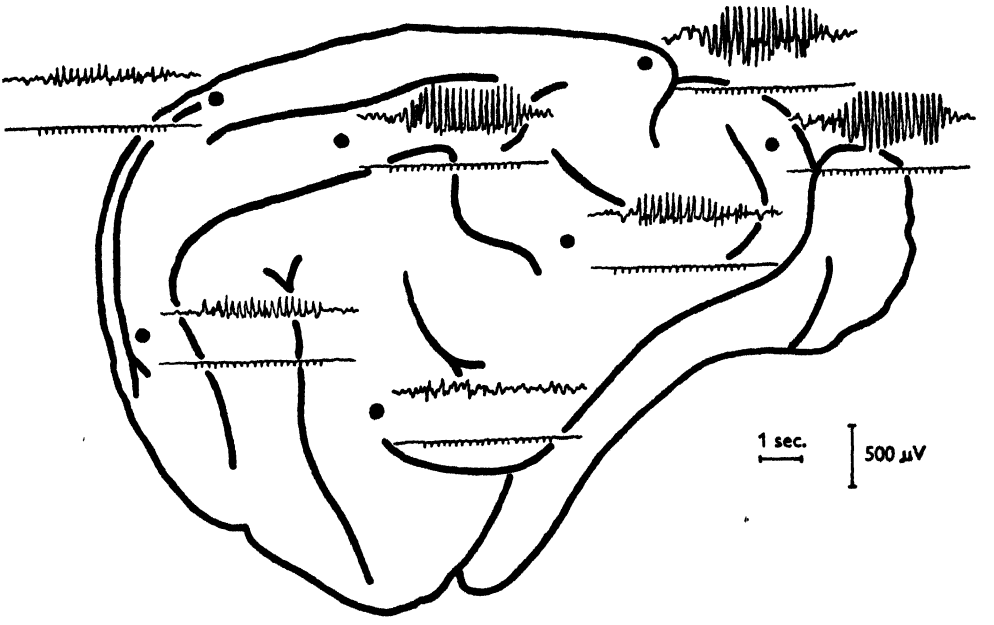


Fig. 202. Recruiting response in different areas of the cerebral cortex in the cat (dial anesthesia). Stimulation of homolateral centrum medianum.

gyrus and the sensorimotor cortex. When stimulating the nucleus ventr. ant., the latency is about 4—5 msec. Stimuli acting on the nucleus centrum medianum or n. centralis med. produce a response only after 20—40 msec.

If a stimulus with a frequency of 200 cycles/sec. is introduced into the thalamic electrodes giving a recruiting response, a typical arousal reaction is elicited. Slow stimulation (6—9/sec.), on the other hand, with electrodes placed in the reticular formation of the mesencephalon or medulla, does not produce a recruiting response nor is usually a clear cut cortical response observed.

Stimulation of the bulbar reticular substance considerably decreases (Fig. 201B) or even suppresses the recruiting response elicited from the homo- or contralateral nuclei of the nonspecific thalamus (Moruzzi and Magoun 1949, Jasper et al. 1955). Stimulation of the reticular activating system also

affects in the same way the so called augmenting response, a reaction similar in character to the recruiting response but produced by stimulation of specific thalamic nuclei (Morison and Dempsey 1942, Gauthier et al. 1956) and limited to the corresponding projection area of the cortex.

Primary cortical responses to stimulation of peripheral nerves are either not fundamentally changed by stimulation of reticular activating system (Moruzzi and Magoun 1949) or are decreased (Gauthier et al. 1956, Hernandez-Péon and Haggbarth 1955). Associative secondary responses, on the other hand, (Albe-Fessard and Rougeul 1955, 1956, Buser and Borenstein 1956) which are especially pronounced in the suprasylvian gyrus after large doses of chloralose, disappear almost completely on reticular stimulation.

Conclusion: The nonspecific system of the thalamus and the bulbo-mesencephalic reticular activating system represent complex polysynaptic structures controlling the functional state of the cerebral cortex and especially of its associative regions.

E. Electrical activity of the hippocampus

Problem: Record the electric responses in the cornu Ammonis of the rabbit to stimulation of different rhinencephalic structures.

Principle: The rhinencephalon is an important part of the telencephalon differing from other structures in the forebrain phylogenetically and morphologically (for literature c. f. Gastaut 1952). It is formed by:

1) bulbus olfactorius, tractus olfactorius, tuberculum olfactorium,
2) gyrus intralimbicus, which among others includes the cornu Ammonis and gyrus dentatus, induseum griseum, striae longitudinales lateralis and medialis,
3) gyrus fornicatus composed of the gyrus hippocampi and gyrus cinguli.

Cytoarchitectonically the rhinencephalic cortical structures are characterised by a preponderance of pyramidal elements and the absence of granular layers. Very favourable conditions for studying electrical activity of different parts of neurones in extracellular recordings are thus created, especially in the hippocampus (allocortex). Ramón y Cajal (1911) and Lorente de Nó (1934) gave a detailed description of the morphology of the hippocampus. Starting from the ependymal surface facing the lateral ventricle, the following layers are found in the cornu Ammonis: the alveus — a 100—200 μ layer of tangential fibres formed partly by axons of pyramidal cells and partly by afferent fibres. A layer of basal dendrites (200—300 μ) follows and a layer of pyramidal cell bodies forming a narrow band (70—100 μ) parallel to the enpendymal surface. The apical dendrites at first have only few branches (250 μ) then, however, they form a dense net reaching into the preceding

layer (600—700 μ). By stimulating afferent fibers ending on different parts of pyramidal neurones it is possible to observe the isolated response of dendrites and cell bodies and to compare the relationship between structure and response (Cragg and Hamlyn 1955, Euler, Green and Ricci 1958). By studying the shape and latency of the responses to stimulation of different rhinencephalic structures, it is possible to clarify the functional and anatomical relations between them (Green and Adey 1956).

A special relation to the ECoG can be found in records of hippocampal activity registered with the conventional EEG apparatus in an unanaesthetised rabbit. The arousal reaction in the cortex is accompanied by very characteristic regular waves of a frequency of 3—6/sec. in the hippocampus (Jung and Kornmüller 1939, Green and Arduini 1954). Transition to sleep ECoG pattern, on the other hand, is accompanied by appearance of low and fast asynchronous activity in the hippocampus.

Object: Rabbit, weight 2—3 kg.

Apparatus: A two channel D. C. or A. C. amplifier with an input resistance of 5 MΩ and a double beam cathode-ray oscilloscope. Overall sensitivity at least 100 μV/cm. A stimulator giving square wave pulses or condenser discharges synchronised with the time base of the oscillograph. An isolation unit. A time marker. An EEG ink writing apparatus with at least four channels.

Other requirements: Surgical instruments, trephines, dental drill. A stereotaxic apparatus with equipment for fixation of the head of the rabbit. Steel semimicroelectrodes with a tip diameter of 30—40 μ pointed electrolytically from the original diameter of 0·2 mm. Wick Ag-AgCl electrodes for corticography. Concentric needle electrodes for recording spontaneous activity of the hippocampus. 10% pentothal. A respiration pump. A vacuum pump and glass pipettes for sucking off brain tissue.

Procedure: The trachea of a rabbit under light pentothal anaesthesia is exposed and a tracheal cannula introduced. After exposing and cleaning the skull bones an extensive craniotomy is performed above both hemispheres and the head is fixed in the holder of the stereotaxic apparatus. The dura is reflected and the larger pial vessels of one hemisphere are sealed with a cautery or with local application of $AgNO_3$. Pia is cut at first and the cortex lying above the lateral ventricle is then removed by suction using a fine pipette and minimal negative pressure. The hippocampus then appears at the bottom of the lateral ventricle. Finally the spinal cord is severed at CI-CII and artificial respiration is given.

By microdrive of the stereotaxic electrode carrier, one steel needle electrode is advanced to a depth of 0·8 mm below the ependymal surface and fixed there. It is connected to the output unit of the stimulator. The second stimulating electrode (with a large surface) is fixed to the muscles or skin of the neck.

Another electrode is then fixed into the electrode carrier and inserted into the hippocampus parallel to the stimulating electrode, 1·5 to 2·0 mm from the latter. The reference electrode is placed on the cut skin. The needle electrode is pushed down in 0·1 mm steps, and at each position a recording is made of responses to a threshold stimulus and a stimulus about three times as strong. We attempt to decrease stimulation artifacts first by changing the mutual position of the stimulating or reference electrodes.

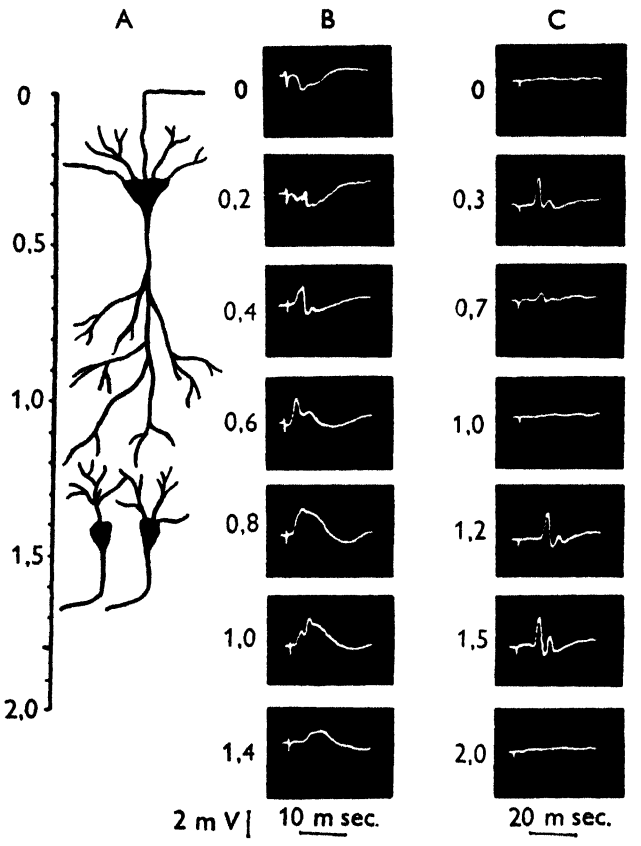


Fig. 203. Hippocampal responses to electrical stimulation. A) diagram of pyramidal cells in the hippocampus and g. dentatus, scale in mm. B) response from different depths below the dorsal surface of the hippocampus to stimulation of a point 0·8 mm deep. C) responses from a depth of 0·3 mm to stimulation at different depths. Figures denote the depth of the recording (B) or stimulating (C) electrode. Negativity of the active electrode upward.

During the next phase of the experiment the connection of the electrodes is reversed. The fixed electrode (0·3 mm deep) is connected to the amplifier input and the movable electrode to the stimulator output. Stimulation is again performed at 0·1 mm intervals with an intensity producing a threshold reaction when the stimulating electrode is at a depth of 0·1 mm.

Finally, without changing the position of the fixed recording electrode, we attempt to determine with this electrode the response to stimulation of other rhinencephalic structures — e. g. area entorhinalis, fornix, gyrus cinguli etc. A similar experiment may be performed in another part of the hippocampus with the fixed electrode in another position.

If the state of the animal is satisfactory and if it has quite recovered

from the effect of pentothal, the arousal reaction can be demonstrated in the other, as yet untouched, hemisphere. Otherwise another rabbit must be used for this experiment. Here no craniotomy is performed. Only small trephine openings are made for introducing the deep needle electrodes and cortical screw electrodes. Using the stereotaxic apparatus, concentric recording electrodes are pushed down into a region of the hippocampus symmetrical to the exposed cornu Ammonis. Wick Ag-AgCl electrodes for recording the

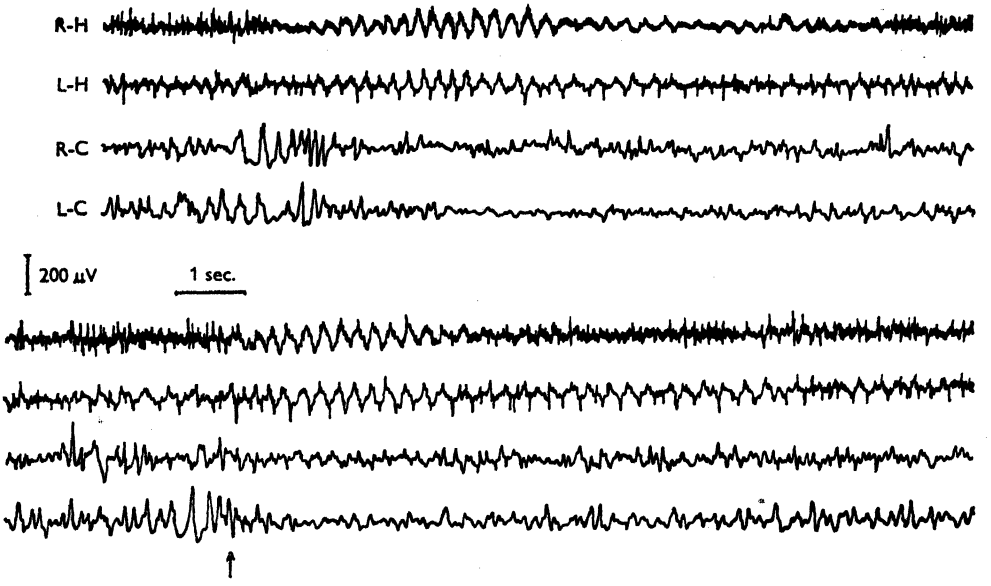


Fig. 204. Bipolar recording of cortical and hippocampal activity in the unanaesthetised rabbit. R–H, L–H — recordings from right and left hippocampus. R–C, L–C — recordings from right and left hemisphere. Top: spontaneous arousal. Bottom: arousal evoked by tactile stimulus (arrow).

ECoG are placed on the exposed surface of the hemisphere. Spontaneous activity of the hippocampus is registered for a few minutes together with the spontaneous ECoG. When the recording from the cortex shows typical sleep activity, sensory stimuli (tactile stimulation of the skin of the cheek, acoustic stimuli, olfactory stimulation — blowing of aromatic substances into the nostrils) are used to produce an arousal reaction, and the electric response in the cerebral cortex and hippocampus is studied.

Results: Fig. 203b shows, in a monopolar recording from various depths, the response to stimulation of a point 2 mm away and 0·8 mm deep. Fig. 203a also shows diagramatically the individual layers of the hippocampal cortex. The shortest latency of the response is at a depth of 0·8 mm. This indicates a tangential course of nerve fibres from the stimulation locus to the

apical dendrites at the recording site. The response is characterised by a negative wave lasting for about 15 msec., which is well in evidence, particularly with weaker stimuli. It is registered as a positive wave in superficial leads (in the region of basal dendrites and axons), evidently because it does not pass beyond the level of cell bodies. If this wave attains a certain amplitude one or more spikes are formed on it. These have an amplitude several times larger and a duration of 2—3 msec. Their spread can be followed down to the layer of basal dendrites. Maximum amplitude is reached immediately above the region of cell bodies. It then decreases, evidently because of the scatter of axons in the alveus. In the region of apical dendrites, the negative spike melts in slow potential waves.

Similar results are obtained when moving the stimulating electrode. The response with the shortest latency is obtained when stimulating at a depth corresponding to the position of the recording electrode. From deeper or more superficial positions the latent period is longer. When using slightly suprathreshold stimuli, hippocampal responses disappear when the stimulating electrode reaches a depth of about 1 mm. If, however, it is pushed further down, the response again reappears when stimulation begins to reach the gyrus dentatus (depth 1·2—2 mm.).

Characteristic responses in the hippocampus may be obtained by stimulating nearly all rhinencephalic structures. From the latent periods at different depths, one may conclude whether termination of corresponding afferent axons is on apical or basal dendrites. The responses to stimulation of the fornix, fimbriae and gyrus cinguli are most easily elicited. The hippocampus also responds to stimulation of peripheral nerves and to acoustic stimuli but responses are obtained only if the intervals between stimuli are very long (Green and Adey 1956).

A characteristic record of spontaneous hippocampal activity and ECoG is shown in Fig. 204. From it the reciprocal relationship between the two structures is evident. While sleep spindles occur in the cortex, the activity of the hippocampus is relatively asynchronous. In the intervals between cortical spindles, however, it is strikingly regular (3—6/sec., 100—200 μV). This activity is even more pronounced and more regular when eliciting the arousal reaction characterised by desynchronisation in the ECoG. This reaction is quite regular and the same for different arousal stimuli, so that it may be regarded as the rhinencephalic equivalent of arousal. At present it is not possible to give a good explanation of its physiological significance, particularly with respect to activity of the cerebral cortex.

Conclusion: A study of electrical activity of the hippocampus permits a better understanding of electrical phenomena in different parts of the neurone and contributes to the elucidation of physiological relationships between the rhinencephalon and the other parts of the telencephalon.

F. Electrophysiological signs of an epileptic seizure

Problem: Record the EEG during an epileptic seizure elicited by electroshock, cardiazol or by sensory stimulation. Demonstrate signs of epileptic irradiation.

Principle: During an epileptic seizure characteristic changes in cerebral electrical activity occur. These differ according to the type of seizure (Penfield and Jasper 1954). Experimental seizures produced in animals usually correspond to the human grand mal, characterised by tonic-clonic paroxysms and a complex of vegetative symptoms. Electrophysiologically, such a seizure appears as a discharge of fast, high voltage activity affecting the whole cortex and extensive subcortical regions. At other times, clear cut spike and wave complexes with a frequency of 2—5/sec. are found in the EEG.

An epileptic seizure is an expression of diffuse spreading of activity in central synapses. This occurs as the result of increased excitability of nervous structures due to drugs (e. g. cardiazol, picrotoxin) or metabolic effects (e. g. hypoglycaemia, hypocalcaemia). That region of the brain in which excitability is especially high, and which consequently may become the starting point of an epileptic seizure through the action of nervous and humoral factors, is termed a focus. Generalisation of a seizure is due to spreading of this pathological activity into regions of the brain stem and thalamus which are diffusely connected with the cortex and other parts of the brain.

Reflex audiogenic epilepsy in rats and mice may serve as an example of focal activation of an epileptic seizure. These are paroxysms occurring in a certain percentage of animals when strong acoustic stimuli act on them (jingling of keys, strong buzzers, bells, Galton's whistle). So-called Amantea's reflex epilepsy permits a detailed analysis of the relationship between the stimulus, the focus and the mechanism of generalisation. Amantea's epilepsy (Amantea 1921) develops in a certain percentage of dogs in whom an epileptogenic focus has been formed artificially by applying strychnine to a cortical projection area. Generalised seizures occur as the result of strong stimulation of the corresponding sense organ.

The ability to outlast the stimulus and to develop from a certain moment independently of it are fundamental characteristics of an epileptic seizure. In addition to automatic activity of centres, this feature may be explained by reverberating circuits permitting auto-re-excitation of activated nerve centres until they become exhausted or inhibited. Afterdischarge evoked by direct stimulation of the cerebral cortex (even of the neurally isolated cortex — Burns 1951) or an epileptic seizure produced by electroshock may serve as examples.

Object: Cat weighing 2—3 kg., rat 200 g.

Apparatus: A multichannel ink writing EEG apparatus. A dual beam cathode-ray oscillograph with A. C. amplification of 50 μV/cm. A stimulator

giving square wave pulses or condenser dischargers synchronised with the time base of the oscillograph. A tone generator or a time marker. A loudspeaker. A regulating autotransformer, a time switch for limiting the electroshock current to 0·1—1·0 sec.

Other requirements: Surgical instruments, trephines, dental drill. Needle electrodes for recording the ECoG in rats, wick Ag-AgCl electrodes with electrode carriers for recording the ECoG in the cat. Animal boards with stereotaxic head holders for the cat and rat. 10% dial, 1% cardiazol, 1% strychnine.

Procedure:

1) Under light ether anaesthesia the skin of the rat's head is cut along the midline and the skull bones are cleaned. The animal is then tied to the board, the head fixed in the stereotaxic head holder and needle electrodes are inserted into the bone as in experiment VIIIB. The fixing screws for the meati end in insulated metallic tips serving as electrodes for administering the electroshock current. The area of the meati is treated with EEG paste before the ear plugs are fixed.

The animal is permitted to recover from the anaesthesia. Fixation is then checked and the experiment begun. Electroshock is given after several minutes of control recording. A. C. current is used, the voltage of which can be changed by the regulating autotransformer. It is led to the electrodes across a serial resistance of 50 kΩ. The shock duration is set at 0·2—0·3 sec. with a bipolar switch. Normally both shock electrodes are grounded, and only during use are they connected to the autotransformer.

The switch and the autotransformer are outside the screened chamber, into which lead only the shielded cables to the shock electrodes. Just before giving a shock, the inputs of the EEG amplifiers are shortcircuited and after the shock has ended, they are again disconnected. Even so, the amplifiers are often blocked because of the large input signal. This can be prevented by means of a special anti-blocking device.

Usually several seconds immediately following the activity is lost for recording. If convulsions do not occur, the voltage (measured with a voltmeter in parallel to the autotransformer) is increased by 10—20 V and, after a pause of 10 min., another shock is given. This is repeated until threshold shock values are obtained. If the threshold is to be determined more exactly, the value of the seizure producing quantity of electricity or the density of this quantity with respect to the size of the head and the brain of the animal is used instead of voltage values.

2) Reflex audiogenic epilepsy in rats. Only animals which, by previous tests, are known to react consistently to epileptogenic acoustic stimuli with a running fit and convulsion are used. The animal is prepared as in paragraph 1. The head is fixed only by the upper jaw and the front teeth. The ears remain free. By dissecting the temporal muscle the bone above the cortical auditory

areas is made accessible, and one or two recording electrodes are placed in it. They serve to determine changes in reactibility of the auditory cortex.

When the effect of anaesthesia has completely worn off, EEG recording is commenced. After a short control period, 50 mg./kg. of cardiazol is given intraperitoneally. Changes in the ECoG are observed and 5 minutes later an acoustic stimulus is given. This is most simply done by jingling a bunch of

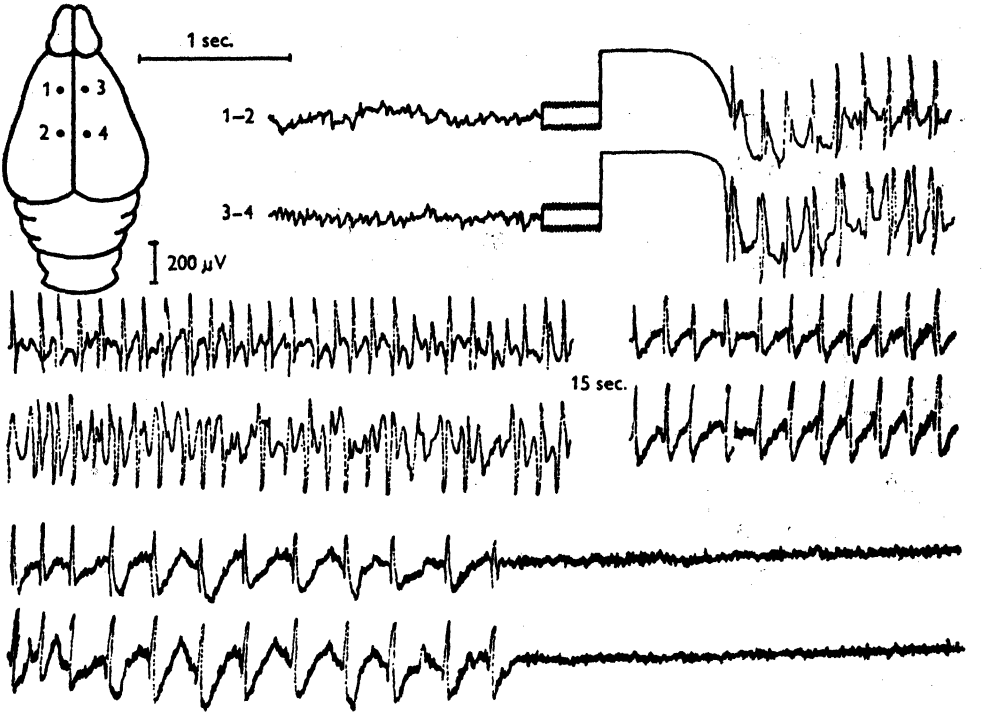


Fig. 205. Epileptic seizure evoked by electroshock in the unanaesthetised rat.

keys at a distance of 20—30 cm from the rat's head. If there is no seizure, the stimulus is discontinued after 2 minutes and the experiment is repeated on another animal. In highly sensitive animals, a seizure may be obtained even without a sensitising dose of cardiazol. Since restraint has an inhibitory effect on audiogenic epilepsy (Bureš 1953), the animal must first be adapted to the conditions of the experiment, i. e. it must be repeatedly fixed in this way before the epileptogenic stimulus is applied.

In addition to recording the spontaneous ECoG, an attempt may be made to record responses to short acoustic stimuli (clicks produced by feeding the stimulator discharges into the loudspeaker) in the auditory cortex with a cathode-ray osciloscope. The effect of cardiazol on primary auditory responses and their changes during and after the epileptogenic stimulus are observed (Fig. 207).

In animals that did not have an epileptic seizure following an acoustic stimulus, the dose of cardiazol is increased to 100—150 mg./kg. and an EEG recording of a series of epileptic seizures thus evoked is made.

3) Elements of epileptic irradiation. A cat under dial anaesthesia (40 mg./kg.) is operated as in experiment VIIID. The extent of the auditory cortex is determined in the usual way. Using bipolar stimulating electrodes

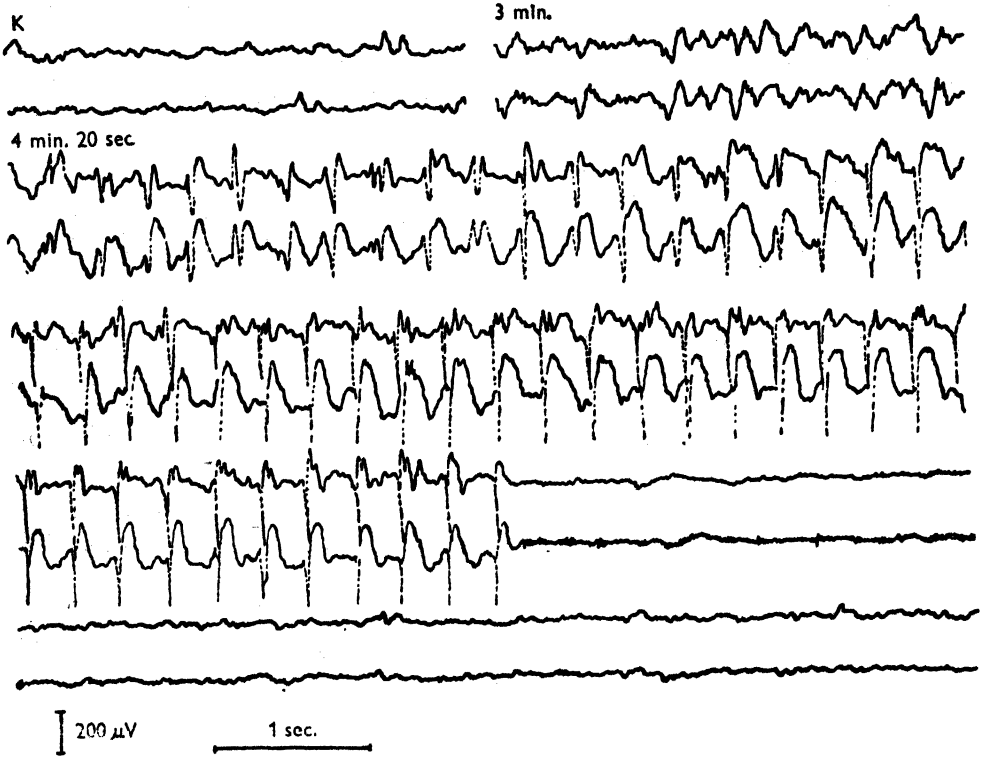


Fig. 206. Audiogenic seizure in a rat given metrazol. Electrodes as in the preceding figure. Figures indicate time following metrazol injection. Epileptogenic stimulus applied after 4 minutes.

(ball-tipped silver wires, diameter 0·5 mm, distance 1·5 mm) the motor area of the front limb is determined with rectangular pulses or A. C. current, regulated by an autotransformer. Care is taken to keep the stimulus duration constant (3—5 sec. preferably). Using threshold or slightly suprathreshold stimuli, a narrow projection is obtained. If a two channel oscillographic apparatus is available one electrode is placed onto the motor and the other onto the auditory area, and the experiment is commenced. After a short control recording of the electrical responses to clicks, a filter paper soaked with 0·5% strychnine is applied on the auditory area. When the effect of strych-

nine is maximal, cardiazol (20—40 mg./kg.) is given and the development of the responses in the motor and auditory areas is observed for 20 minutes. If responses are clear in the motor areas, the strychnine focus is cut around with a subpial knife and the effect of this intervention on responses in the motor areas is studied.

Results:

1) Fig. 205 shows a typical EEG recording before and after a suprathreshold electroshock. The motor reaction corresponds to the EEG tracing. After subthreshold stimuli, this is only a jerk of a defensive character which is sometimes followed by a short clonus of facial muscles. Suprathreshold stimuli, on the other hand, produce a typical seizure with a very regular course — initial clonus, followed by a tonic phase which is finally replaced by coarse clonic convulsions of both fore- and hind limbs.

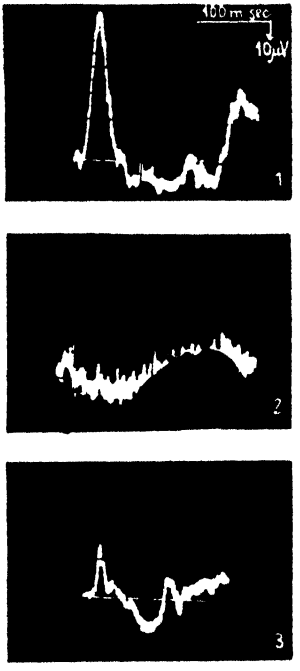


Fig. 207. Primary cortical responses to acoustic stimuli in the rat after metrazol injection (1), during seizure (2), and 1 min. afterwards (3). Monopolar recording. Positivity of active electrode upward.

2) Within several minutes after subthreshold doses of cardiazol, pronounced changes in the EEG occur (Fig. 206). The amplitude increases rapidly, synchronous slow waves of high voltage appear, first singly and later in groups. In general there is a change in frequency towards slower rhythms. After 3—5 minutes pathological rhythms predominate in the EEG (amplitude more than 500 μV, frequency about 3/sec.). At this stage the epileptogenic stimulus usually does not elicit pronounced EEG changes. Pathological slow rhythms of high amplitude continue to be present, sometimes during the whole duration of the acoustic stimulus. At other times, when manifest convulsions occur, they are replaced by another type of activity. The first sign of seizure is a rapid decrease or even disappearance of the slow waves. This may either occur suddenly or in several successive periods. During the seizure itself, this decrease in activity is even more pronounced, so that in all leads we find nearly iso-electric lines with superimposed low voltage, rapid activity. In some rats a shorter or longer period of the spike and wave activity with a frequency of about 3/sec. (Fig. 206) precedes this "electric silence". This

type of activity is characteristic mainly for clonic convulsions. The tonic phase, on the other hand, is usually accompanied by disappearance of electric potentials (Ajmone-Marsan, Marossero 1950).

After an injection of cardiazol, primary responses to an acoustic stimulus, poorly discernible in unanaesthetised rats, begin to increase rapidly and soon attain an iterative character (Bureš 1953). During the epileptogenic stimulus, primary responses to simultaneously applied "clicks" disappear, evidently

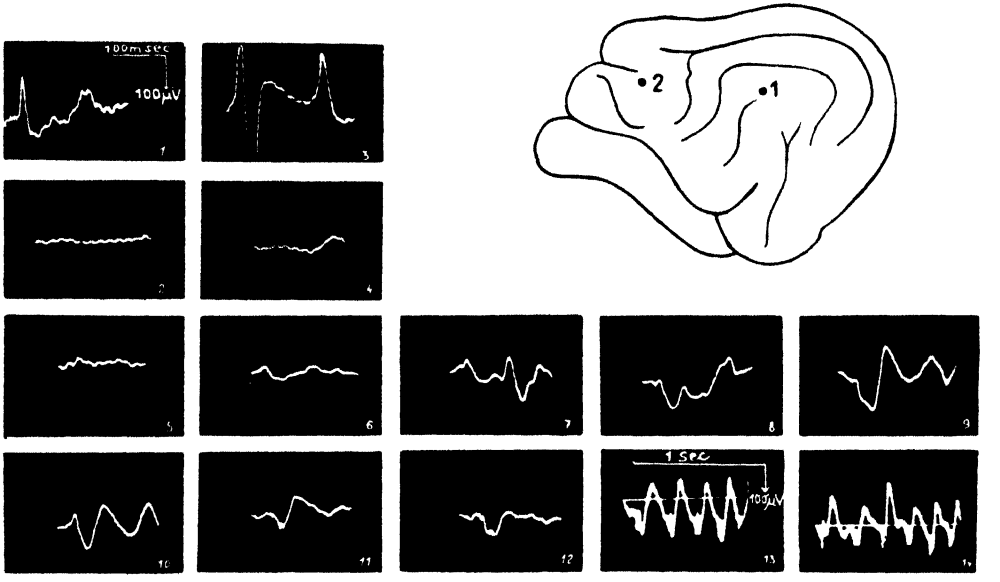


Fig. 208. Spreading of auditory responses into cortical motor areas following metrazol. Responses to acoustic stimulus in the auditory (1) and motor (2) areas. 3, 4 — the same responses after application of strychnine to the auditory area. 5–10 — responses in the motor area at 5 min. intervals after metrazol injection (40 mg./kg. i. p.). 11—12 — after circumcision of the cortical strychnine focus (depth 4 mm). 13—14 — recording from the motor area during clonic convulsions.

because they are masked by the stronger epileptogenic stimulus. If convulsions occur the primary responses remain suppressed even after the epileptogenic acoustic stimulus is off and reappear only after 2—3 minutes (Fig. 207).

3) No responses to acoustic stimuli can be recorded in motor areas of the cat's cerebral cortex before or after application of strychnine to auditory areas (Fig. 208). If, however, cardiazol is injected when the effect of strychnine is maximal, first weak and later more and more pronounced potentials of irregular shape and with a longer latency appear in the motor areas. A second wave usually follows the first one with an interval of about 100 msec. Potentials in the motor cortex begin to be accompanied by spasms of the ears, limbs and trunk. With a sufficient dose of cardiazol, repeated acoustic stimuli may

produce a clonic convulsion with the characteristic spike and wave activity of a frequency corresponding to that of clonus. A subpial incision interrupting intracortical connections between the auditory cortex and the other cortical areas usually only produces a certain decrease but not suppression of responses to acoustic stimuli in motor areas. This indicates that corticosubcortical connections between the strychnine-treated focus in the auditory area and motor areas are involved. Such connections may be regarded as elements of the mechanism of an epileptic seizure (Gastaut and Hunter 1950, Bureš 1953, Hunter and Ingvar 1955).

Conclusion: An experimental epileptic seizure manifests itself in the ECoG by a characteristic high voltage activity indicating synchronous discharge of cortical neurones. An analysis of the paths along which epileptic activity spreads from the focus, shows that subcortical centres are essential in the mechanism of generalisation of the fit. An epileptic seizure is terminated by deep depression of spontaneous and evoked cortical activity.

Appendix I

Stereotaxic atlases for the cat, rabbit and rat

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I. Material and methods

Three cats, rabbits or rats were used for determining the stereotaxic coordinates. The animals were exsanguinated under pentothal anaesthesia. The brain was fixed by perfusion through the carotid arteries with saline, followed by a solution of two parts of 96% ethanol and one part of 40% formalin. The soft tissues were dissected away, trephine openings made and the skull was immersed in 10% formalin for an additional 8 days. During this procedure, only insignificant weight changes occur (Flatau 1897).

After satisfactory fixation was attained, the skull was rigidly clamped into the head holder of a stereotaxic apparatus and the reference planes necessary for calculation of coordinates were determined (see also chapter IX A).

In the cat, the head was rigidly held by steel plugs introduced into the auditory canals, and by bars holding the upper jaw. A plane passing through the auditory meatus and the lower orbital margins is the basal reference plane. A parallel plane lying 10 mm dorsally was used as the horizontal zero plane. Needles were introduced perpendicularly to the basal plane 7 mm laterally from the sagittal zero plane at the level of the bregma and 16·5 mm occipitally. The needles introduced at the level of bregma determine the frontal zero plane. The sagittal coordinates of structures lying rostrally or caudally from this plane are marked AP— or AP+. The deep coordinates are determined with respect to the horizontal zero plane: above this level they are marked V+, below it V—. Distances to the left from the sagittal zero plane are marked S, to the right D. All values for stereotaxic coordinates are given in mm. The corresponding enlargement is given in the diagrams.

After additional fixation in ascending alcohol series, the brains were embedded in celloidine. Complete serial frontal sections (50 μ) were made and stained by the method of Nissl. The plane of section was parallel to the zero frontal plane in all animals used.

In the cat the stereotaxic atlas of Jasper and Ajmone-Marsan (1952b) was used as the basic guide for topographic orientation of subcortical structures. The data in papers of Rioch (1929) and Ingram et al. (1932) were used for the diencephalon and those of Fox (1940) for the septum, basal ganglia and amygdala. To make the determination of diencephalic lesions easier for neurophysiologists, the nomenclature of Jasper and Ajmone-Marsan was principally adhered to. Only those structures in which a secondary division can easily be found even with low magnification, were localised more precisely.

In the hypothalamus, the regio preoptica (RPO in the atlas of Jasper and Ajmone-Marsan) was divided into two parts having a different cellular structure. The part lying near to the medial wall of the III ventricle (of its most rostral part) is marked as area

praeoptica medialis (APM), the part lying more laterally and composed of disperse cells is called area praeoptica lateralis (APL). It joins the area hypothalamica lateralis caudally.

In the tuberal part of the hypothalamus, Jasper and Ajmone-Marsan distinguished two nuclei, nucleus periventricularis hypothalami and nucleus filiformis. As can be seen from their microphotographs and diagrams, these are two parts of the nucleus paraventricularis. The term "periventricularis" should be reserved for cellular groups directly adjoining the ependyma of the III ventricle, which form in many nammals true periventricular nuclei with typical small round cells. The cytoarchitectonic structure of the nucleus paraventricularis, on the contrary, is characterised by large, intensively-stained cells, clearly different from those of the periventricular nuclei.

In the septal region, described by Jasper and Ajmone-Marsan briefly as "septum" (SPT), the nucleus septalis medialis, nucleus septalis lateralis, nucleus septalis triangularis, nucleus septalis fimbrialis and nucleus fasciculi diagonalis (Broca) were distinguished. The nomenclature of thalamic nuclei remained unchanged; only the nucleus lateralis dorsalis of Jasper and Ajmone-Marsan is termed nucleus lateralis anterior (Kappers 1947).

In the rabbit, the skull is immobilised in the stereotaxic head holder in such a way that the bregma is 1·5 mm higher than the lambda (Sawyer et al. 1954). A plane passing through the bregma and an imaginary point 1·5 mm above the lambda corresponds to the basal horizontal plane. A parallel plane lying 12 mm lower is the horizontal zero plane. Structures above this plane have the depth coordinates marked V+, below it V—. Needles were introduced perpendicularly to the basal plane into brain, 5 mm. to the left of the midline at the level of the bregma and 3·5 mm. and 7·5 mm caudally. The plane passing through the bregma and perpendicular to the above basal plane is the frontal zero plane. Structures lying rostrally to the zero frontal plane have the stereotaxic coordinates AP—, those lying caudally AP+. Distances to the left and right from the sagittal zero plane are marked in the same way as in the cat (SD).

In describing subcortical structures, the stereotaxic atlas of Sawyer et al. was used. In the thalamic region the data of this atlas were elaborated, however. A part of the midline nuclei desribed by Sawyer at al. briefly as massa intermedia (M) was subdivided according to d'Hollander (1913) into nucleus reuniens and nucleus rhomboidalis rostrally and nucleus centralis medialis caudally. In the medial group of thalamic nuclei, nucleus centralis lateralis (nucleus magnocellularis of d'Hollander) and nucleus paracentralis (nucleus lamellaris of d'Hollander) were further distinguished. The nucleus parafascicularis, which accompanies the tractus habenulointercrulis also belongs to this group and is the most occipital of the medial group of thalamic nuclei (Gurdjian 1927).

In the rat the skull was fixed in a similar way. The bregma, however, was only 1 mm higher than the lambda (Krieg 1946b). The plane perpendicular to the sagittal plane and passing through the bregma and a point 1 mm above the lambda is the basal horizontal reference plane and at the same time the horizontal zero plane. 3 mm laterally from the sagittal zero plane needles were inserted into the brain perpendicularly to the basal plane at the level of bregma and 2·5 and 5·5 mm occipitally. The frontal zero plane passes through the bregma perpendicularly to the basal plane. The marking of the stereotaxic coordinates with respect to the reference planes is the same as in the cat and rabbit.

The subcortical structures were described according to the stereotaxic atlas of Krieg (1946b), and according to the paper of Gurdjian (1927). Instead of the nucleus lateralis (Gurdjian 1927), the group of cells lying rostrally in the dorsalateral region of the thalamus is termed nucleus lateralis anterior (Kappers 1947). In describing basal ganglia and nucleus amygdalae the nomenclature of Gurdjian (1928) is used.

Abbreviations

A	= aqueductus mesencephali
AAA	= area amygdalaris anterior
AB	= nucleus basalis amygdalae
AC	= nucleus centralis amygdalae
AD	= nucleus antero-dorsalis
AI	= nucleus intercalatus amygdalae
AL	= nucleus lateralis amygdalae
AM	= nucleus anteromedialis thalami
AV	= nucleus antero-ventralis
ACB	= nucleus accumbens septi
ACO	= nucleus corticalis amygdalae
AHA	= area hypothalamica anterior
AHL	= area hypothalamica lateralis
AME	= nucleus amygdalae medialis
APL	= area praeoptica lateralis
APM	= area praeoptica medialis
ARC	= nucleus arcuatus
BP	= brachium pontis
BCI	= brachium colliculi inferioris
BCS	= brachium colliculi superioris
CA	= commissura anterior
CD	= nucleus caudatus
CF	= commissura fornicis
CH	= commissura habenularum
CI	= capsula interna
CL	= claustrum
CM	= centrum medianum
CP	= commissura posterior
CS	= colliculus superior
CCS	= commissura colliculi superioris
CDP	= caudate-putamen complex
CPYR	= cortex pyriformis
CEXT	= capsula externa
CHO	= chiasma opticum
DA	= nucleus Darkschewitsch
DB	= nucleus fasciculi diagonalis (Broca)
DS	= decussatio supramammillaris
DBC	= decussatio brachium conjunctivum
DTD	= decussatio tegmenti dorsalis
DTV	= decussatio tegmenti ventralis
E	= epiphysis
EN	= nucleus entopeduncularis
EW	= nucleus Edinger-Westphal
FF	= fimbria fornicis
FI	= fossa intercruralis
FO	= fasciculus opticus
FLM	= fasciculus longitudinalis medialis
GL	= corpus geniculatum laterale
GM	= corpus geniculatum mediale
GP	= globus pallidus
GLV	= corpus geniculatum laterale, pars ventralis
H	= hypophysis
HL	= nucleus habenulae lateralis
HM	= nucleus habenulae medialis
HDM	= nucleus dorsomedialis hypothalami
HIP	= hippocampus
HVM	= nucleus ventromedialis hypothalami
IP	= nucleus interpeduncularis
IS	= nucleus interstitialis
IV	= nucleus interventralis
IAM	= nucleus inter-antero-medialis
LA	= nucleus lateralis anterior
LL	= lemniscus lateralis
LM	= lemniscus medialis
LP	= nucleus lateralis posterior

LIM = nucleus limitans
LME = lamina medullaris externa

MD = nucleus medio-dorsalis
ML = nucleus mammillaris lateralis
MM = nucleus mammillaris medialis

NIII = nucleus originis nervi oculomotorii
NIV = nucleus originis nervi trochlearis
NP = nuclei pontis
NR = nucleus ruber
NAM = nucleus anteromedialis
NCM = nucleus centralis medialis
NCL = nucleus centralis lateralis
NCP = nucleus commissurae posterioris
NFS = nucleus fimbrialis septi
NHD = nucleus hypothalamicus dorsalis
NHP = nucleus hypothalamicus posterior
NLL = nucleus lemnisci lateralis
NPC = nucleus paracentralis
NPL = nucleus paralemniscalis
NRP = nucleus reticularis pontis
NRT = nucleus reticularis tegmenti
NSL = nucleus septalis lateralis
NSM = nucleus septalis medialis
NTS = nucleus triangularis septi
NCAST = nucleus commissurae anterioris et striae terminalis
NTOLFLAT = nucleus tractus olfactorii lateralis

P = putamen
PF = nucleus parafascicularis
PC = pedunculus cerebri
PM = pedunculus mammillaris
PR = praetectum
PU = pulvinar
PV = nucleus paraventricularis
PTH = nucleus posterior thalami
PVA = nucleus paraventricularis anterior
PVP = nucleus paraventricularis posterior

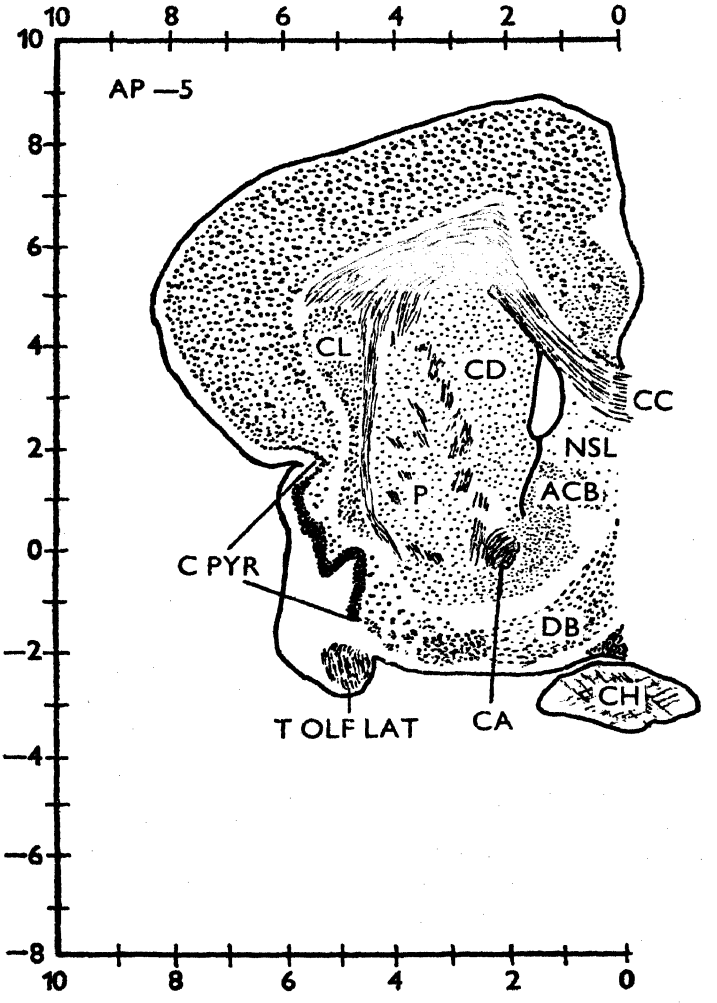
R = nucleus reticularis
RA = raphe
RE = nucleus reuniens
RH = nucleus rhomboidalis

S = stria medullaris
SG = nucleus supragenicularis
SM = nucleus submedius
SN = substantia nigra
SO = nucleus supraopticus
ST = stria terminalis
SCH = nucleus suprachiasmaticus
SGC = stratum griseum centrale
SOD = nucleus supraopticus diffusus
SPF = nucleus subparafascicularis
SPM = nucleus supramammillaris
STH = nucleus subthalamicus

TA = taenia anterior
TCS = tractus cortico-spinalis
THP = tractus habenulo-pedicularis
TMT = tractus mammillo-thalamicus
TMG = tractus mammillo-tegmentalis
TUBOLF = tuberculum olfactorium
TTC = tractus tegmentalis centralis
TOLFLAT = tractus olfactorius lateralis

V. = radix mesencephalica nervi trigemini
V = ventriculus lateralis
VIII = ventriculus tertius
VA = nucleus ventralis anterior
VE = nucleus ventralis thalami
VL = nucleus ventralis lateralis
VM = nucleus ventralis medialis
VDM = nucleus ventralis pars dorsomedialis
VPL = nucleus ventralis posterolateralis
VPM = nucleus ventralis posteromedialis

ZI = zona incerta



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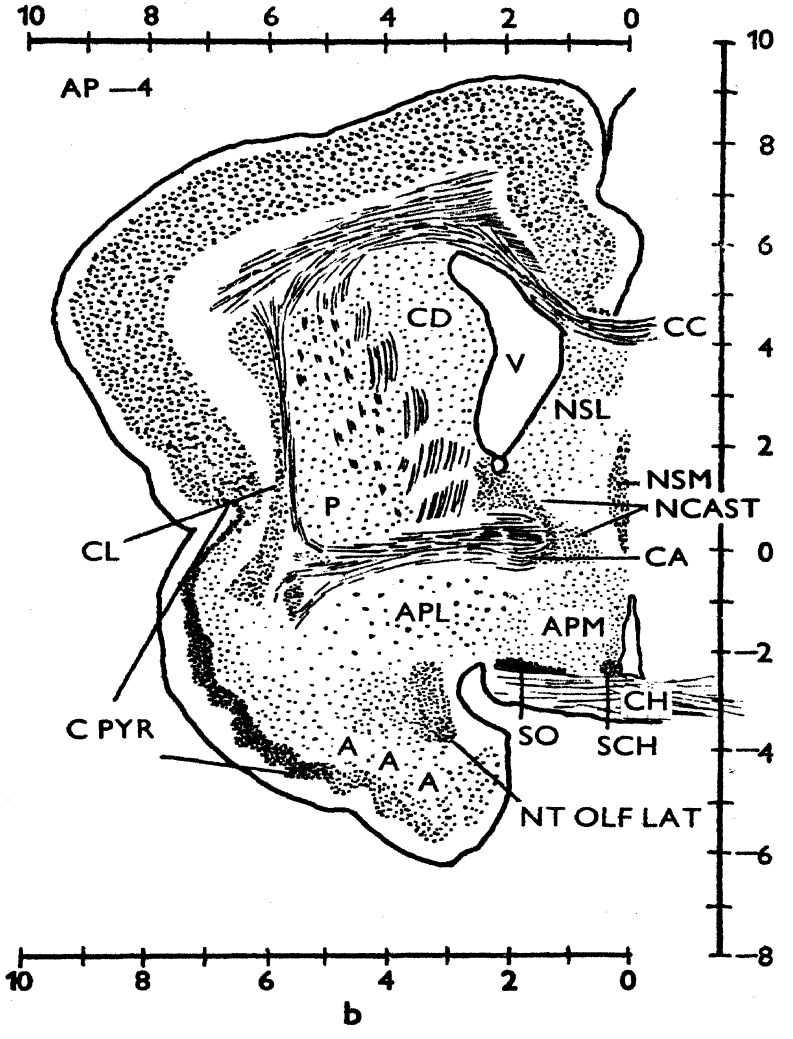
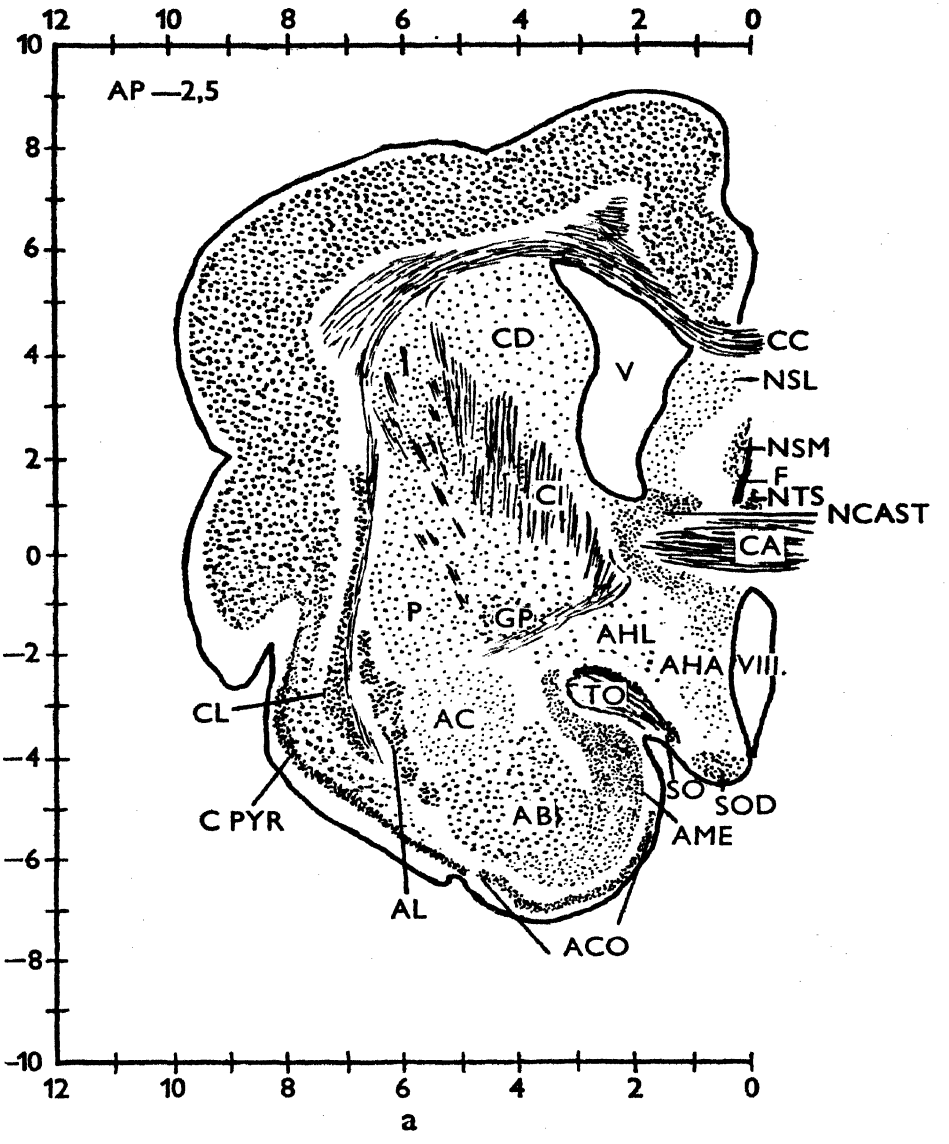


Fig. 209a, b. Coronal sections of brain. Rabbit.


AP —2,5
CD
V
CC
NSL
NSM
F
NTS
NCAST
CAE
CI
P
GP
AHL
AHA
VIII.
TO
CL
AC
SO
SOD
AME
C PYR
ABL
AL
ACO

a

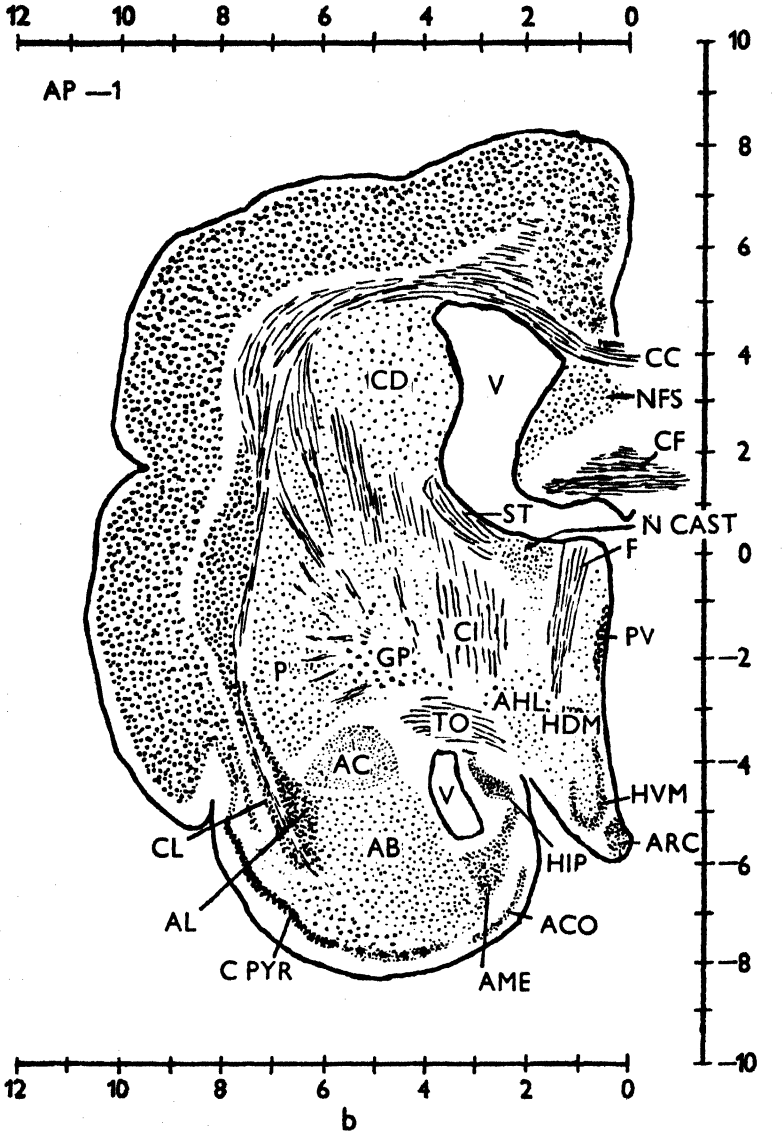
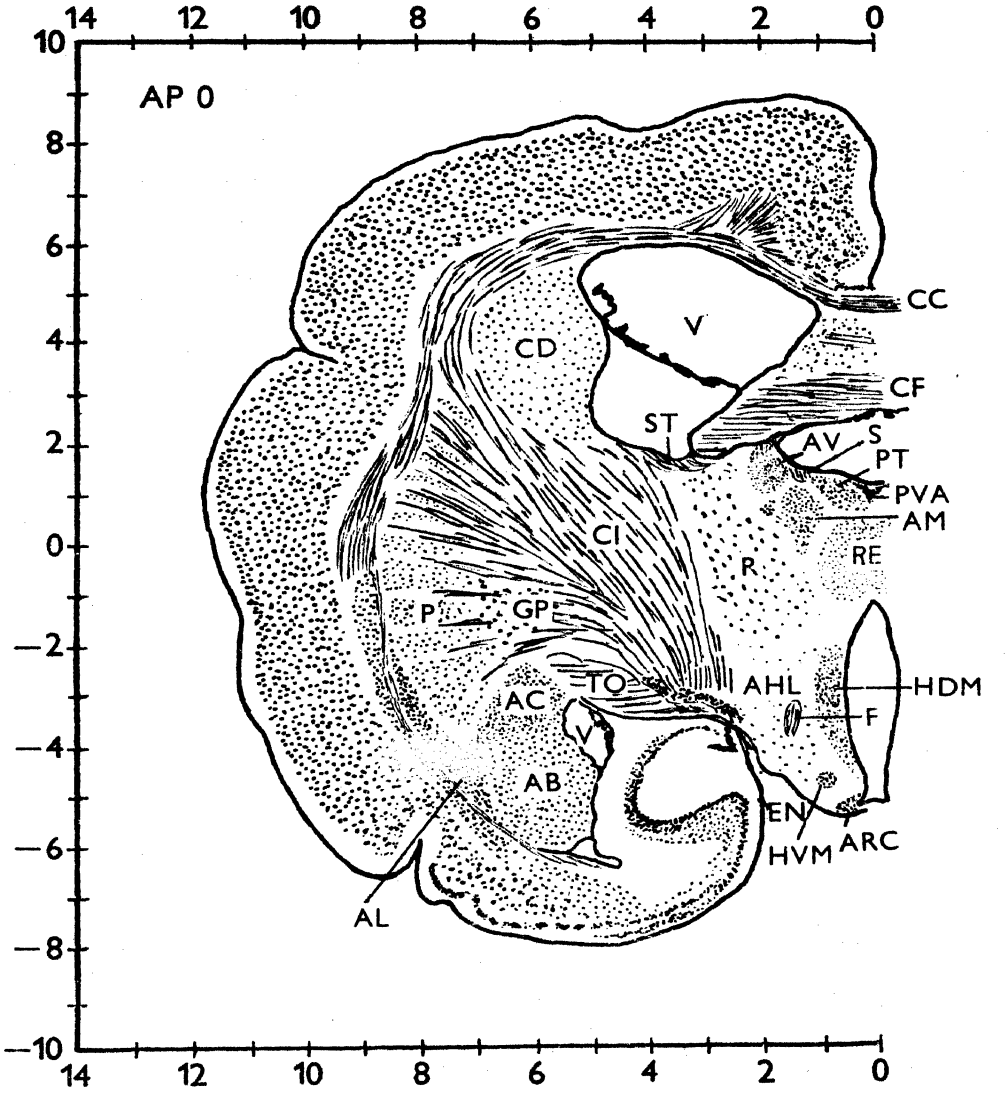


Fig. 210a, b. Coronal sections of brain. **Rabbit.**


AP 0
14
12
10
8
6
4
2
0
10
8
6
4
2
0
−2
−4
−6
−8
−10
CC
V
CD
CF
ST
AV
S
PT
PVA
AM
CI
R
RE
P
GP
TO
AHL
HDM
AC
F
V
AB
EN
HVM
ARC
AL

a

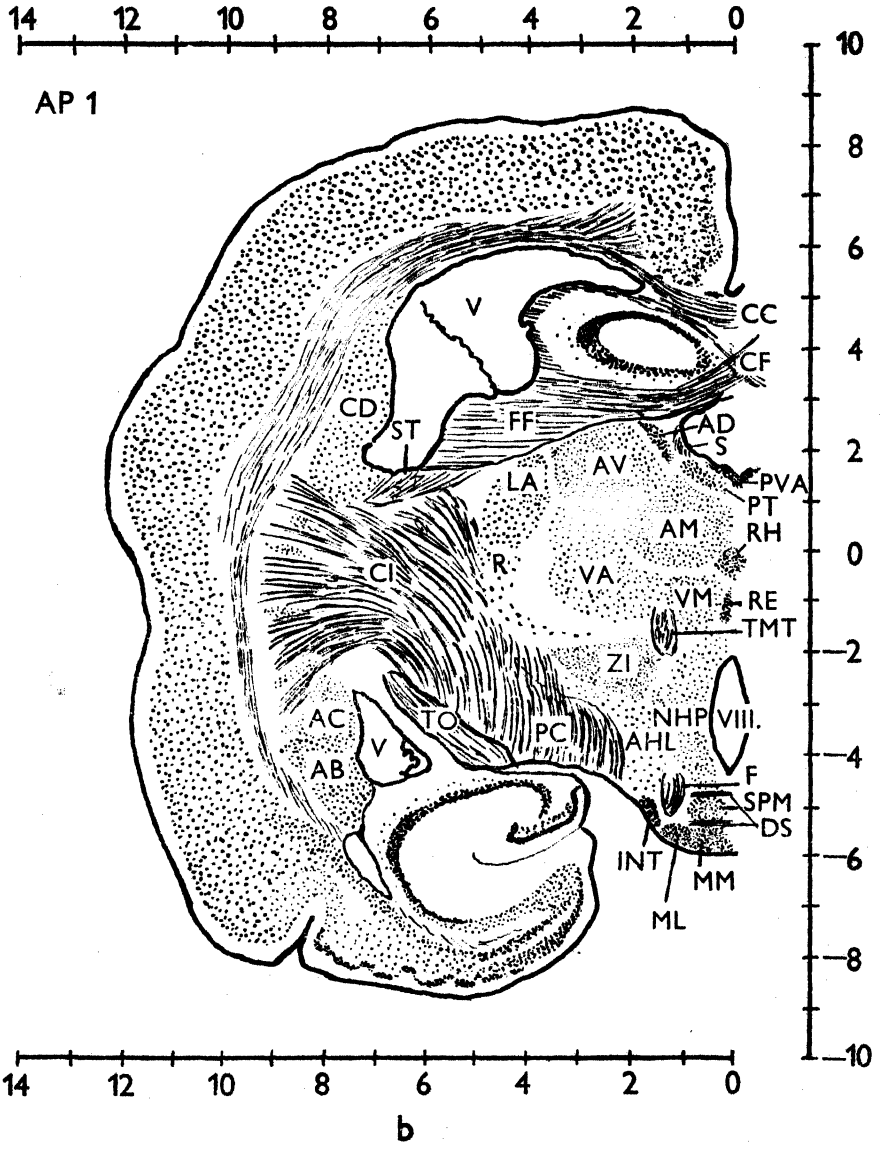
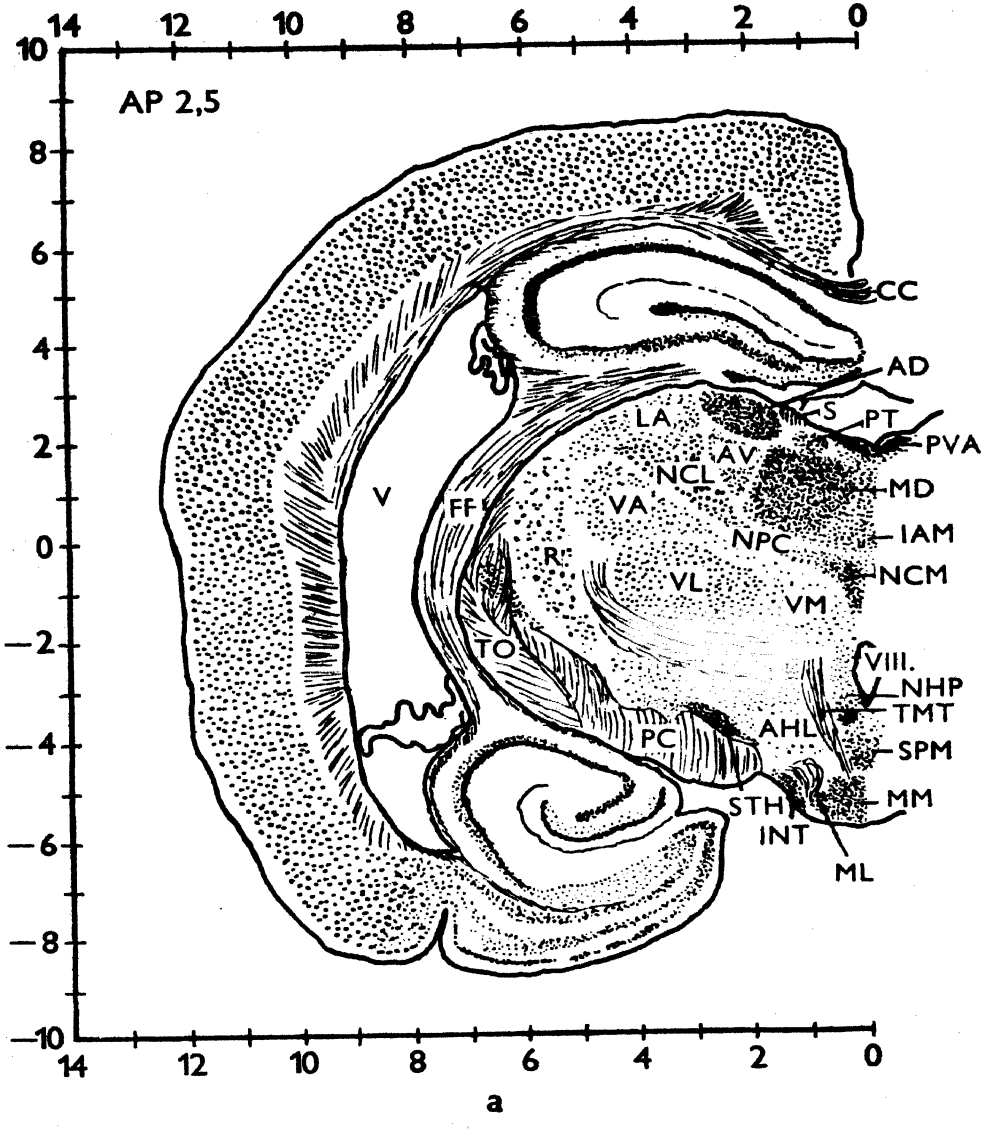


Fig. 211a, b. Coronal sections of brain. Rabbit.


AP 2,5
14
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6
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2
0
10
8
6
4
2
0
−2
−4
−6
−8
−10
CC
AD
S
PT
PVA
LA
AV
NCL
MD
V
FF
VA
IAM
NPC
R
NCM
VL
VM
TO
VIII.
NHP
TMT
AHL
PC
SPM
MM
STH
INT
ML

a

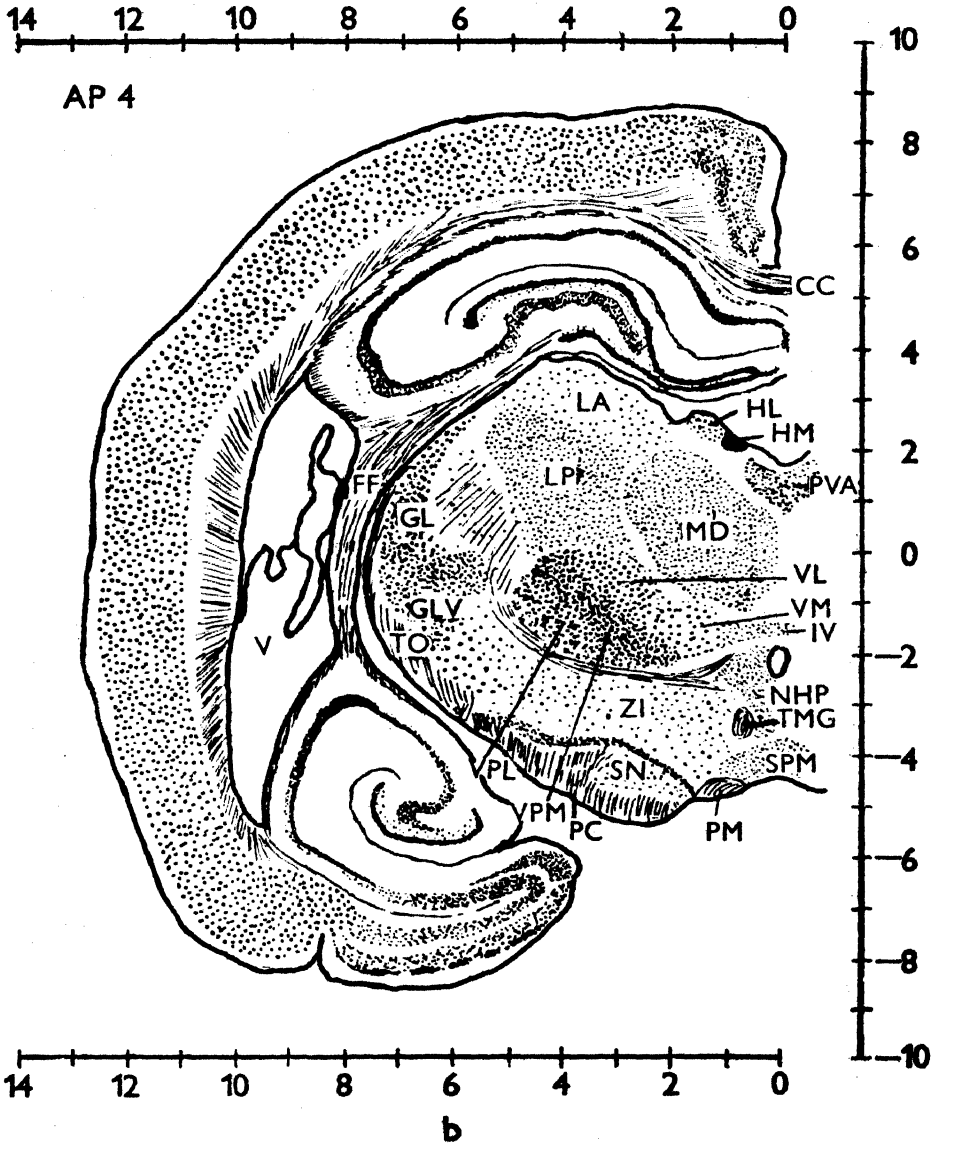
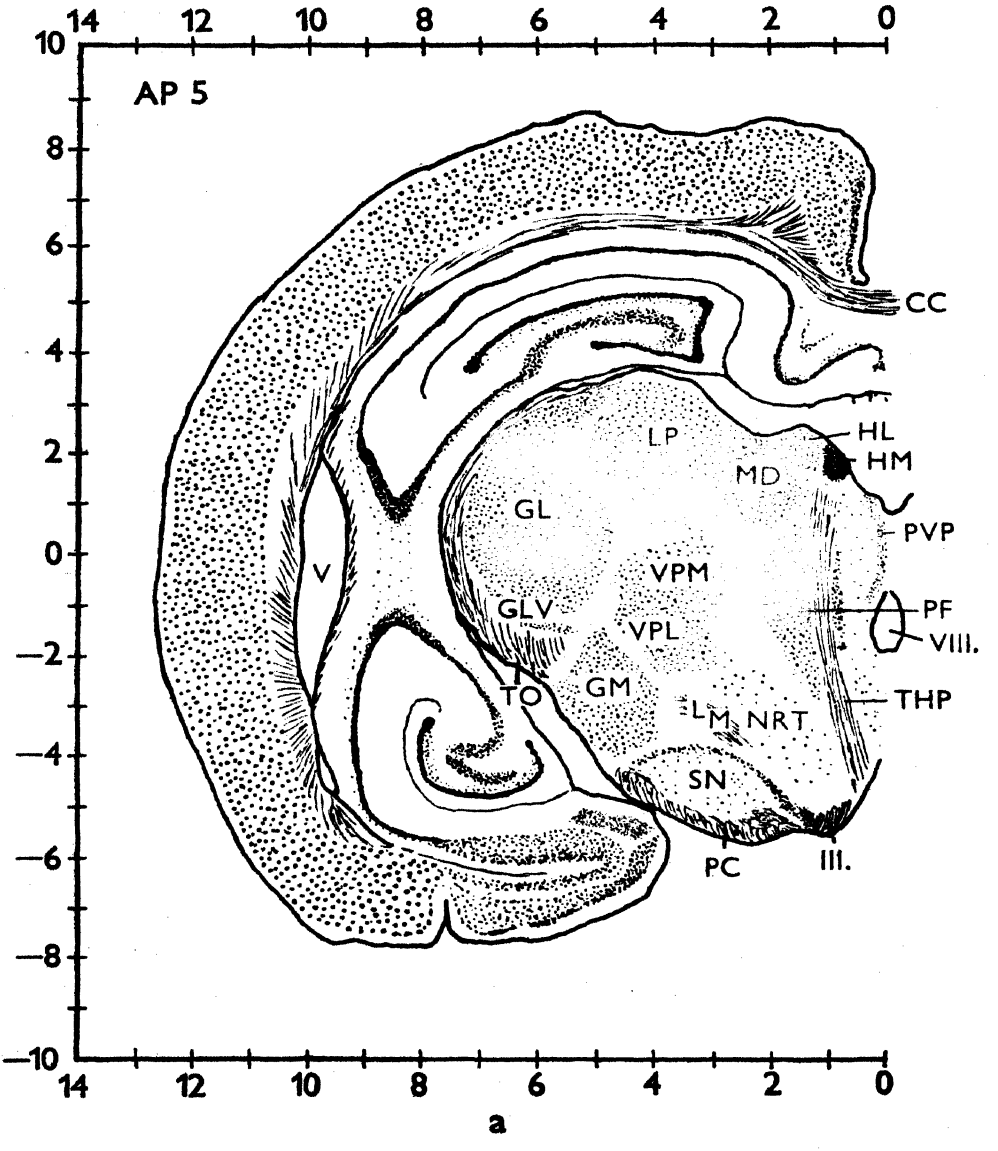


Fig. 212a, b. Coronal sections of brain. Rabbit.


AP 5
14
12
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6
4
2
0
10
8
6
4
2
0
−2
−4
−6
−8
−10
CC
LP
HL
HM
MD
GL
PVP
V
VPM
GLV
VPL
PF
VIII.
TO
GM
THP
LM
NRT
SN
PC
III.

a

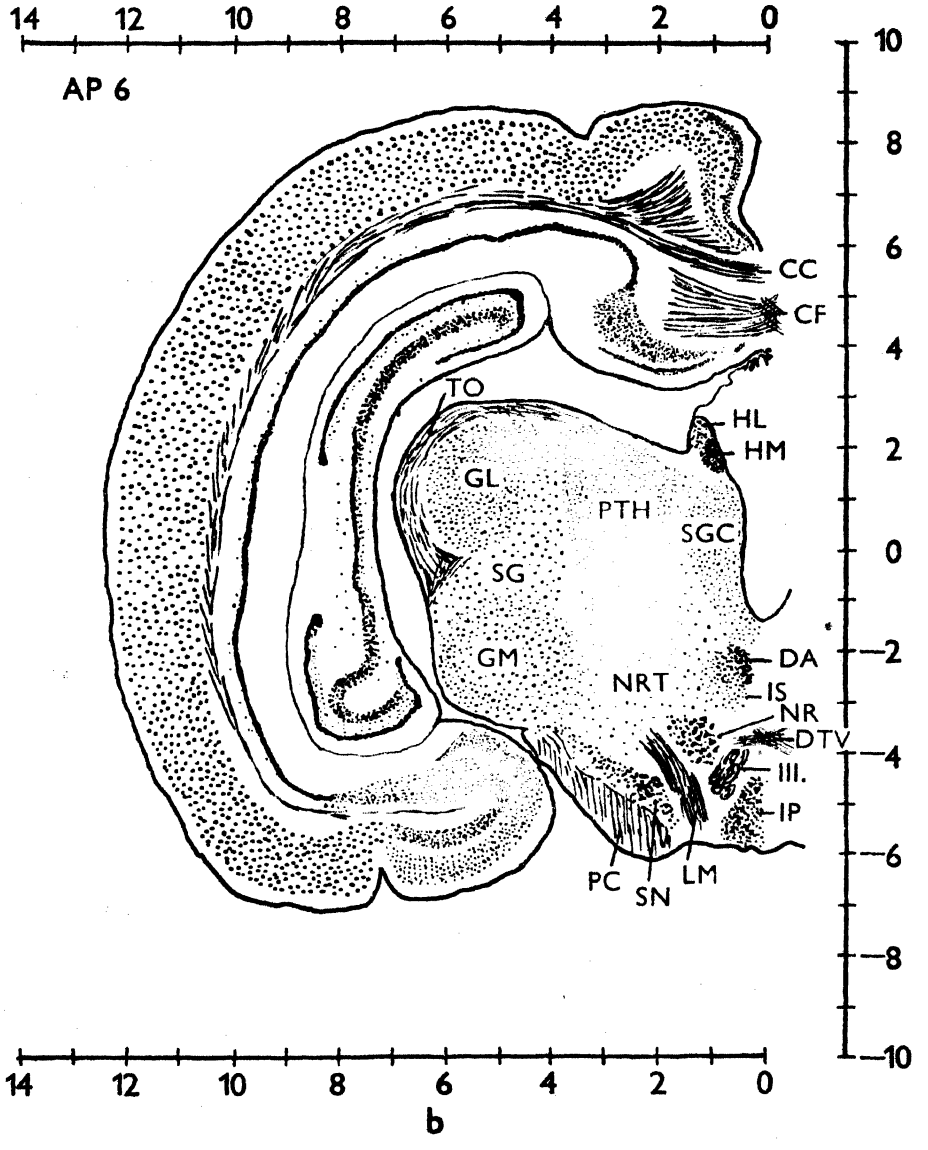
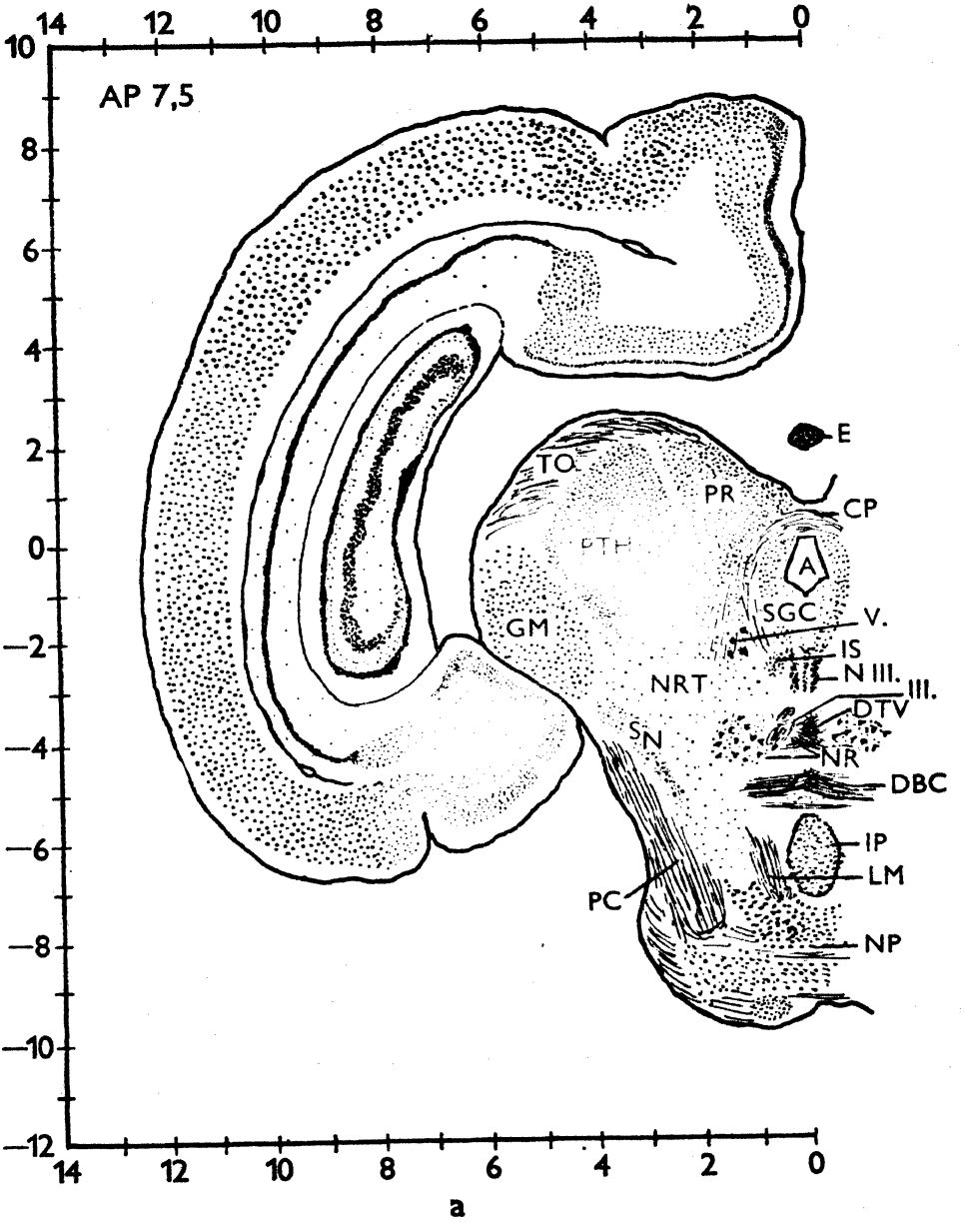


Fig. 213a, b. Coronal sections of brain. Rabbit.


AP 7,5
14 12 10 8 6 4 2 0
10 8 6 4 2 0 −2 −4 −6 −8 −10 −12
E
TO
PR
CP
PTH
A
SGC
V.
GM
IS
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NRT
III.
DTV
SN
NR
DBC
IP
LM
PC
NP

a

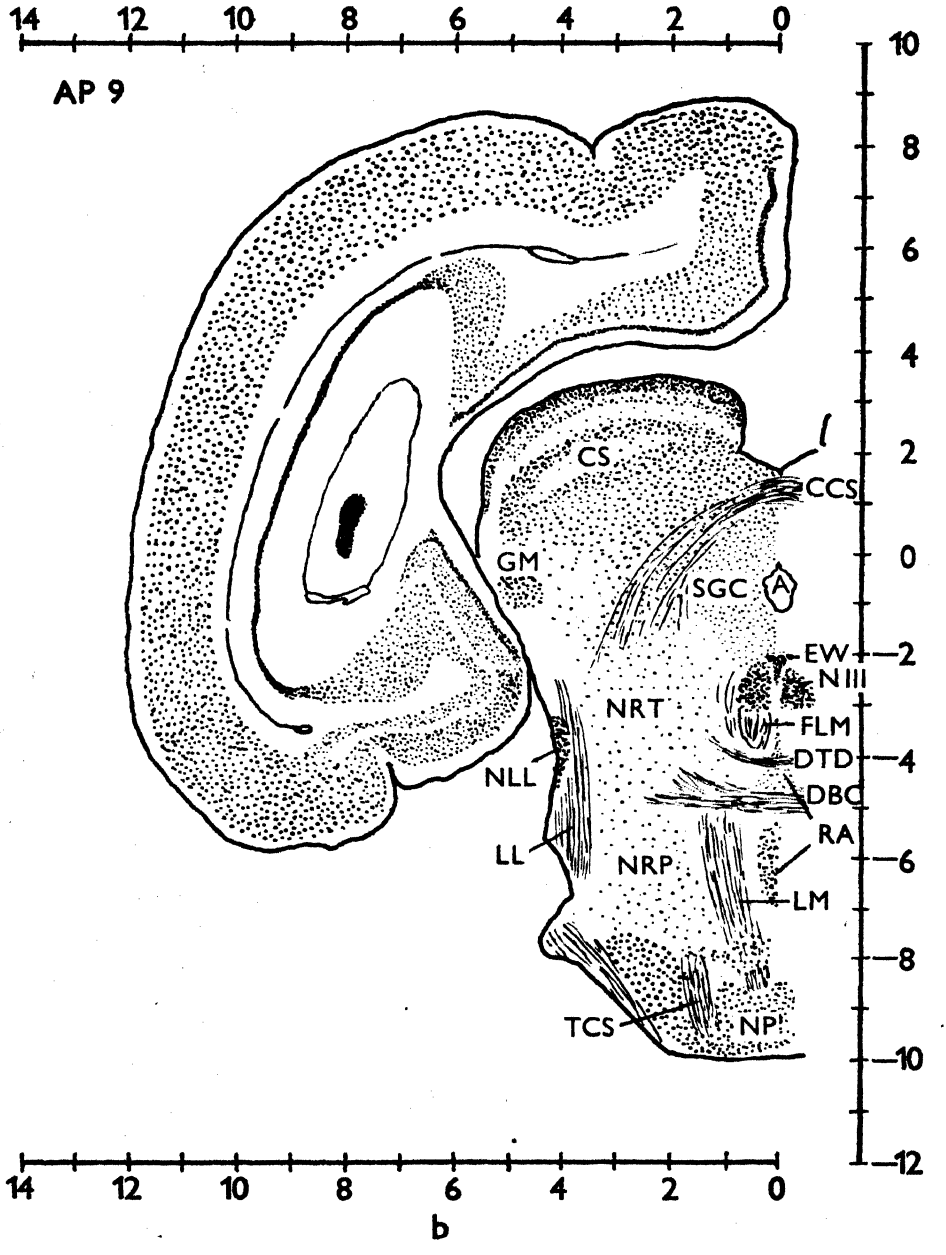
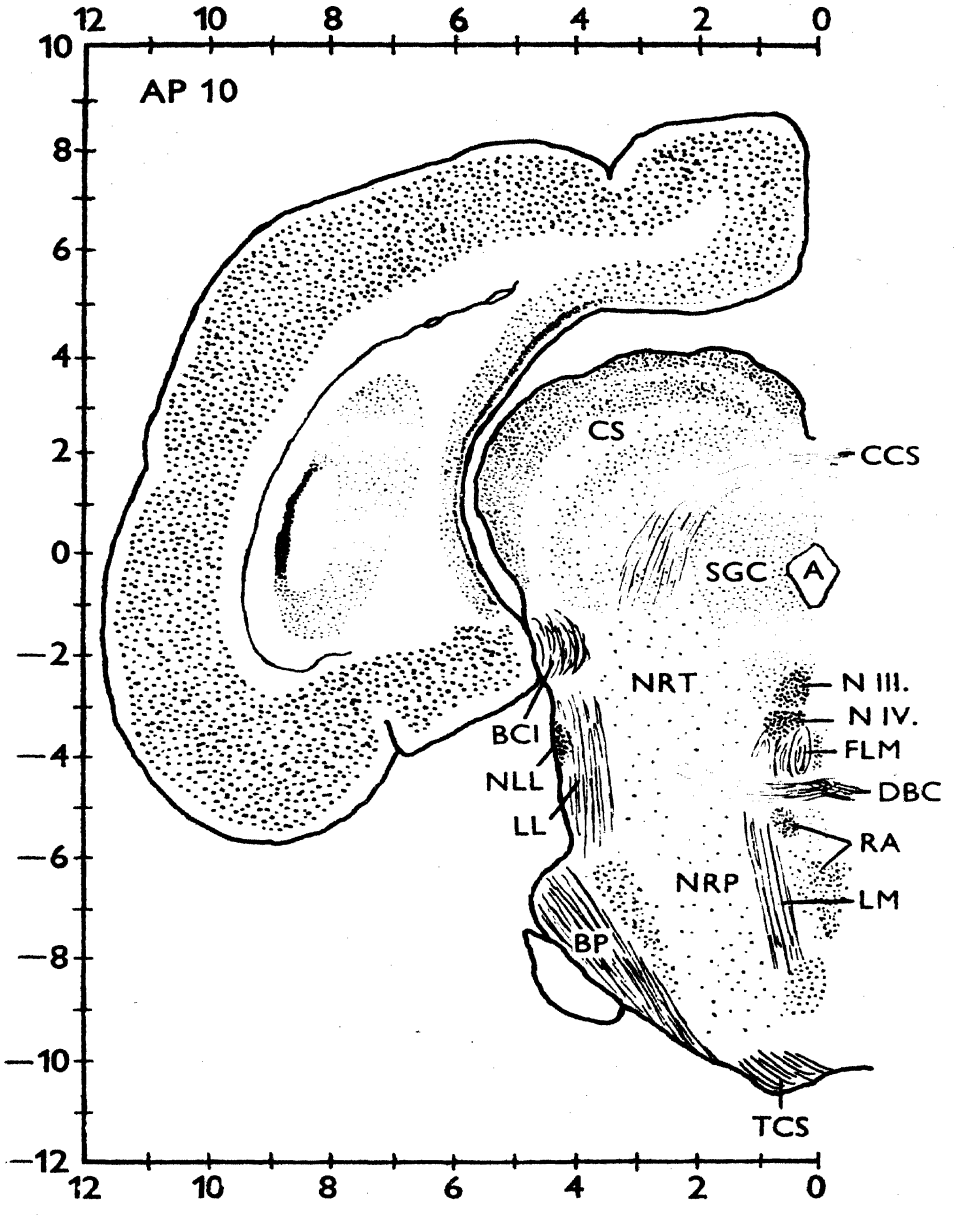


Fig. 214a, b. Coronal sections of brain. Rabbit.



a

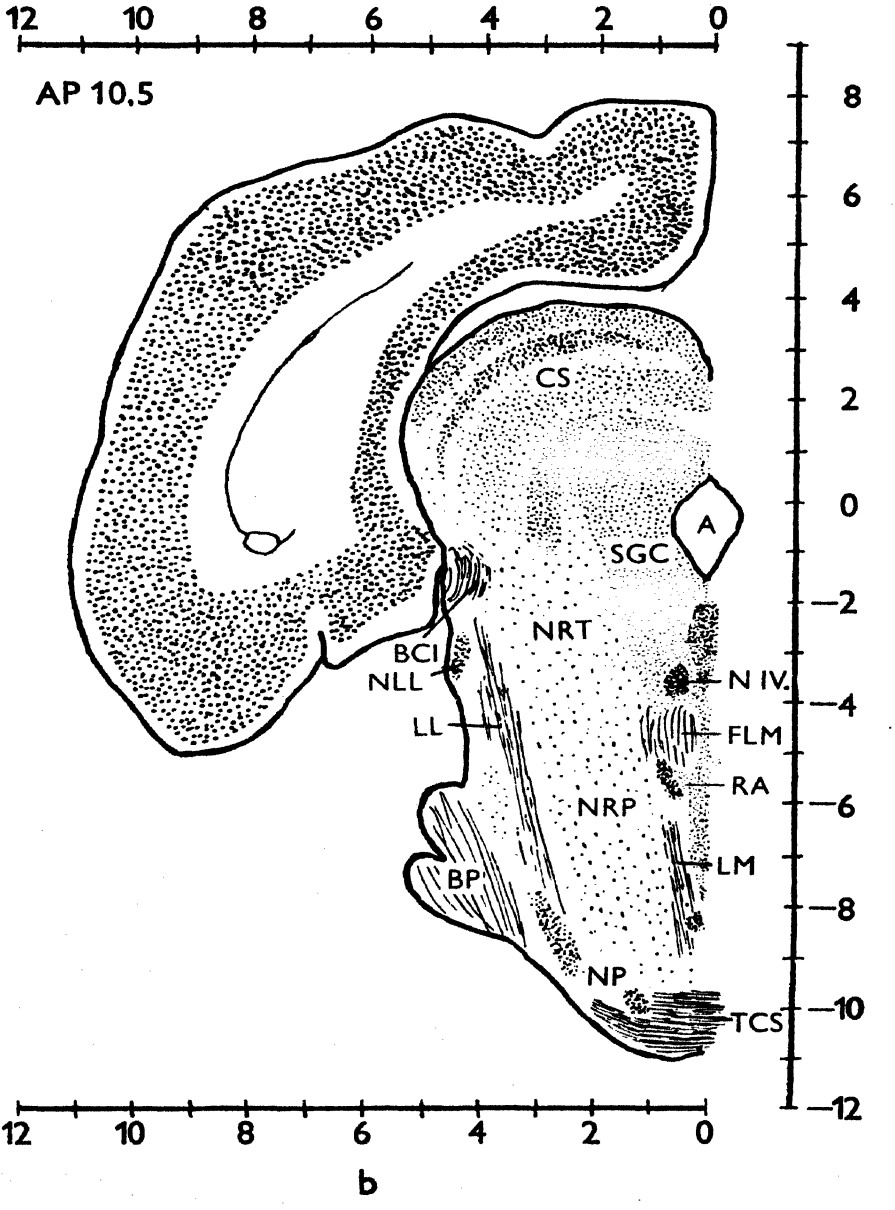
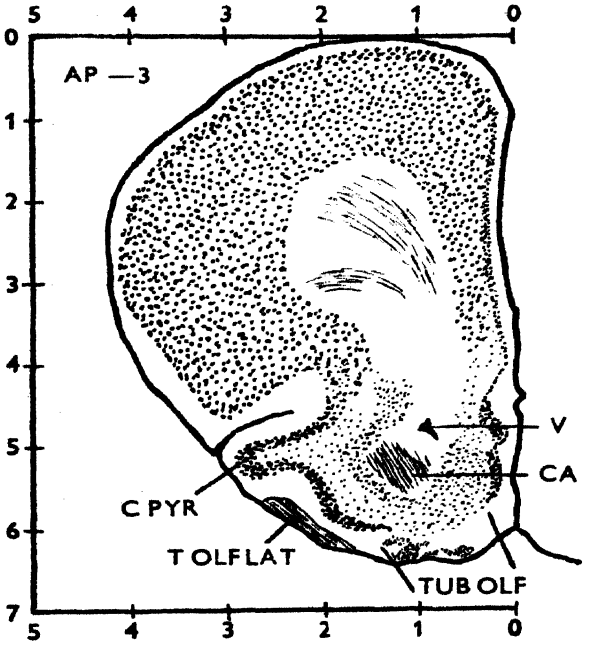


Fig. 215a, b. Coronal sections of brain. Rabbit.



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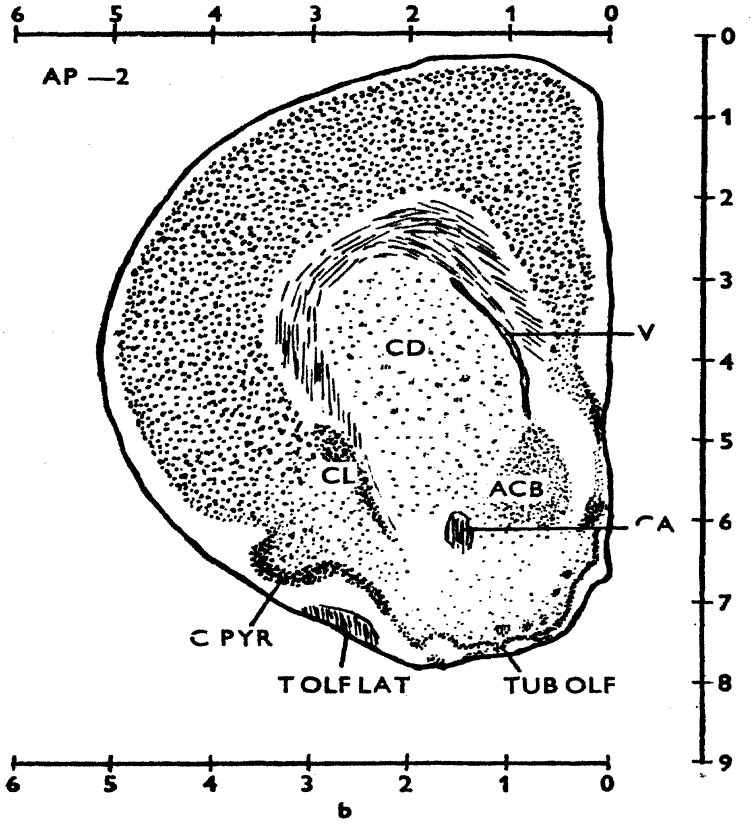
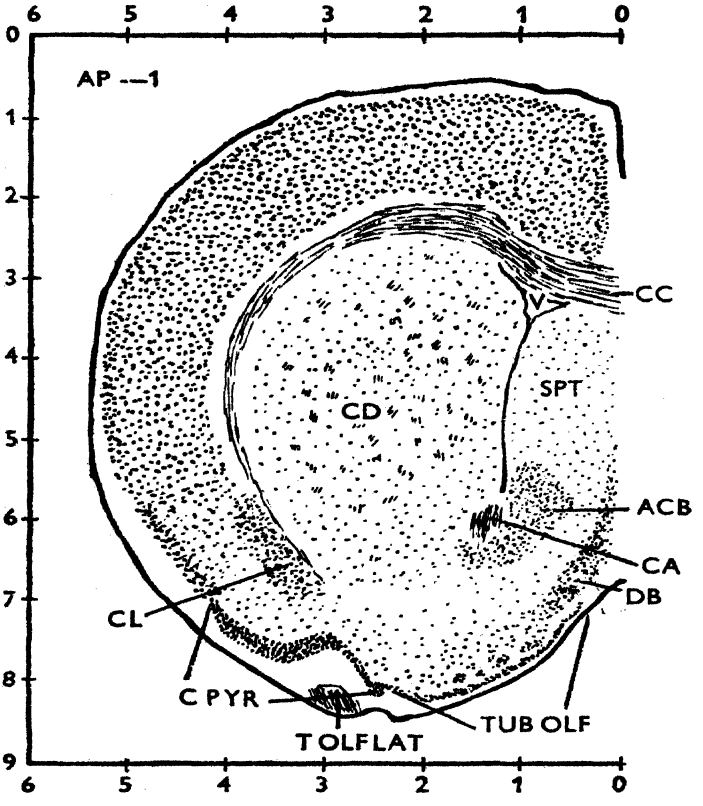


Fig. 216a, b. Coronal sections of brain. Rat.


6 5 4 3 2 1 0
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AP —1
1
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C PYR
TUB OLF
T OLFLAT
6 5 4 3 2 1 0

a

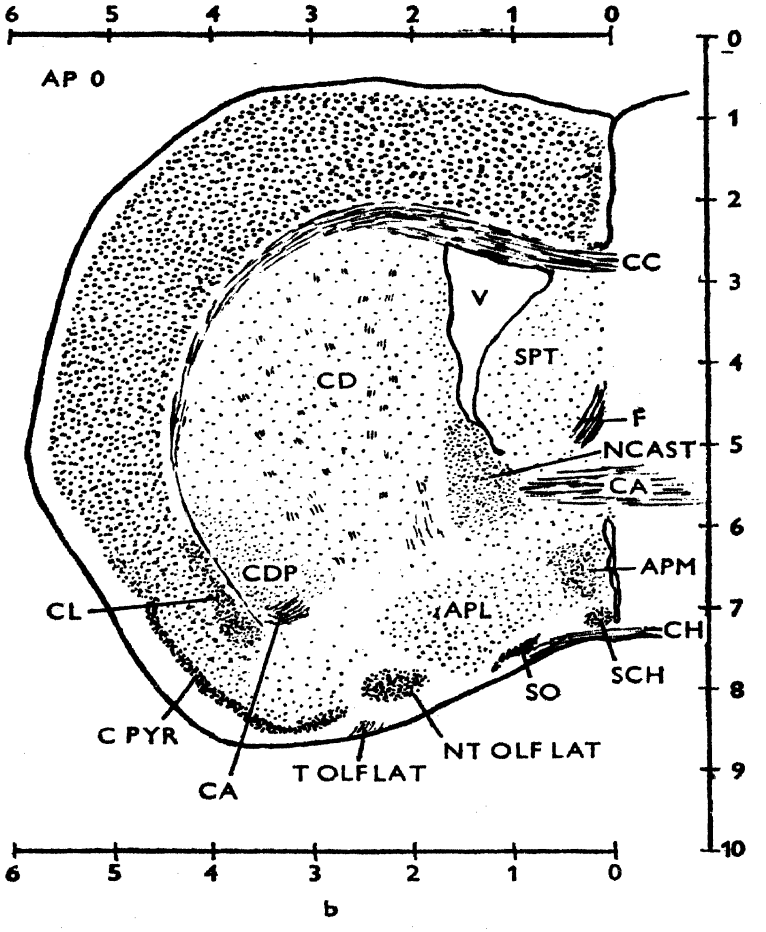
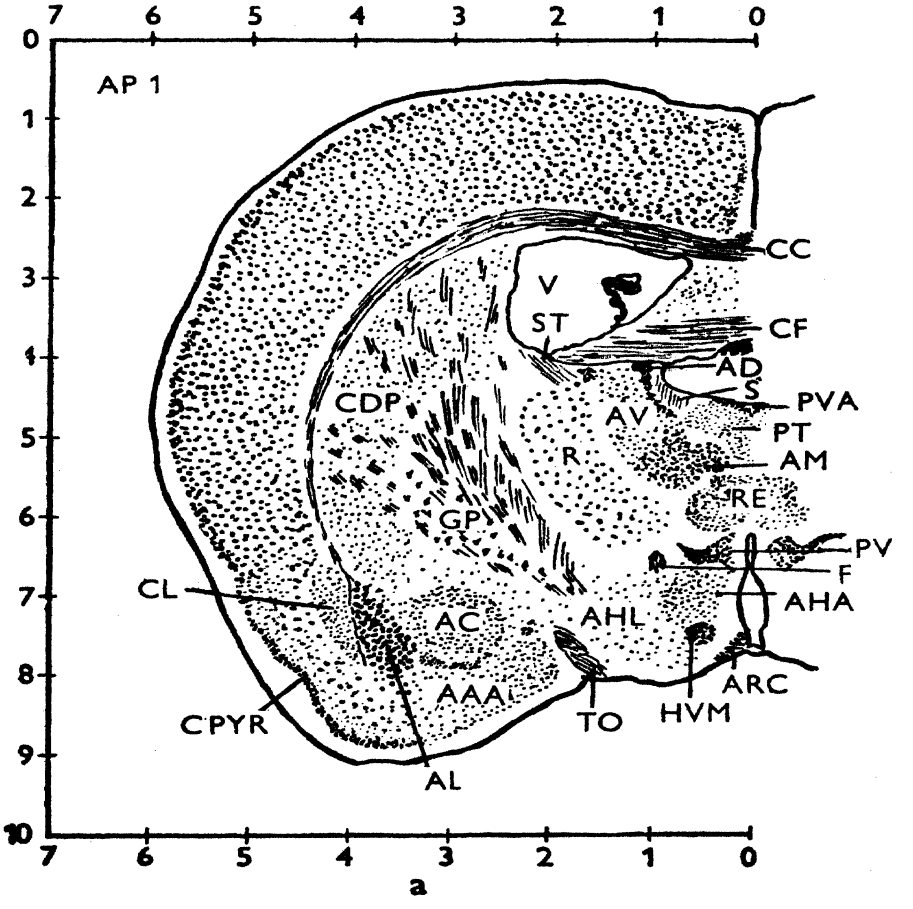


Fig. 217a, b. Coronal sections of brain. Rat.


AP 1
CC
V
ST
CF
AD
S
PVA
CDP
AV
PT
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AM
RE
GP
PV
F
CL
AC
AHL
AHA
ARC
AAA
CPYR
TO
HVM
AL
a

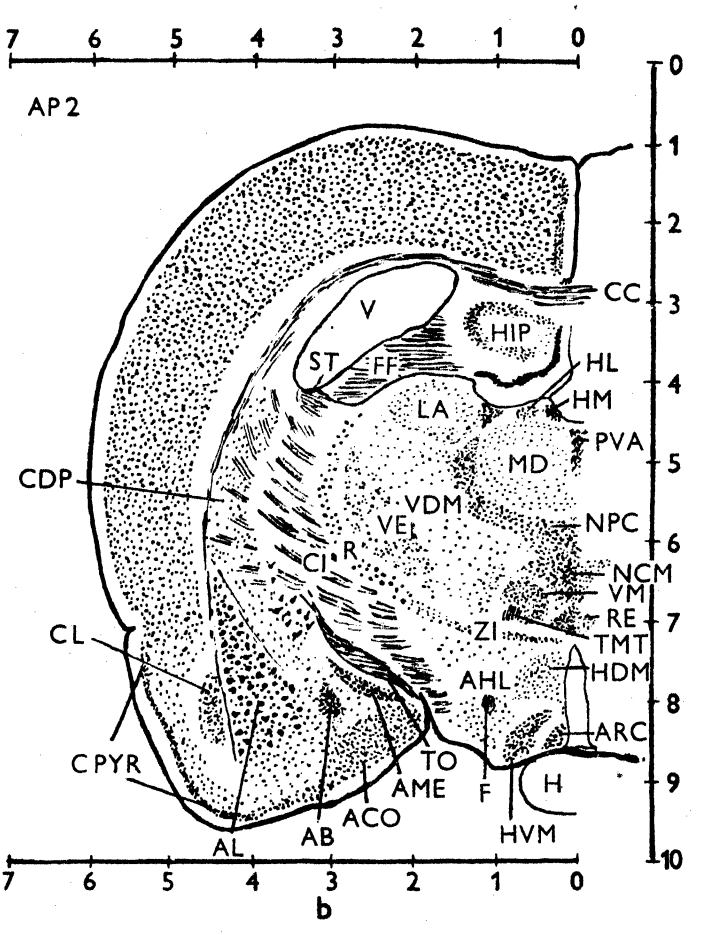
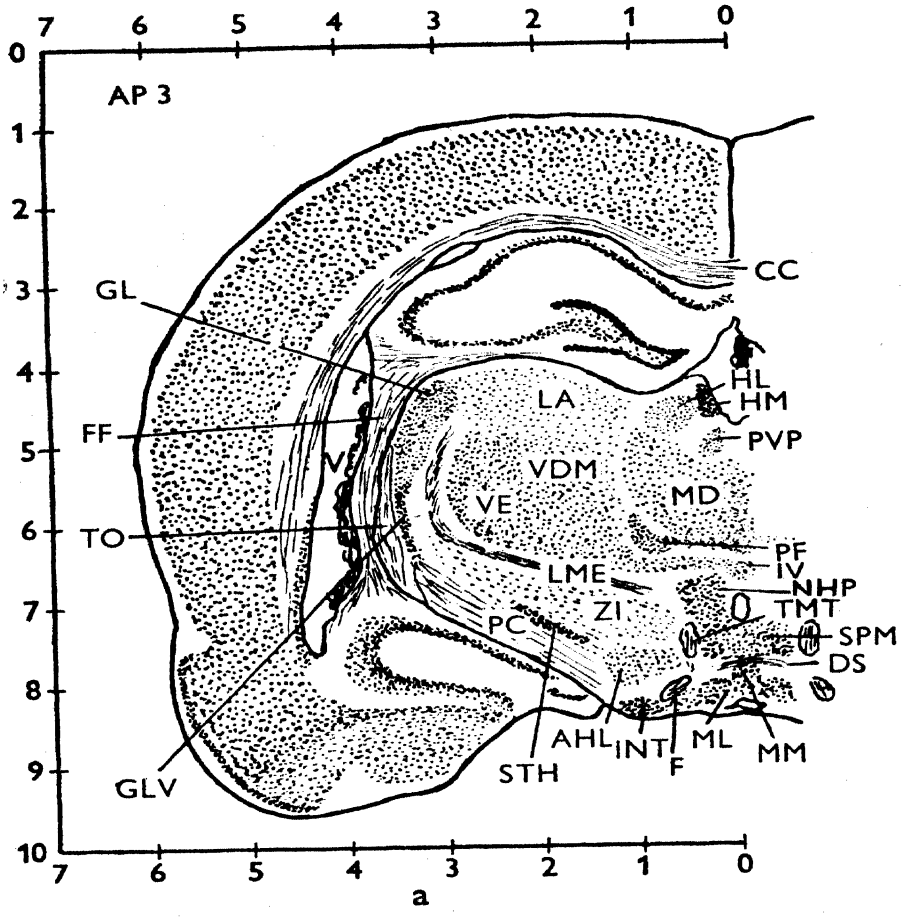


Fig. 218a, b. Coronal sections of brain. **Rat.**


7 6 5 4 3 2 1 0
0
AP 3
1
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3
4
5
6
7
8
9
10
7 6 5 4 3 2 1 0
GL
FF
TO
GLV
V
CC
HL
HM
LA
PVP
VDM
VE
MD
PF
IV
LME
NHP
ZI
TMT
PC
SPM
DS
AHL
INT
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MM
F
STH

a

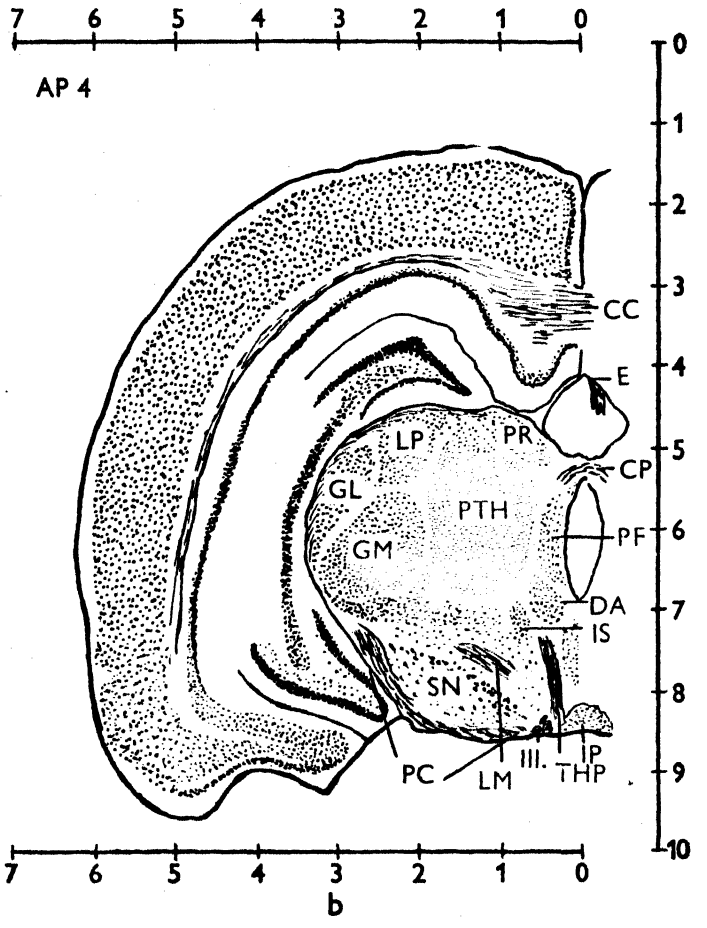
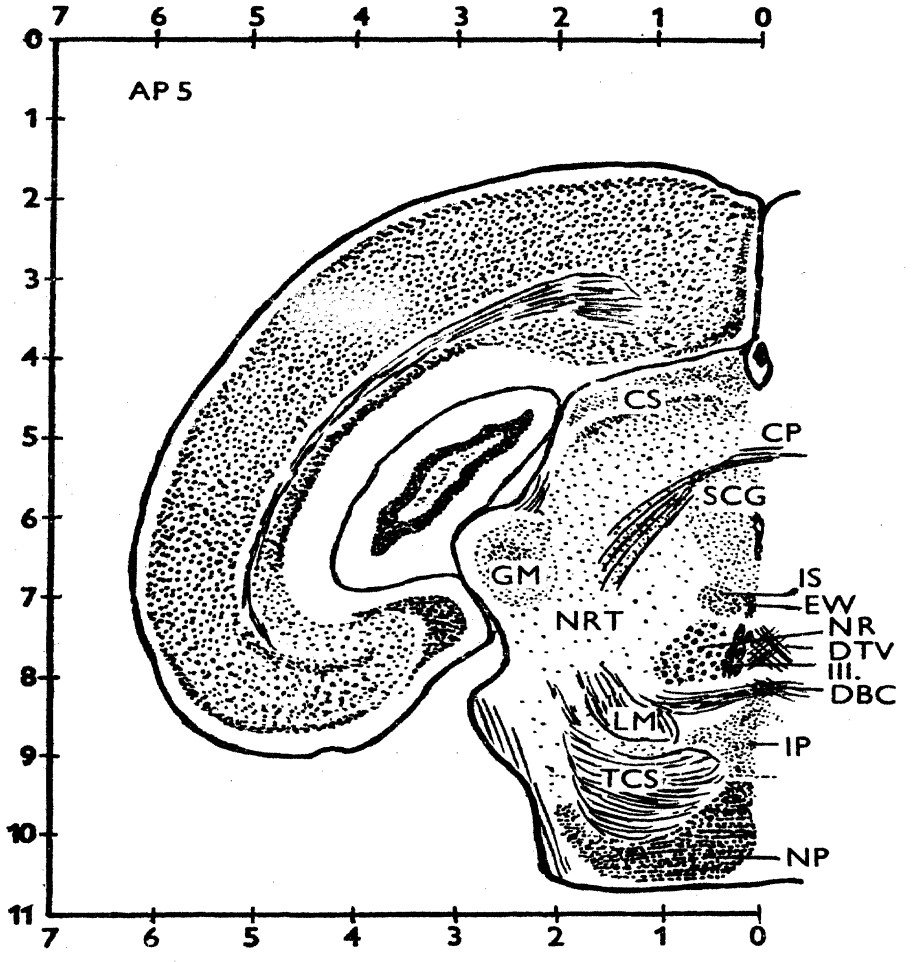


Fig. 219a, b. Coronal sections of brain. Rat.



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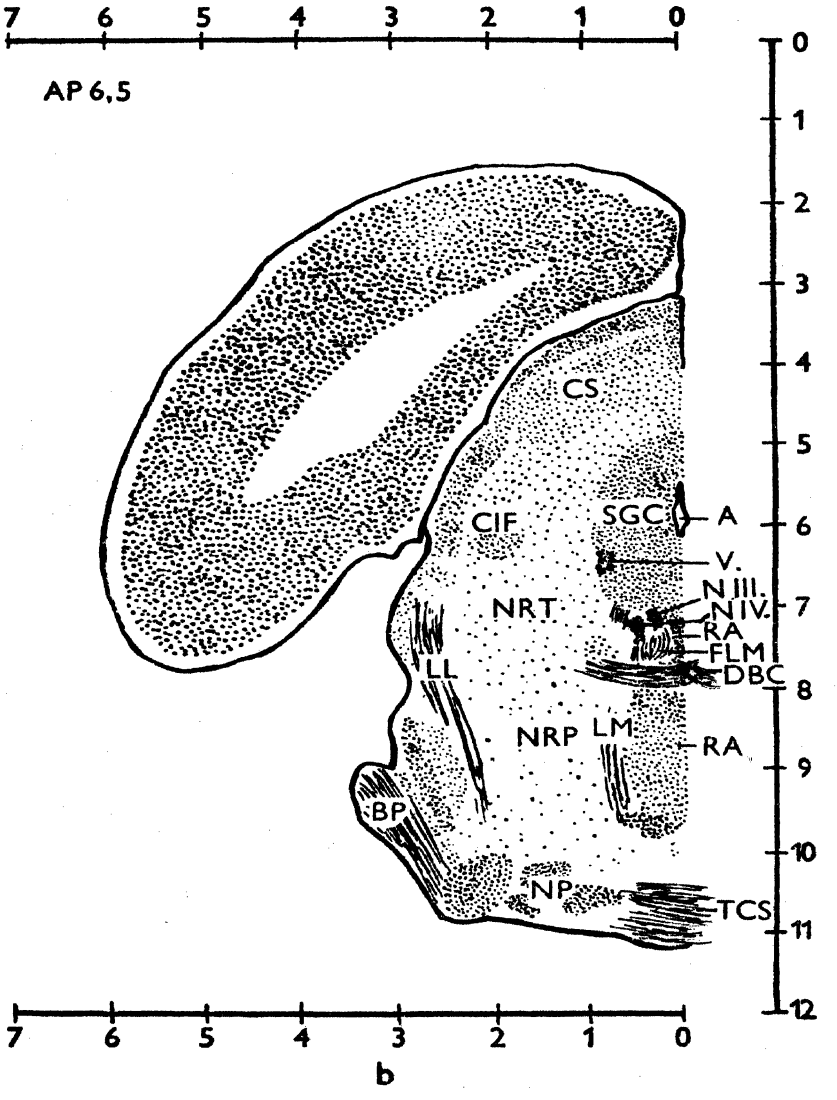
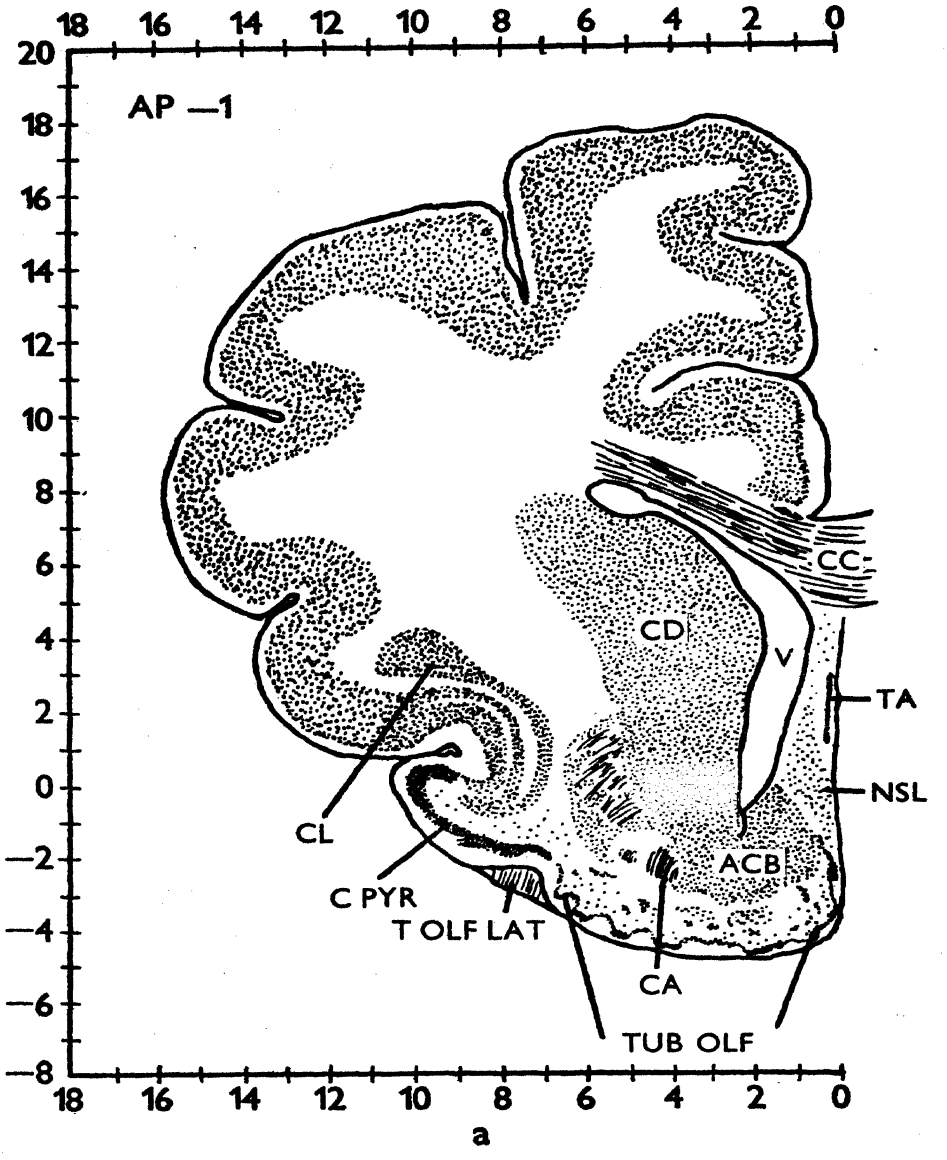


Fig. 220a, b. Coronal sections of brain. Rat.



a

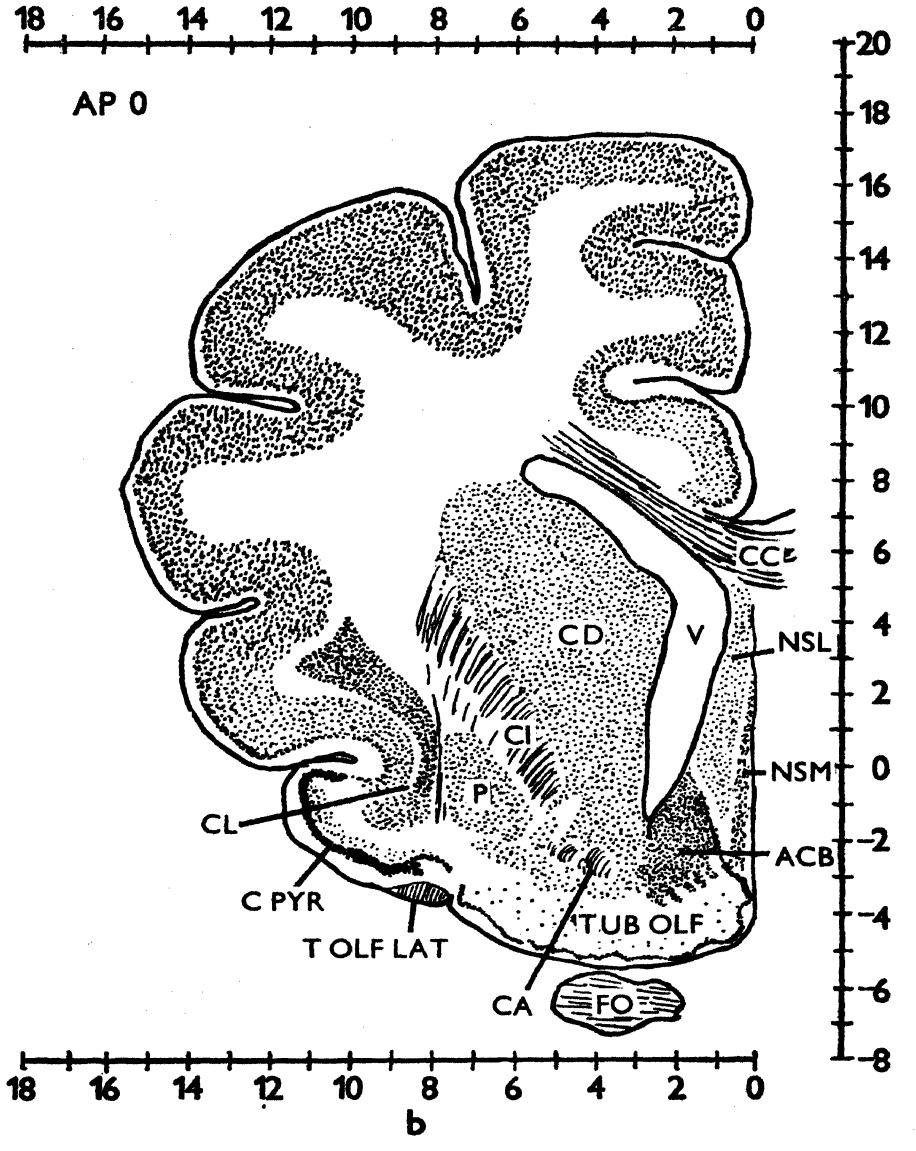
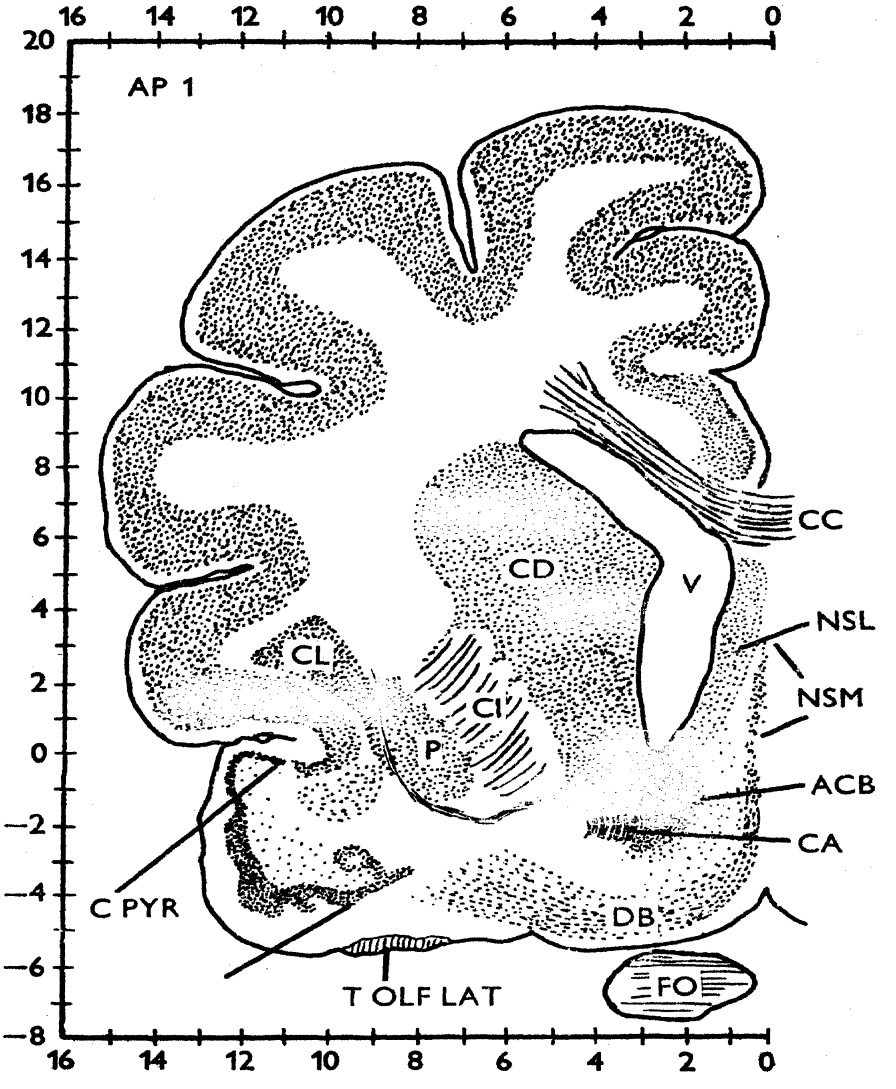


Fig. 221a, b. Coronal sections of brain. Cat.


16 14 12 10 8 6 4 2 0
20 18 16 14 12 10 8 6 4 2 0 −2 −4 −6 −8
AP 1
CC
CD
V
NSL
NSM
CL
CI
P
ACB
CA
C PYR
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T OLF LAT
FO

a

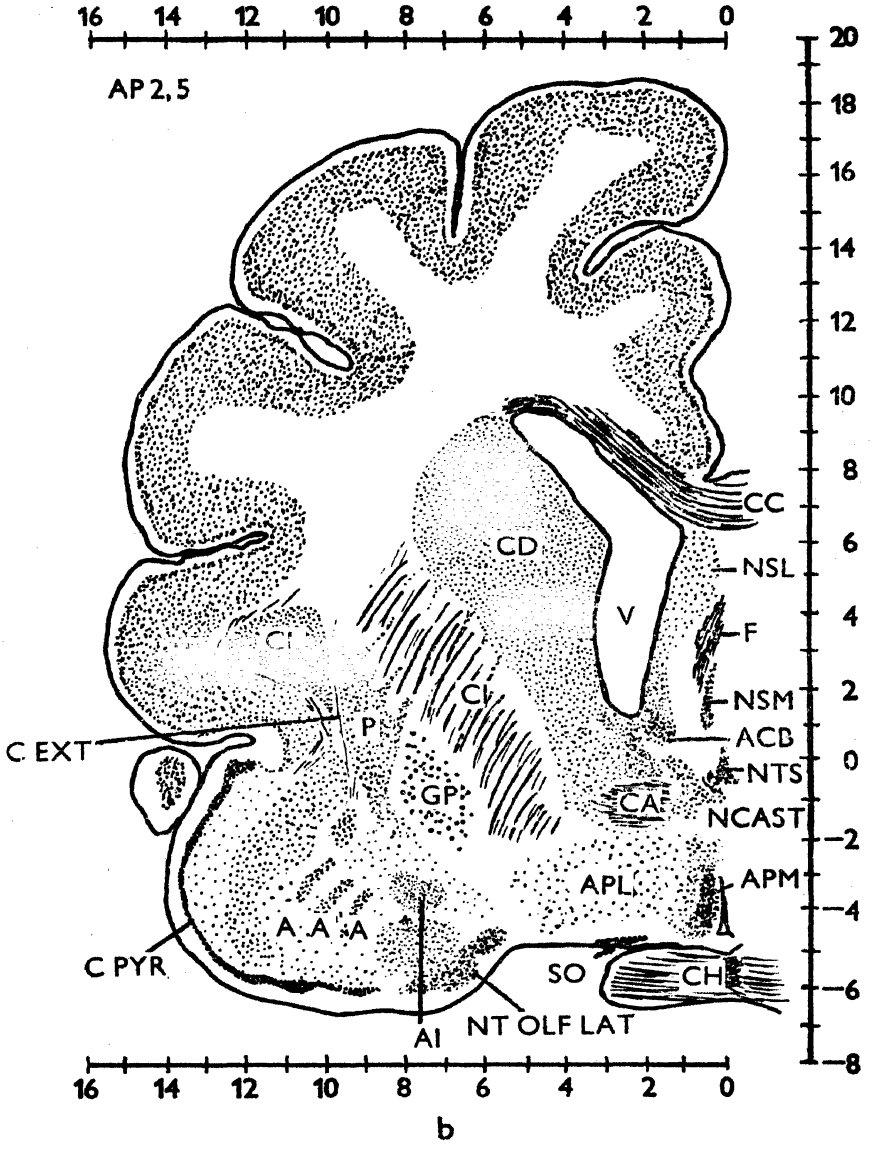
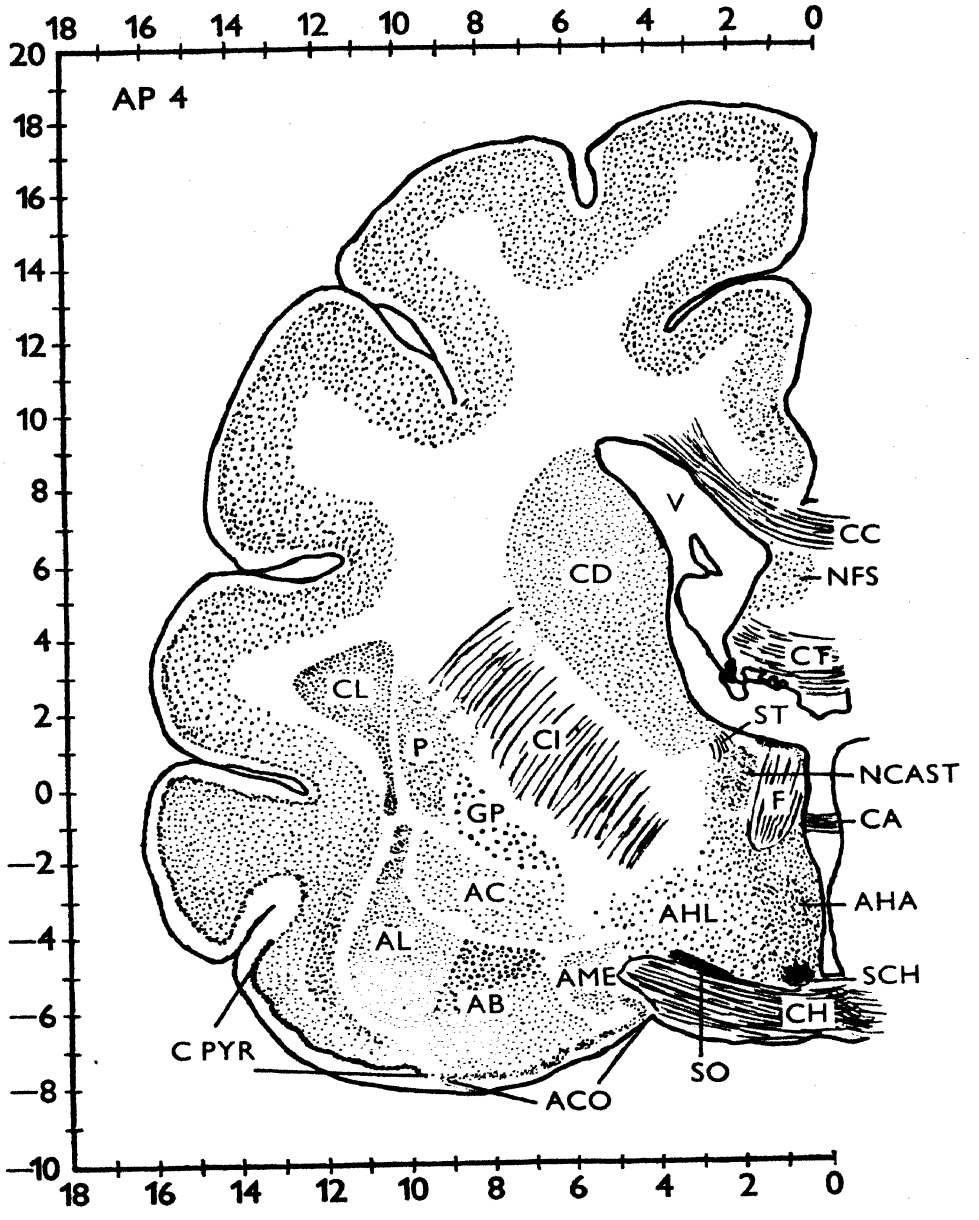


Fig. 222a, b. Coronal sections of brain. Cat.


AP 4
18 16 14 12 10 8 6 4 2 0
20 18 16 14 12 10 8 6 4 2 0 −2 −4 −6 −8 −10
V
CC
NFS
CD
CT
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ST
P
CI
NCAST
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CA
AC
AHA
AHL
AL
AME
SCH
AB
CH
C PYR
SO
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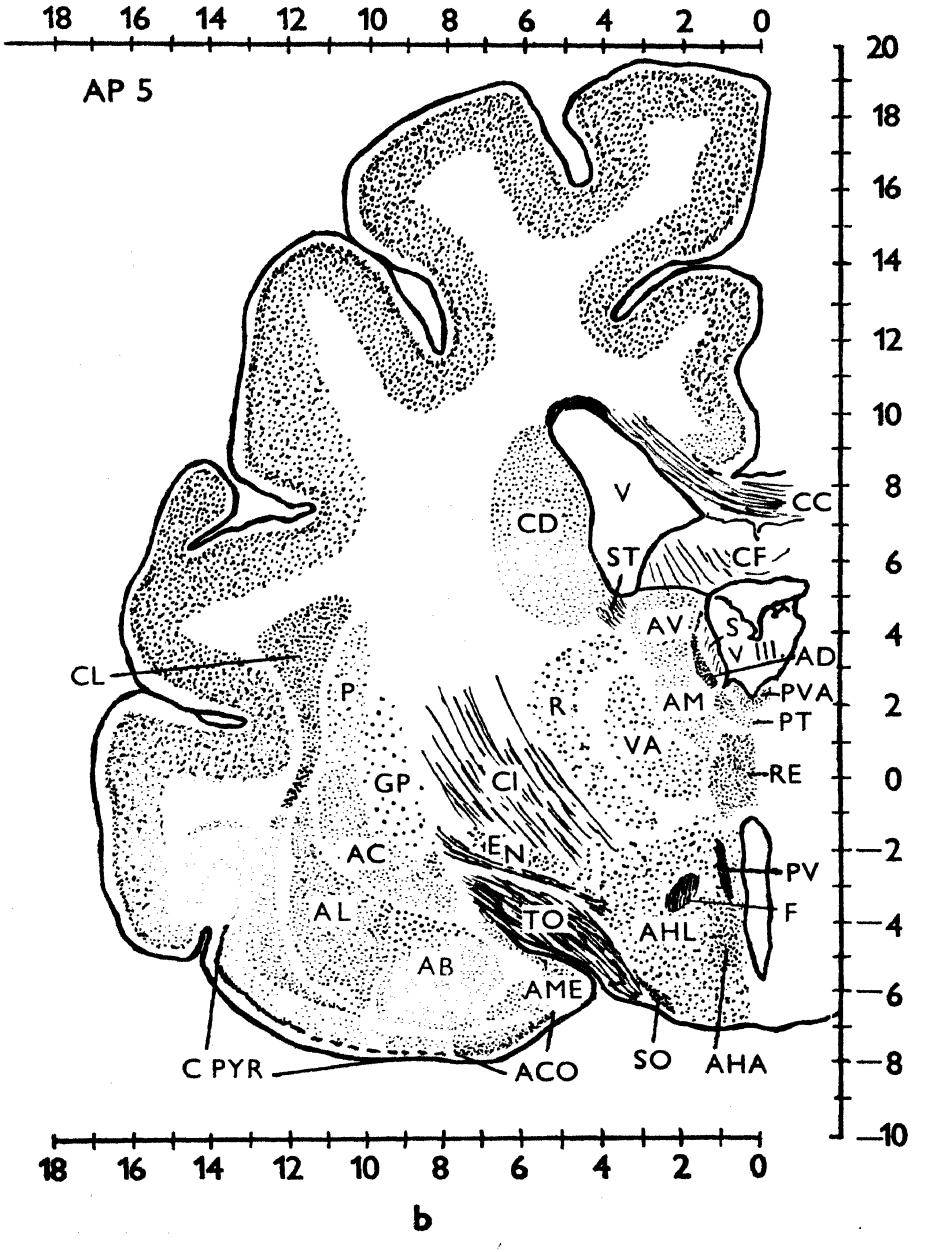
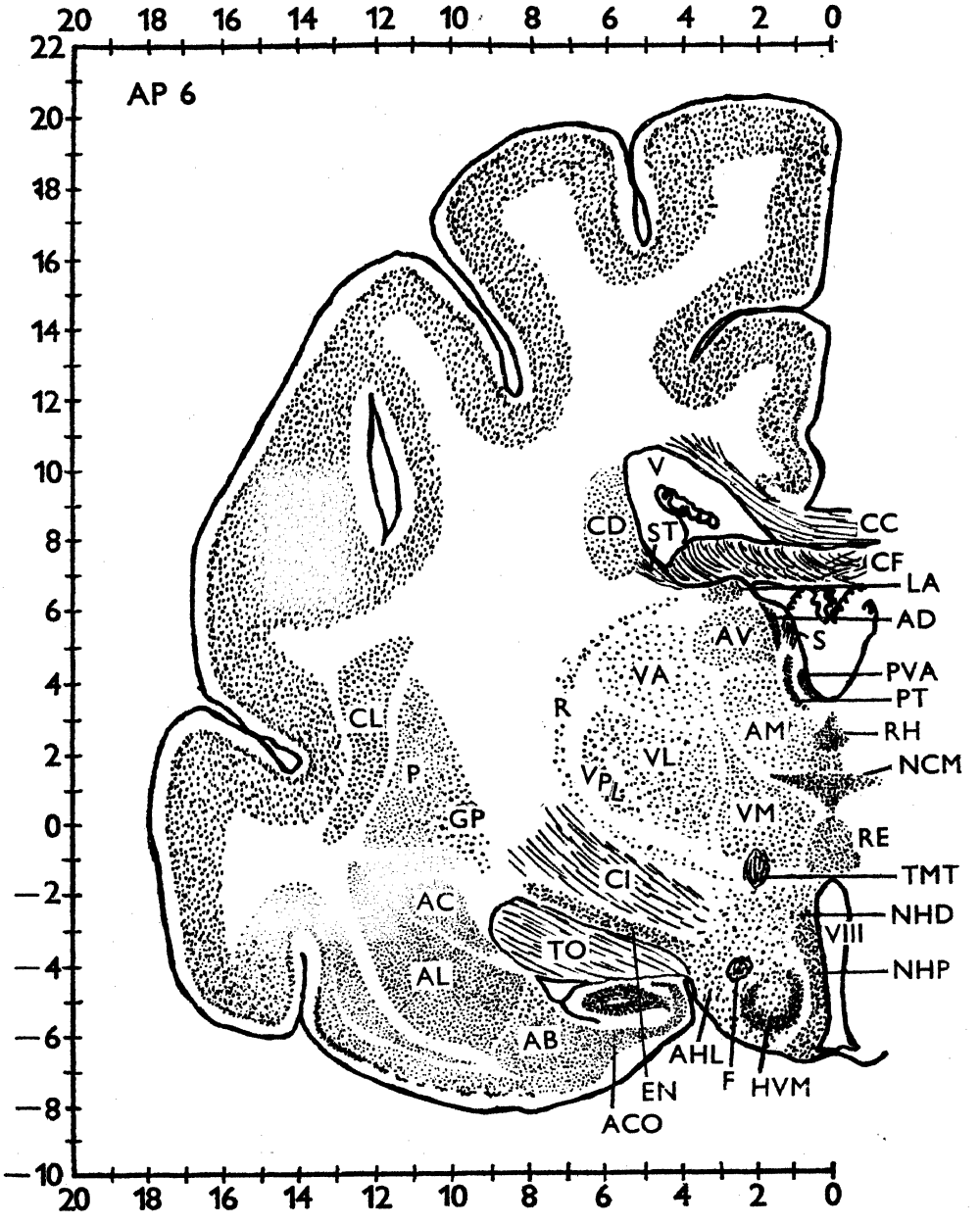


Fig. 223a, b. Coronal sections of brain. Cat.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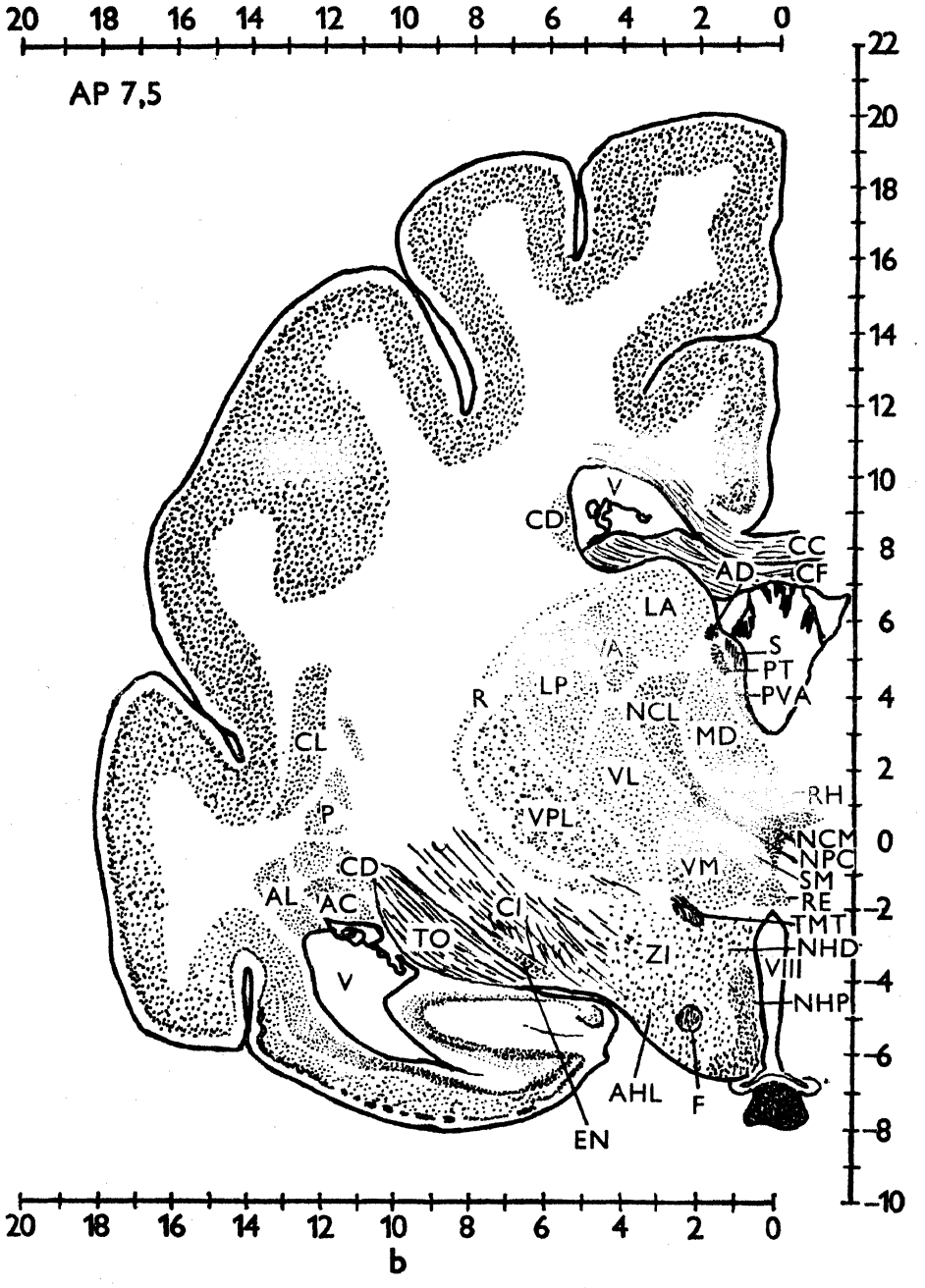
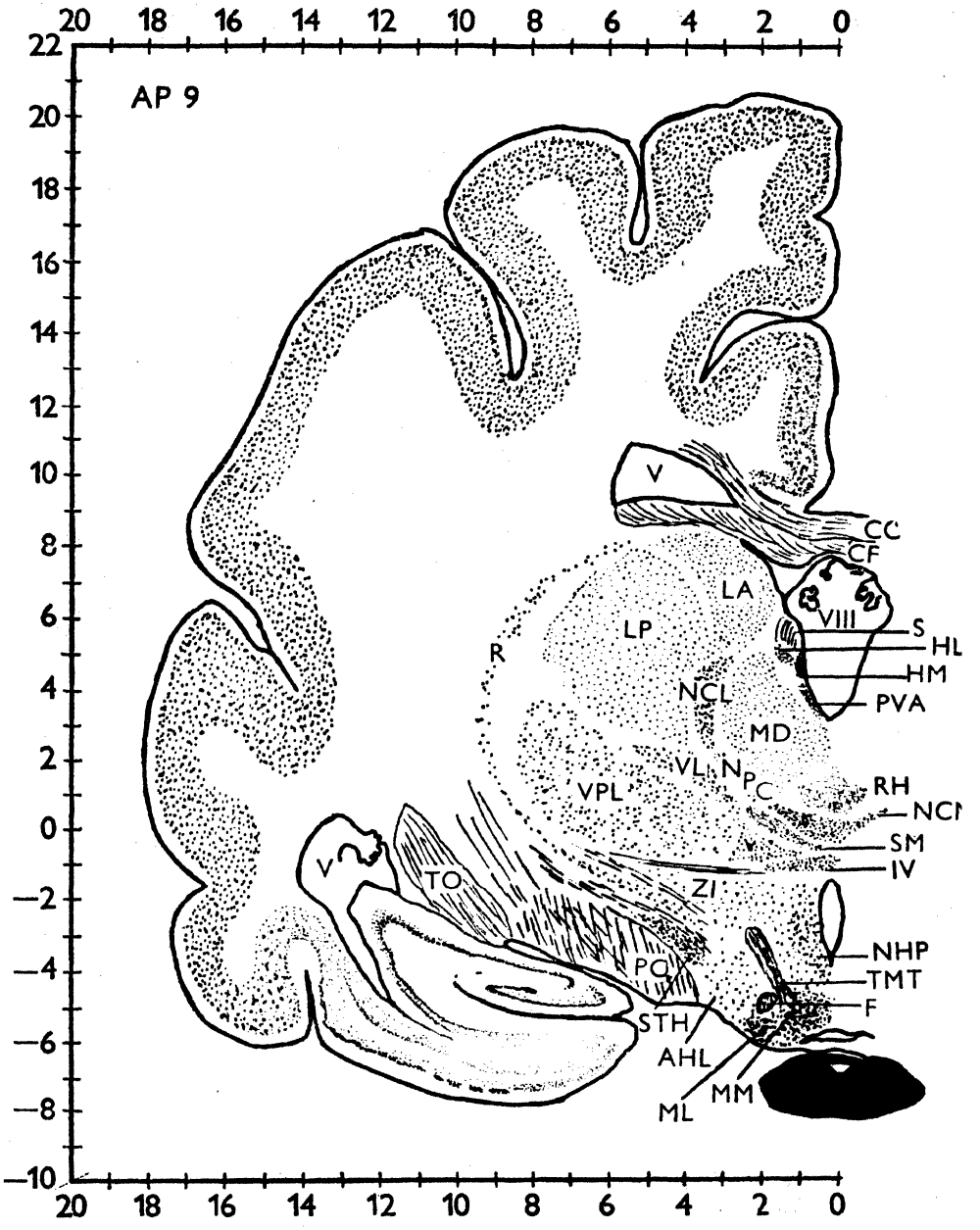


Fig. 224a, b. Coronal sections of brain. Cat.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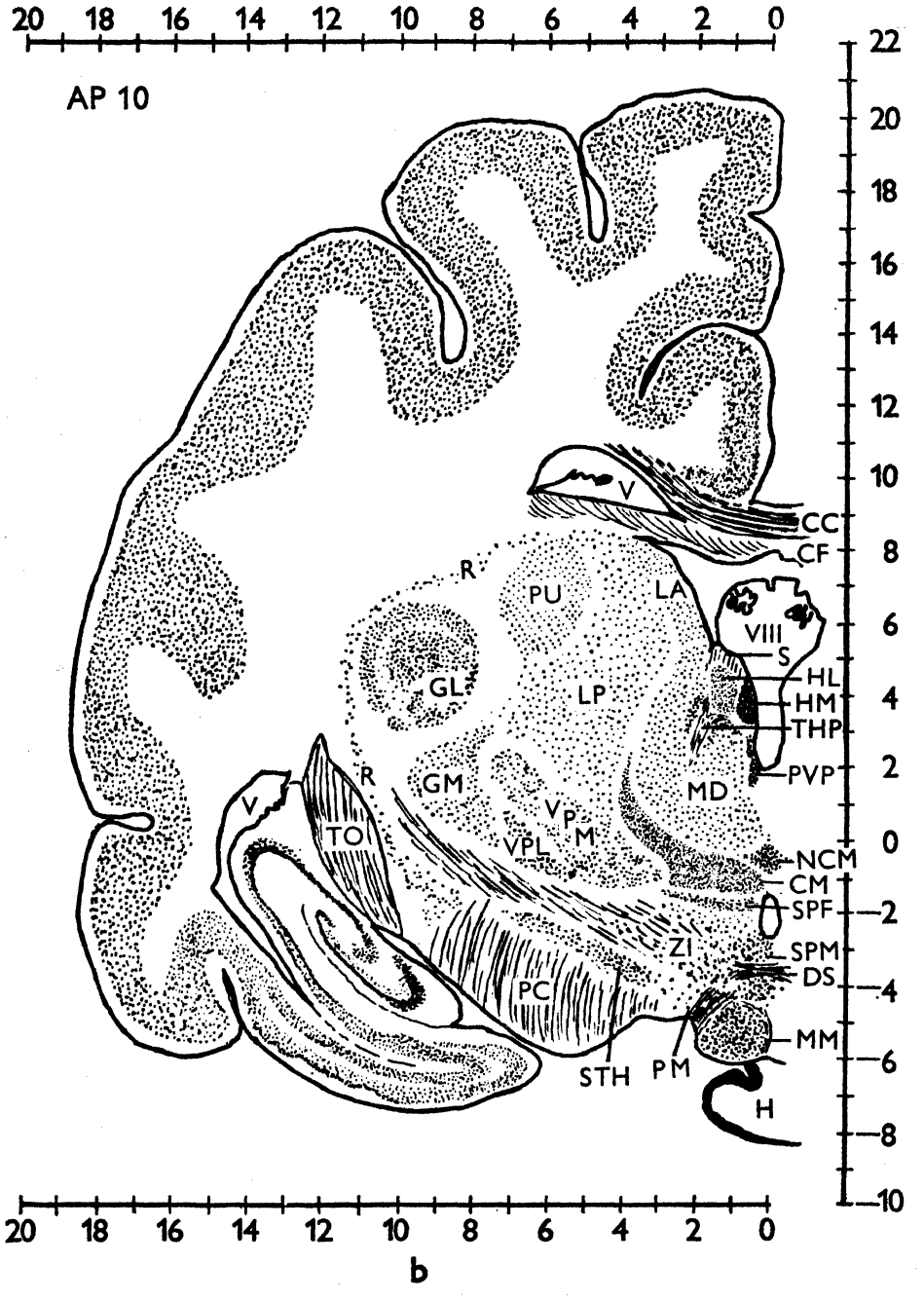
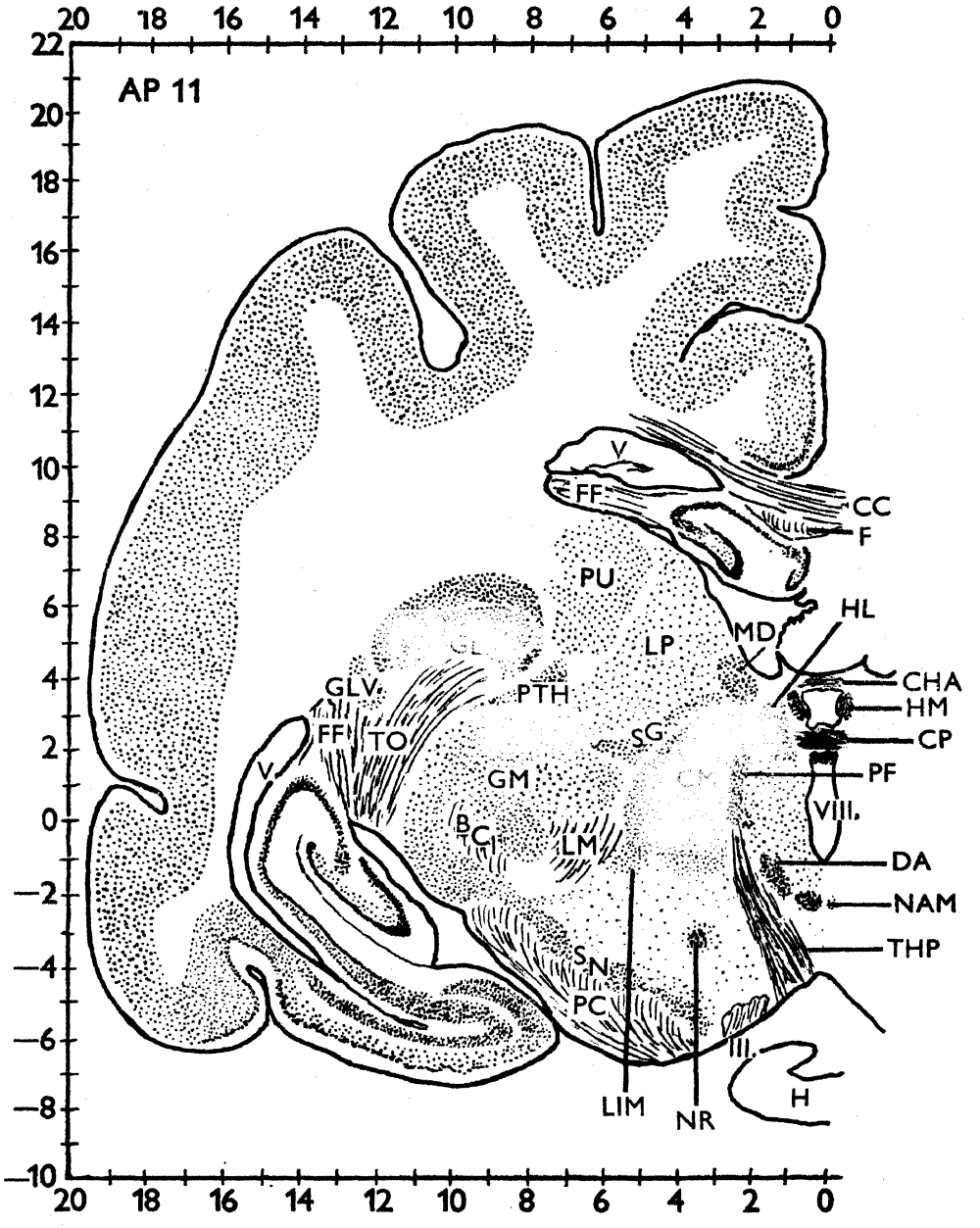


Fig. 225a, b. Coronal sections of brain. Cat.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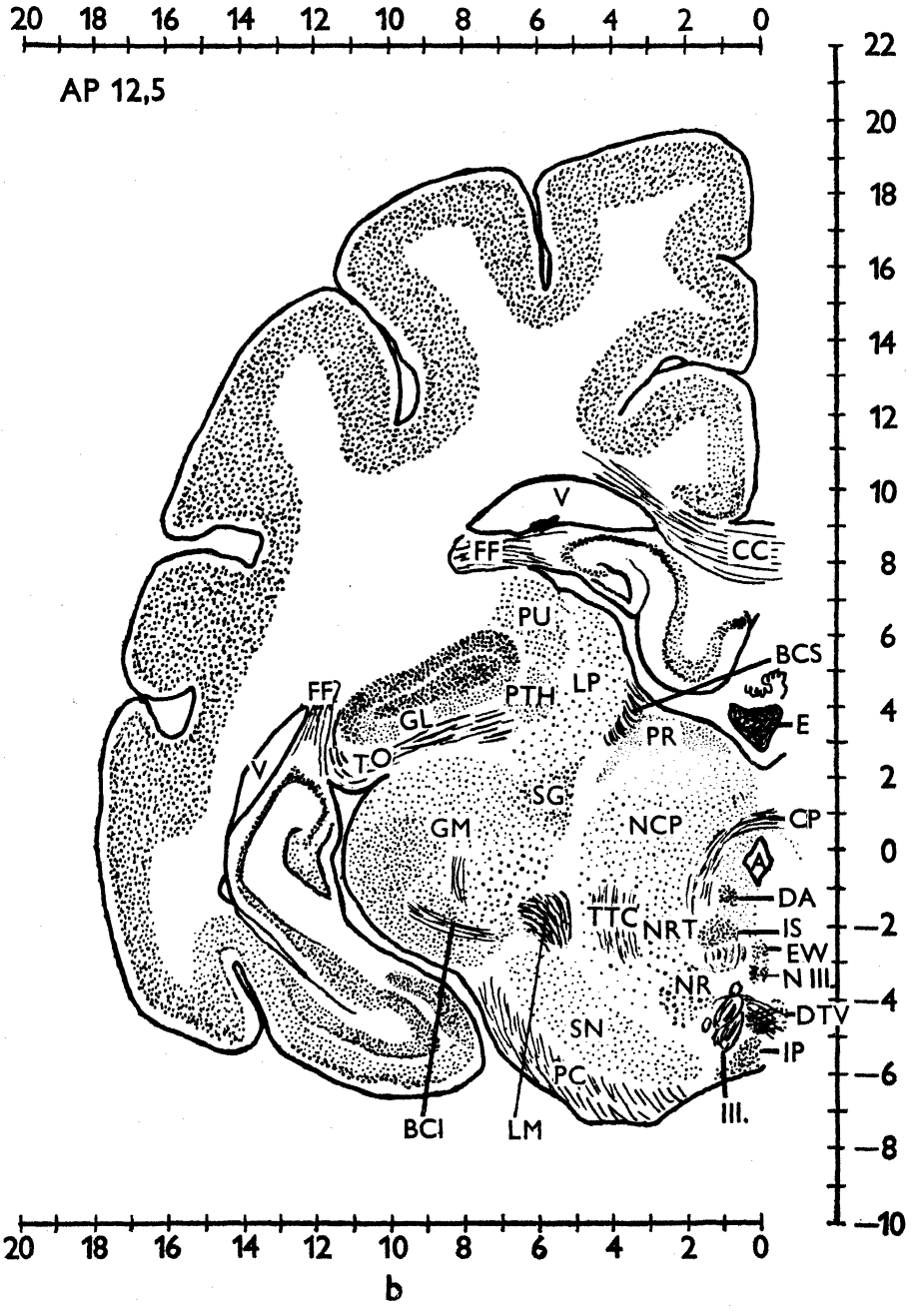
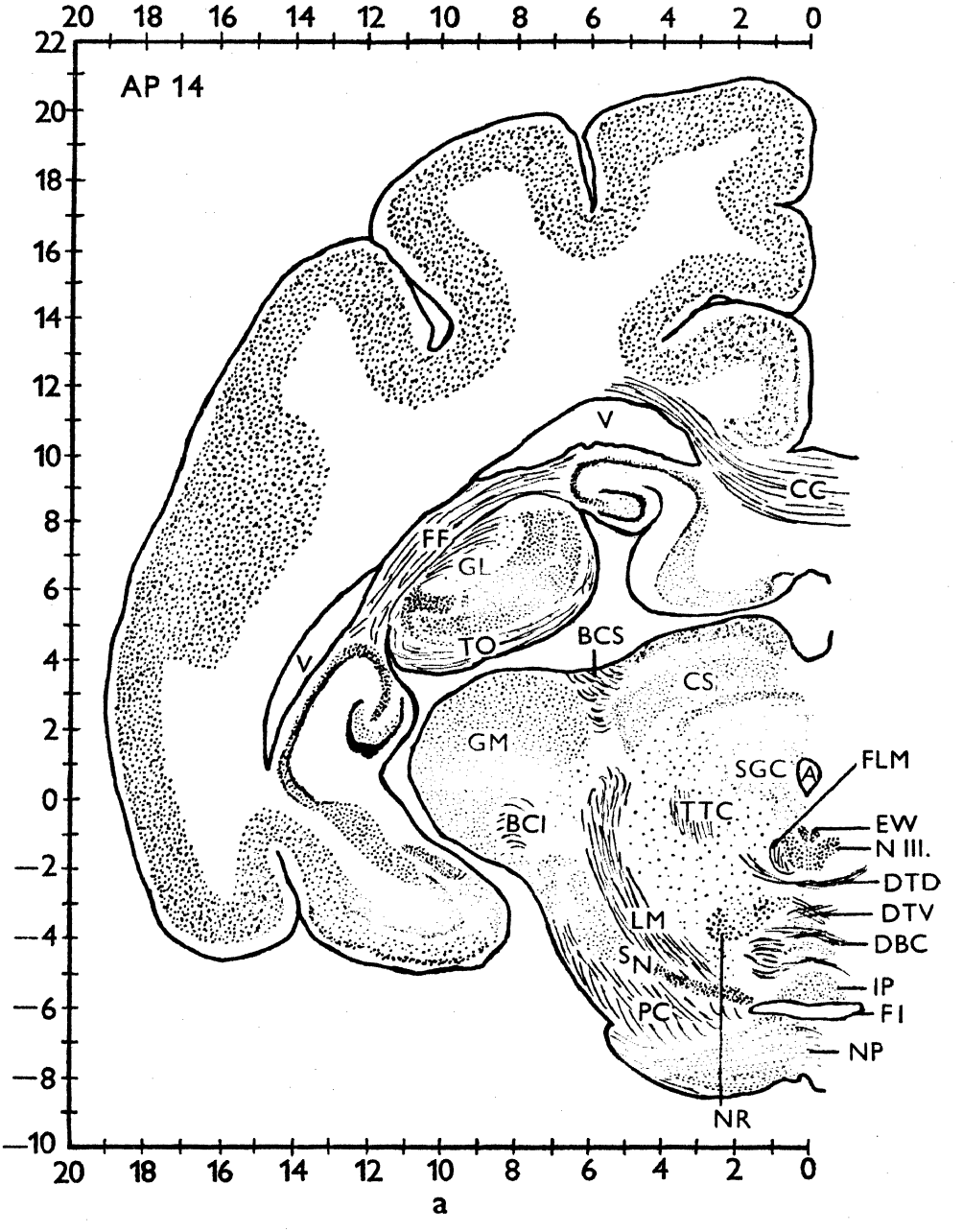


Fig. 226a, b. Coronal sections of brain. Cat.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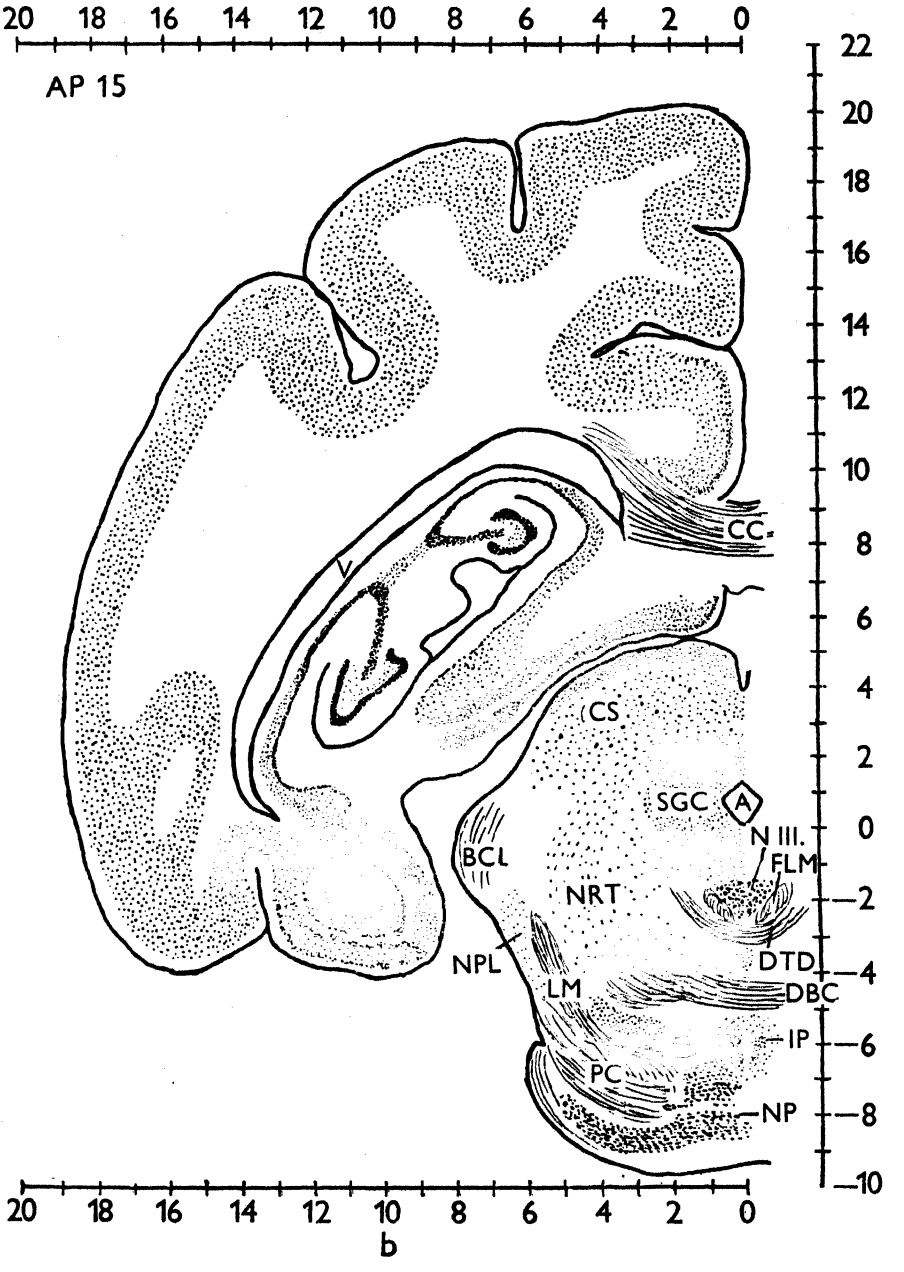

Fig. 227a, b. Coronal sections of brain. Cat.

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