The Physiology of Nerve Cells

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FOR MY WIFE, *Irene Frances*

PREFACE

This book was developed from three lectures that were delivered at The Johns Hopkins University in the fall of 1955. The Johns Hopkins University had honoured me by an invitation to deliver the Twenty-ninth Course of Lectures on the Herter Foundation. The three lectures were entitled:

1. The motor neurone and excitatory synaptic action
2. Inhibitory synaptic action
3. Pathways and transmitter substances in the central nervous system

Each lecture has been amplified considerably and is represented by two chapters of the book. The account given in the lectures has been substantially modified in two fields where more recent investigation has shown that it was erroneous. Otherwise, the changes have consisted merely in the addition of more experimental illustrations of the essential ideas expressed in the lectures.

It is not unreasonable to maintain that nerve cells are more interesting and important than any other cells, being, as they are, the unitary constituents of the nervous system and the functional units responsible for all its multifarious activities, including the amazing performance of the human brain; yet until recently the nerve cell in itself has been understood too little to warrant a monograph. In the last few years, however, the situation has been changed by the application of new and powerful methods, the microtechniques. The greater part of the present monograph is concerned with the intracellular investigation of nerve cells, not only the mere recording of the intracellular potentials evoked in their various reactions, but also the modifications of these potentials that occur during and after the passage of current through an intracellular microelectrode. Such

intracellular investigations have been of particular significance in studying the synaptic responses of the individual cell. On the other hand, the extracellular recording of potential fields by microelectrodes has been of great value in elucidating the propagation of impulses over the various components of the individual nerve cells and along pathways formed by two or more cells in synaptic series. Finally, electron-microscopy already has revealed extremely fine structural details which may be correlated with the functional behaviour of the synaptic junctions.

Though these three types of microinvestigations are still at an early stage, so much information has been obtained already that it has seemed opportune to organize it into a monograph. This present account can be regarded as being complementary to a monograph entitled "The Neurophysiological Basis of Mind: The Principles of Neurophysiology," which was written about four years ago, and which gave an account of some of the earliest investigations with intracellular microelectrodes. Already these sections of that monograph have been superseded.

In the earlier book it was recognized that synaptic transmission in the central nervous system was mediated by chemical transmitter substances, but little could be added to this general concept. Even now there has been an identification of only one transmitter at one type of synaptic junction in the central nervous system. Nevertheless, recent experimental work makes it possible to devote a whole chapter to investigations and problems concerning pathways and transmitter substances.

The final chapter is frankly much more speculative, for here the attempt is made to develop ideas that may be of significance in the further investigation and understanding of the nervous system. The title of this chapter is derived from a remarkable book, *Features in the Architecture of Physiological Function,* by Sir Joseph Barcroft, in which the theme was not only that form and function are closely correlatable in living tissues, but also that a form or pattern may be discovered in the functional processes themselves.

There can be no doubt that these concepts will prove particularly fruitful in the central nervous system, where fundamental significance attaches both to the functional and to the structural patterns. The final chapter does not attempt a systematic survey of this whole field. It is merely an attempt to illustrate it by a few examples.

I wish to thank the Committee of The Herter Lectureship, Drs. Lehninger, Harvey, and Rich, of The Johns Hopkins University, for kindly inviting me to give the lectures and for their efforts in making the lectures a success. In addition, I wish to express my gratitude to my numerous friends at Johns Hopkins and in particular to Drs. Bard, Magladery, Kuffler, and Mountcastle. I have been helped greatly in writing this monograph by my neurophysiological colleagues from many countries. The ideas expressed herein were developed not only in discussions with my collaborators here in Canberra, but also with neurophysiologists during the meetings, the colloquia, and the more informal occasions that were so memorable during my overseas visit in 1955. In particular, I would like to thank Drs. Palade, Palay, Bullock, and Hagiwara for kindly allowing me to reproduce illustrations from their unpublished work. I also wish to thank my colleagues P. Fatt, A. Lundberg, J. S. Coombs, D. R. Curtis, A. W. Liley, V. B. Brooks, and Rosamond Eccles, not only for granting me the use of some of the figures, but also for reading and criticizing the manuscript. Finally, I wish to thank Mr. Winsbury, Mr. Daynes, Mr. Chapman, and Mr. Paral, who have helped so much in the design and construction of equipment as well as in the preparation of the illustrations, and Miss R. Burkitt for all her work in the preparation of the manuscript.

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CONTENTS

CHAPTER I

THE NERVE CELL AND ITS SURFACE MEMBRANE

A. INTRODUCTION

Cajal (1934, 1954) has told in his characteristically vigorous style the history of the concept that the nervous system is composed of discrete units or nerve cells. The concept was first proposed by His and Forel and then independently by Cajal, while later the name "neurone" was suggested by Waldeyer for the nerve cell and "neurone theory" for the concept of independence of nerve cells. Although all the great neurohistologists of that classical era were ranged for or against the neurone theory, it was pre-eminently the achievement of Cajal to establish that the functional connections between individual nerve cells, or neurones, are effected by close contacts and not by continuity in a syncytial network, as proposed in the rival reticular theory of Gerlach and Golgi. Appropriately Cajal's last great contribution (1934) was devoted to a critical survey of the evidence for and against the neurone theory, which has not been seriously challenged since that time, at least for the vertebrate nervous system.

Sherrington (1897) gave the name "synapse" to these functional connections that are made by close contact between nerve cells. His magnificent contribution to neurology was concerned largely with showing how the reactions of the nervous system could be explained by the integrated behavior of individual nerve cells, each of which functioned as a unit and exerted graded excitatory or inhibitory synaptic actions on other nerve cells (Sher-

rington, 1906; 1925; 1929; 1931). This functional unity derived from two kinds of reaction. First, the cell integrated the various synaptic excitatory and inhibitory influences, inhibition acting as a quantitative antagonist to excitation. Second, if the unbalanced excitatory influence was sufficiently intense, the cell generated an all-or-nothing impulse which traversed its axon to exert in turn excitatory or inhibitory synaptic influences on other nerve cells, or, if the cell was a motoneurone, to cause contraction of its motor unit. Essentially we can consider the behaviour of the nervous system as being built up from the behaviour patterns of each of its myriad nerve cells, of which the human central nervous system contains more than 10^{10}. This behaviour pattern is defined at any instant by the two possible states of a cell, activation by an impulse or quiescence.

B. THE STRUCTURE AND DIMENSIONS OF NERVE CELLS

Since much of the experimental investigation already performed has dealt with the motor nerve cells (motoneurones) of the mammalian spinal cord, special reference will be made to them. Other nerve cells of widely differing type, however, have now been studied sufficiently to give us assurance that the mammalian motoneurone is providing valid information about nerve cells in general, though in some respects it will be found to exhibit specialized behaviour.

The essential structure of a motoneurone and the synaptic contacts thereon are shown in Figure 1. The diagrammatic representation of the whole motoneurone is derived not only from serial sections, as in the models constructed by Haggar and Barr (1950), but also from remarkable preparations of isolated motoneurones (Chu, 1954). Typically the motoneurone has a cell body or soma approxi-

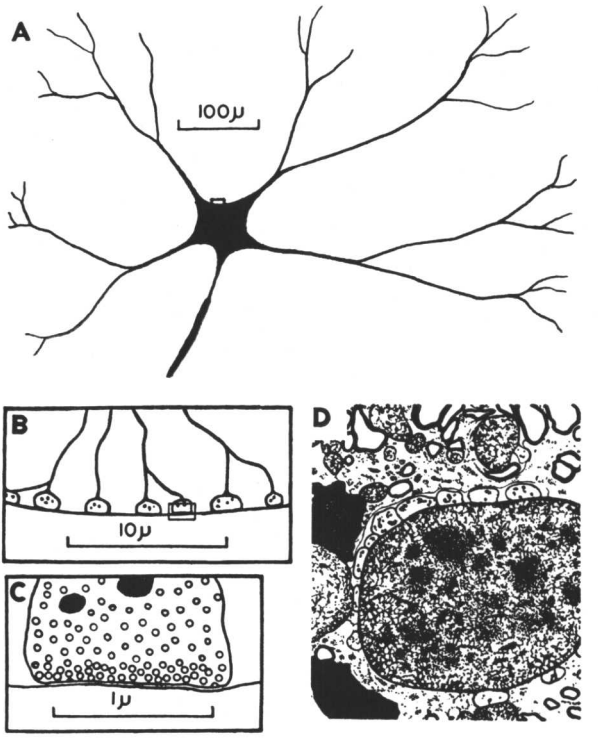

Figure 1 A–D

Drawing of a motoneurone to illustrate general relationships of dendrites and axon to the soma. The small surface area that is outlined is drawn at 20 times higher magnification in *B* to illustrate the relationship of the synaptic knobs to the surface (cf. *D*). The small area outlined in *B* is drawn at 10 times further magnification in *C* to show the width of the synaptic cleft and the thickness of the surface membranes of the synaptic knob and the nerve cell (cf. Figure 1E). Also shown are the synaptic vesicles and mitochondria of the synaptic knob. *D*. Drawing of a low-power photograph obtained with an electronmicroscope showing twelve synaptic knobs in contact with a large dendrite, and also smaller dendrites, on one of which there is a synaptic knob. Note characteristic mitochondria of the knobs (Wyckoff and Young, 1956).

mately 70 μ across, from which radiates a number of branching processes (dendrites) that extend for long distances, as much as 1 mm, before breaking up into fine terminal branches. Also arising from the soma is the axon,

or motor nerve fibre, which gradually narrows before assuming a myelin sheath at a distance of some 50 μ to 100 μ. The twenty fold higher magnification of Figure 1B shows the density with which the expanded axonal terminals (synaptic knobs) of other nerve cells encrust the surface of the soma and the basal regions of the dendrites, the distribution being progressively sparser as the dendrites are followed more peripherally (Lorente de Nó, 1938; Barr, 1939; Bodian, 1952). This is well shown in Figure 1D, which is a drawing from an electron microphotograph (Wyckoff and Young, 1956). Synaptic endings have also been described on the axon hillock and the non-medullated segment of the axon (Barr, 1939), though Hoff (1932a) and Lorente de Nó (1938) do not report their presence there with motoneurones. Certainly the density is much less than on the soma, and there is no sign of the specialized synaptic terminals that surround the axonal origin from the Mauthner cells of teleosts (Bodian, 1937; 1940; 1942; 1952).

Finally, in Figure 1C a further ten fold magnification shows schematically the fine structure of a synapse, as described by Palade and Palay (1954) and de Robertis and Bennett (1955), for the synapses on several different types of cells. As revealed in the electron microphotograph (Figure 1E) continuous membranes approximately 50 Å thick cover the synaptic knob and the neuronal soma, there being a cleft approximately 200 Å in width between the two membranes. Within the synaptic knob there are numerous vesicles about 300 Å in diameter, which occasionally are seen opening on the synaptic surface. The mitochondria indicate that there is a high level of metabolic activity in the synaptic knob, which contrasts with the cytoplasm beneath the subsynaptic membrane. Probably, as with the motor nerve endings on muscle (Robertson, 1956; Palade and Palay, 1954), the vesicles of the synaptic knobs may be regarded as containing the chemical substances that are responsible for transmission across the synaptic junctions (cf. Chap. V). It should be noted that,

in all the following discussions on the responses of neurones, the fine internal structure (cf. Palay and Palade, 1955) has been neglected. The immediate electrical responses and the specific actions of transmitter substances appear to be surface phenomena, while the deep structures seem to be concerned with recovery processes, metabolism, protein manufacture, etc. (cf. Hydén, 1943).

The word synapse, as proposed by Sherrington, may be applied to the synaptic knob with its chemical mechanism, the synaptic cleft of 200 Å, and the subsynaptic membrane with its specific receptive and reactive mechanism. On analogy with the end-plate membrane of the neuromuscular junction (Kuffler, 1943; Fatt and Katz, 1951; Castillo and Katz, 1954e; 1955a), it is probable that the subsynaptic membrane has very different properties from the remainder of the soma-dendritic membrane, being specifically affected by the transmitter substance, and probably being unable to respond to an impulse.

Even in the unstained preparation, the axon hillock and the non-medullated axon of motoneurones may be distinguished from the soma and dendrites by the absence of Nissl substance and pigment granules (Chu, 1954); and as noted above there is much less coverage by synaptic knobs. Moreover, it will appear later (Chap. II) that on physiological grounds there is a very remarkable distinction between these two zones of the motoneurone. For one zone, therefore, it is proposed to make use of the collective term "initial segment of the axon" or simply "initial segment," which generally has been applied to the non-medullated segment of the axon and the axon hillock from which it arises (cf. Lloyd, 1951a, Lorente de Nó, 1953).

When inserting a microelectrode, by far the largest target is presented by the soma and the adjacent large dendritic branches. In all but a few exceptional experiments we may, therefore, assume that the microelectrode is implanted therein. It is improbable that with our technique a microelectrode could be implanted satisfactorily in

such tenuous structures as the more distal dendritic branches. Furthermore, on analogy with muscle fibres and giant axons, it is probable that the intracellularly recorded electric potentials arise across the surface membrane of the nerve cell. Investigations on large peripheral nerve cells have the great advantage that the microelectrode actually can be seen within the cell (Tauc, 1954; 1955b; Eyzaguirre and Kuffler, 1955a; Arvanitaki and Chalazonitis, 1955). Such investigations show further that all parts of the cytoplasm are virtually isopotential, which is to be expected, because the cytoplasm is likely to have such a low specific resistance that the currents flowing within the soma would develop only a very small potential. We may, therefore, conclude that the whole surface membrane of the soma has virtually the same potential difference across it, which is that recorded between the intracellular electrode and the indifferent external electrode.

On the contrary the dendrites are so long, relative to their diameter, that changes in the membrane potential of more distal regions would make a negligible contribution to potentials recorded by a microelectrode implanted in the soma. An approximate calculation based on probable values for the specific membrane resistance and for the specific resistance of the cytoplasm reveals that, if a steady change is produced in the membrane potential of a dendrite 5 μ in diameter, only one-half of that potential change will be recorded at a distance of 200 μ along the dendrite from that zone, i.e., the length constant is about 300 μ (Coombs, Eccles, and Fatt, 1955a). Similarly, when currents through the microelectrode are employed to change the ionic composition of the motoneurone, these changes will be restricted largely to the soma and adjacent segments of the dendrites.

Approximate values for the effective volume and surface area of a standard motoneurone may therefore be derived by neglecting the dendrites beyond 300 μ and assuming that the standard motoneurone is a sphere 70 μ in diameter with 6 cylindrical dendrites (cf. Balthasar, 1952) of 5 μ in

diameter radiating therefrom for 300 μ, and an axon arising from a conical axon hillock. The volume and surface areas so calculated for a standard motoneurone are approximately 2.5 x 10^{-7}cm^3 and 5 x 10^{-4}cm^2 respectively if allowance is made for the conical origin of the dendrites from the soma.

Pyramidal cells of the cerebral cortex have a much smaller volume, 2 x 10^{-8}cm^3 being the largest soma volume measured by Sholl (1953), and interneurones would be smaller still. The largest sympathetic ganglion cells have a volume of no more than 1 x 10^{-8}cm^3. Presumably the relatively small size of interneurones and sympathetic ganglion cells accounts for the difficulty in recording intracellularly from them and for their rapid deterioration under such conditions (R. M. Eccles, 1955). For example, diffusion from the microelectrode would be expected to cause a rapid change in the ionic composition, and there would be an accompanying rapid swelling due to influx of water. On the other hand many nerve cells of invertebrates are quite large. For example, the crustacean stretch receptor cells are at least as large as motoneurones (Figure 32A; Alexandrowicz, 1951; 1952; Florey and Florey, 1955, Eyzaguirre and Kuffler, 1955a), and many ganglion cells of *Aplysia* are very much larger (Tauc, 1954; 1955b; Arvanitaki and Chalazonitis, 1955).

C. TECHNICAL PROCEDURE

Nerve cells of the central nervous system have complex branches, which interlace with the branches of multitudes of other nerve cells from many of which they receive synaptic contacts; hence for physiological investigations it is not feasible to attempt anatomical isolation, as has been done post mortem by Chu (1954). It is possible, however, by intracellular microelectrode techniques (Brock, Coombs, and Eccles, 1952a; 1953; Woodbury and Patton,

1952; Araki, Otani, and Furukawa, 1953; Albe-Fessard and Buser, 1954; Araki and Otani, 1955; Coombs, Eccles and Fatt, 1955a, b, c, and d; Phillips, 1955; 1956a, b; Frank and Fuortes, 1955a, b; 1956a, b) to secure all the advantages that would accrue from anatomical localization, and yet at the same time to have this cell lying virtually unmolested in the central nervous system and being normally supplied with blood. These techniques are so effective that they are the methods of choice for peripheral nerve cells that could be isolated anatomically, e.g., the crustacean stretch receptor cells (Eyzaguirre and Kuffler, 1955a, b) and the ganglion cells of *Aplysia* (Tauc, 1954; 1955b; Arvanitaki and Chalazonitis, 1955).

Essentially the technique requires (cf. Grundfest, 1955) that the surface membrane of the cell be punctured by a very fine glass tube which usually is filled with a salt solution and which acts as an insulated lead from the interior of the cell. The microelectrode, as it is called, has a tip diameter measuring from 0.5 μ to 1 μ and a resistance usually of from 10 MΩ to 20 MΩ. The other electrical lead is an indifferent lead from a large area of the animal. If mechanical disturbances are reduced by extreme precautions in fixation, the microelectrode is sealed into the surface membrane and the cell may behave normally for several hours. In order to secure these favourable conditions, the animal must be fixed rigidly on a heavy steel frame. A special micromanipulator is used to insert the microelectrode (Figure 2). During the insertion procedure, electrical responses are evoked from the motoneurones so that there is displayed on the cathode ray screen a standing wave which provides information about the proximity of the various groups of motoneurones to the tip of the microelectrode. Penetration of a neurone results in the immediate appearance of the membrane potential and in a drastic change in the signals so recorded, which are inverted and greatly increased in size. The recorded potentials are produced practically entirely by the impaled cell, the reactions of which can thus be studied in isolation from