JOHN CAREW ECCLES

THE PHYSIOLOGY OF SYNAPSES

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With 101 Figures

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For Irene Frances

PREFACE

I must thank my friend, Professor HANS WEBER, for being, as it were, the prime mover in causing this book to be written. He persuaded me in 1960 to contribute a review to the Ergebnisse der Physiologie. As originally planned, it was to be relatively short. However, the interest and scope of the whole subject of synapses stimulated me to write a much more comprehensive and extensive account. I was not even then satisfied, particularly as so many new and attractive investigations and ideas were being evolved during and after the writing of this review; and during the writing of this book most interesting developments are occurring in so many centres of research. Through the kind cooperation of my friends I have been given the opportunity to quote and even to illustrate from these new and fascinating developments before their final publication.

There would be some justification if the author were to make the claim that this book is the fruit of a life-time of enquiry into the physiology of synapses. In 1927 the subject of Excitatory and Inhibitory Synapses was chosen for investigation in the course leading to the Oxford D. Phil. But there have been such remarkable developments during the last 12 years that in this book very little reference will be made to work earlier than 1951 except in the historical introductions. Yet the influence of three great scientists has been with me as I have striven to give a coherent account of synapses, which we may regard as the key structures of the nervous system. There is firstly RAMON Y CAJAL whose achievement was to show that the nervous system was made up of independent structures, the neurones, cells of the utmost diversity, yet amazingly organized in their relationship to each other. Secondly is SHERRINGTON who linked this structural separateness with neuronal integration by the concept of the synapse and who defined the essential properties of the synapse with great insight. Thirdly is DALE who was the leader in developing the theory of chemical transmission across synapses, which has been so fruitful in all recent developments, structural, physiological, pharmacological and neurochemical.

The enormous advances in knowledge and understanding have been brought about largely as a result of the microtechniques: electron-microscopy; electrical

investigations by microelectrodes, particularly by intracellular electrodes; and microinjection procedures both intracellular and extracellular. Relatively few types of synaptic actions have yet been subjected to intensive investigation by these new procedures; nevertheless we can have confidence that general principles of synaptic action are being established because there has been a remarkable uniformity of the essential features of synaptic actions for a wide variety of synapses in invertebrates as well as vertebrates.

It is noteworthy that the same general principles of synaptic action have been expressed in a book "Synaptic Transmission" by Dr. H. McLENNAN (1963) that was published after the manuscript of this book had been sent to press. Though there is an essential similarity in the two accounts of synaptic transmission, Dr. McLENNAN's book is also in part complementary to the present book. This is especially so in his extensive and very well documented accounts of neuropharmacology and neurochemistry.

We may provisionally define a synapse as a structure that is formed by the close apposition of neurone either with neurone or with effector cell and that is specialized for the transmission of excitation or inhibition. Presumably this definition will have to be extended to include transmission from some receptor cells to afferent terminals, but it seems best to defer this development until there is more precise information on the nature of this transmission (cf. DAVIS 1961; GRAY 1959). It may be objected that the word, ephapse, has commonly been used for many electrically transmitting junctions that would come within this broad definition of the synapse, as has been done for example by GRUNDFEST (1959) in his valuable review. However ARVANITAKI (1942) first introduced "ephapse" for a quite different situation in which under certain conditions of spatial arrangement the electrical currents generated by an impulse in a giant axon excited another axon. The requisite spatial arrangement of the ephapse could be that naturally occurring or it could be artificial, but the distinction was made by ARVANITAKI that the synapse "designates surfaces of contact (whether axo-somatic, axo-dendritic or axo-muscular) anatomically differentiated and functionally specialized for the transmission." The further distinction was made that at the synapse transmission is irreciprocal, but this irreciprocity of transmission is now known to occur for junctional transmissions indubitably ephaptic, such as the electrical excitation of intramuscular nerve fibres by the muscle spike potential (LLOYD 1942; LEKSELL 1945; BROWN and MATTHEWS 1960), or even between two giant axons when specially treated. On the other hand reversibility of transmission is very well developed at junctional regions that because of the criteria of design and of function will be classed as synapses (Chapter IX)—for example the septa of some giant axons and the electrically transmitting bridges between nerve cells or between giant axons, and with the large synapses of avian ciliary ganglia.

It is assumed that the reader of this book already will be informed about the general physiological properties of nerve fibres, nerve cells and muscle fibres. In particular it is assumed that the reader has a knowledge of the electrical properties of excitable cells that is at a level equivalent to that given in the initial chapters of my previous books "The Neurophysiological Basis of Mind" and "The Physiology of Nerve Cells." Though the symbols used as abbreviations are defined at the time of their introduction, a list is given immediately after the Table of Contents.

The stories and ideas that are expressed in this book have derived in large part from the numerous discussions and symposia that have been such a feature of these recent years. It has been a singular advantage to meet and to know personally almost all of the scientists whose work forms the basis of this book. I am particularly indebted to my colleagues in Canberra. They have come from all parts of the world and have given me and each other conditions in the laboratory that have been both happy and fruitful. During the actual labour of writing this book I was helped particularly by my colleagues: D. R. CURTIS, J. S. COOMBS; J. I. HUBBARD; P. ANDERSEN; M. ITO; J. C. WATKINS; and ROSAMOND ECCLES who read chapters and made most valuable suggestions. I wish also to thank Mr. L. M. DAVIES for his expert technical assistance; V. PARAL, R. WESTON, A. CHAPMAN, Miss C. MACPHERSON and Miss S. WILLIAMSON for their expert assistance with all the illustrations; and Miss I. SHEAFFE and Miss R. MACDONALD for all their work in preparation of the manuscript.

Dr. R. F. SCHMIDT has given most valuable help in the indexing. My special thanks go to the publishers for their unfailing courtesy and for their extraordinary efficiency in producing this volume in such a short time. As a consequence, in a book published in 1963, there are nearly 100 references to papers published in this same year.

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SYMBOLS AND ABBREVIATIONS

Physical and Chemical

mm	= millimetre	mM	= 10^{-3} M
μ	= 10^{-3} mm	V	= volt
Å	= Ångstrom = 10^{-7} mm	mV	= 10^{-3} volt
min	= minute	μV	= 10^{-6} volt
sec	= second	Ω	= ohm
msec	= 10^{-3} second	MΩ	= 10^{6} ohm
c/sec	= frequency	A or amp	= ampere
g	= gram	mA	= 10^{-3} ampere
mg	= 10^{-3} gram	μA	= 10^{-6} ampere
μg	= 10^{-6} gram	mμA	= 10^{-9} ampere
mμg	= 10^{-9} gram	mho	= reciprocal ohm
M	= mole (one gram molecule) per litre	F	= farad

Physiological

EPP	= endplate potential (intracellular)
min. EPP	= miniature endplate potential
EPC	= endplate current
EPSP	= excitatory postsynaptic potential (intracellular)
IPSP	= inhibitory postsynaptic potential (intracellular)
DRP	= dorsal root potential
PAD	= primary afferent depolarization (intracellular)
EHP	= extracellular hyperpolarizing potential
E_R	= resting membrane potential
E_{Cl}	= equilibrium potential for chloride ions
E_K	= equilibrium potential for potassium ions
E_{IPSP}	= equilibrium potential for IPSP
$(K^+)_i$	= intracellular concentration of K^+ ions
$(Cl^-)_i$	= intracellular concentration of Cl^- ions

Anatomical

IS	= initial segment at axonal origin from neurone
SD	= soma plus dendrites of neurone
Group Ia	= afferent fibres from annulospiral endings of muscle spindles
Group Ib	= afferent fibres from Golgi tendon organs of muscle

Group I = Group Ia + Ib
Group II = afferent fibres from secondary endings of muscle spindles
Group III = small medullated afferent fibres from muscle
DSCT = dorsal spinocerebellar tract
VSCT = ventral spinocerebellar tract
L_7VR = ventral root of lumbar seven segment
S_1VR = ventral root of sacral one segment
PBST = posterior biceps-semitendinosus
SMAB = semimembranosus-anterior biceps

Pharmacological

ACh = acetylcholine
AChE = acetylcholine esterase
dTC = d-tubocurarine
DFP = di-isopropyl fluorophosphate
TEPP = tetraethylpyrophosphate
GABA = γ-amino butyric acid
TMA = tetramethyl ammonium

CONTENTS

CHAPTER I

THE DEVELOPMENT OF IDEAS ON THE SYNAPSE

A. The conflict between the neurone theory and the reticular theory

When neurohistologists began to study the nervous system in detail, the complexity of the interlacing fibre structure led them to postulate that the nervous system was a complex net-like structure, which is the reticular theory of GERLACH (1871). The nerve cells were believed to be at the nodes of this reticular structure, and the nerve fibres originating from them branched profusely and anastomosed, so forming the fibre meshwork characteristic of grey matter (GOLGI 1885).

This interpretation was first challenged by HIS (1886, 1889) and FOREL (1887) who proposed instead that each nerve cell was an independent unit and that their branches did not anastomose, but merely entered into close contacts. His arrived at this conclusion from a study of the development of the nervous system from the individual neuroblasts, while FOREL was impressed by the selectivity of the atrophy of nerve cells after nerve fibres had been destroyed. Independently, RAMÓN Y CAJAL (1888, 1890a, 1890b, 1890c) had reached the same conclusions as a result of his investigations of embryonic material and of the intensive application of the Golgi technique, which stained specifically a very few nerve cells, that were thus revealed in their entirety and in isolation from all others. Other neurohistologists such as KÖLLIKER (1890), VAN GEHUCHTEN (1891) and V. LENHOSSÉK (1892, 1893) also strongly supported the theory of the independence of the nerve cells, which, following the suggestion of WALDEYER (1891), came to be known as the neurone theory, in contrast to the reticular theory.

Implicit in the neurone theory was the assumption that nerve cells must enter into functional connection with one another by contiguity, not continuity. As originally described by the neurohistologists this contiguity was achieved by the profusely branching nerve terminals which embraced nerve cells to form the baskets (corbeilles) and terminal brushes of RAMÓN Y CAJAL (1890a) and the "Fasernkörbe" that HELD (1891) first described in the

trapezoid body. Fibres were also described interlacing with dendritic processes, such as for example the climbing fibres around the dendrites of the Purkinje cells (RAMÓN Y CAJAL 1890a) and in sympathetic ganglia (RAMÓN Y CAJAL 1909; DE CASTRO 1922, 1932). At first no differentiated terminals were distinguishable, probably because they were not developed in the very young animals that were investigated, and also because of the ineffectiveness of the Golgi technique in displaying the ultimate terminals. It remained for HELD (1897), AUERBACH (1898), RAMÓN Y CAJAL (1903), and WOLFF (1905) to demonstrate the characteristic "Endkörbe," "Endfüße," or "boutons" by which actual functional contact is achieved. It is convenient to use the term "synaptic knob" for the differentiated terminals of all kinds. Subsequently there was an intensive investigation of synaptic knobs with st udies of the details of morphology and distribution on a wide variety of neurones (cf. WINDLE and CLARK 1928; BARTELMEZ and HOERR 1933; BARR 1939; BODIAN 1937, 1940, 1942).

Nevertheless, despite the wealth of evidence against it (cf. RAMÓN Y CAJAL 1909), the reticular theory lingered on with the support of GOLGI (1890, 1891) and later of HELD (1905, 1909), who wrote in defence of it as recently as 1929. For example HELD (1905) believed that continuity between neurones was established by the fine neurofibrils that were described as passing from the synaptic knob to the underlying nerve cell. At that time neurofibrils were often believed to form the structural basis for transmission of impulses. Much detailed histological evidence was also adduced (cf. BIELSCHOWSKY 1928) that the synaptic knobs gave origin to a fine pericellular network of fibres, which established continuity with fine fibrils in the underlying nerve cell. This interpretation closely paralleled the periterminal reticulum which BOEKE (1911, 1932, 1940) described as subserving continuity across the neuromuscular junction. It is now recognized that these fine pericellular networks are non-nervous, and consequently they do not provide evidence for continuity between nerve cells (HINSEY 1934; NONIDEZ 1944).

In view of this continued support for the reticular theory, RAMÓN Y CAJAL (1934) was constrained to write his memorable last work in which he examined critically the whole controversy between the exponents of the neuronal and reticular theories. So effectively did he do this that the neuronal theory has not been seriously challenged since that time, though many of the old reticularists continued in their beliefs, or at least continued to claim that the neuronal theory was dead (cf. BOEKE 1940). The subsequent unassailable position of the neurone theory has been well described by BODIAN (1942, 1952) and NONIDEZ (1944). It has received strong support from degeneration experiments which showed that after section of a presynaptic pathway there was degeneration of the synaptic knobs, but not of the postsynaptic structure (HOFF 1932; FOERSTER, GAGEL and SHEEHAN 1933; SCHIMERT 1939), and

that after axon section the retrograde degeneration did not involve the synaptic knobs in contact with the degenerated neurone (BARR 1940; SCHADEWALD 1941, 1942).

The resolving power of conventional light microscopy was inadequate to reveal the fine structure of the synapse at a level that was required to explain the physiological mechanism. As techniques improved, the synaptic knobs appeared to be more closely attached to the postsynaptic membrane (cf. WYCKOFF and YOUNG 1956); so much so that there was thought to be just one membrane shared by the pre- and post-synaptic structures (BODIAN 1952). Such an arrangement was not acceptable as an efficient device for chemical transmission.

However, as described in Chapter II, the higher magnification given by electron microscopy has revealed that the presynaptic and postsynaptic membranes are two separate membranes about 70 Å thick, and that they are separated by a cleft about 200 Å in width which may be termed the synaptic cleft. This cleft is in direct communication with the interstitial spaces between the neurones (PALADE and PALAY 1954; PALAY 1956; DE ROBERTIS 1956; DE ROBERTIS and FRANCHI 1956). Similar features are also displayed at the vertebrate neuromuscular junction (ROBERTSON 1956). An important consequence of this pioneer electron-microscopy was that the neurone theory was definitively established for the vertebrate central nervous system.

B. Early developments in the functional concept of the synapse

Meanwhile the physiological implications of the neurone theory were being realized, as has been so well described by LIDDELL (1960) in "The Discovery of Reflexes." SHERRINGTON in particular suggested that the characteristic features of the reflex arc may be satisfactorily explained by the special properties of the transverse membranes that separate two neurones in regions of close juxtaposition, and consequently in 1897 he introduced for this junctional region the term "synapse," which is derived from the Greek word *σύνάπτω*, to clasp. For example the one-way conduction in the reflex arc was attributed by SHERRINGTON (1900, p. 798) to the valve-like behaviour of the synapse and not to a one-way conduction within a nerve cell, from dendrite to axon, such as was postulated by RAMÓN Y CAJAL (1895, 1909) and VAN GEHUCHTEN (1892) in their concept of dynamical polarization. Another important property of the reflex arc was the delay additional to that attributable to conduction time in the nerve pathway (SHERRINGTON, 1906, p. 22). Values as brief as 2 msec were assumed for the delay in traversing one synapse (cf. SCHÄFER 1900, p. 609). However, it was not until 1922 that HOFFMANN described the monosynaptic reflex and was thus able to derive from experimental data (cf. JOLLY 1911) the first reliable estimate (about 1 msec) for transmission time across a single

synapse. Subsequent investigations (LORENTE DE NÓ 1935, 1938a; ECCLES and PRITCHARD 1937; RENSHAW 1940; LLOYD 1943) have given values ranging from 0.5 to 1.3 msec for the delay in transmission of an impulse across a synapse.

SHERRINGTON (1906, p. 141—142) also pointed out that interaction would be likely between the many synapses situated on the surface of a single motoneurone. Activated excitatory synapses would give mutual reinforcement, while inhibitory synapses would antagonize the excitatory. He also listed many other differences between conduction in nerve-trunks and in reflex arcs and suggested that these differences were attributable to the synapses across which reflex transmission must pass. For example, reflexes are characterized by such features as fatigability, after-discharge, and greater sensitivity to oxygen lack and to anaesthetics.

Though having a structural basis, SHERRINGTON always used "synapse" in its functional sense, restricting it to those areas of close contact that were specialized for effective transmission from one neurone to another. Until the advent of electronmicroscopy there was much uncertainty about the specificity of the areas of close contact. For example it was widely held that any areas of sufficiently close contact between neurones would act effectively as synapses. There was also much speculation on the nature of the synaptic membrane, and neurohistologists as recently as 1952 referred to it as a single membrane, the "synaptolemma," which was composed by fusion of the juxtaposed membranes of the two neurones. In almost every respect SHERRINGTON'S conceptual developments with regard to the synapse during the early decades of this century were on the direct path to the present position. So much so that the significant development of ideas can be very effectively illustrated from his writings on excitatory and inhibitory actions on reflexes. For example in 1908 he wrote:

> From the observations it seems clear that the reflex effect of concurrent stimulation of excitatory afferent nerve with inhibitory afferent nerve on the vastocrureus nerve-muscle preparation is an algebraic summation of the effects obtainable from the two nerves singly, as v. CYON has maintained for the heart... One inference allowable from this is that in the case before us the two afferent arcs employed act in opposite direction at one and the same point of application in the excitable apparatus... As to the common locus of operation, the point of collision of the antagonistic influences, it seems permissible to suppose either that it lies at a synapse ... or that it lies in the substance of the "central" portion of a neurone. The net change which results there when the two areas are stimulated concurrently is an algebraic sum of the *plus* and *minus* effects producible separately by stimulating singly the two antagonistic nerves.

Here we have a clear statement of the algebraic summation of central excitatory and inhibitory actions, but still vagueness about the location and nature of these antagonistic actions.

In 1925 SHERRINGTON wrote a theoretical paper on central excitation and inhibition from which we may extract the following key statements.

Following the view that with the nervous impulse a short-lived local change ... is propagated along the course of the nerve-fibre, it may be supposed that the arrival of that change at the central terminals of the afferent fibre is an essential element in the central excitation process... There the nervous impulse resulting directly from the external stimulus may be regarded as ending, for there through an intermediary process and mechanism it generates, not inevitably though commonly, a new impulse.

SHERRINGTON then goes on to state that in order to account for summation of repetitive stimulations we must postulate that:

at some central situation there be a structure which is something other than a nerve-fibre, and has, unlike nerve-fibre, no absolute refractory phase. In such a structure the production of an exciting agent, in response to a previous stimulus, would on receipt of a second stimulus before subsidence of it be augmented by further production, so that its amount would be increased. In this way the central exciting state might by repetition of successive stimuli to the afferent nerve-fibre rise from below liminal amount or concentration to above liminal value.

Here we have the first clear statement of the concept of the central excitatory state. And later the locus of the state was defined as post-synaptic:

Of the terminals themselves histological evidence (CAJAL and others) shows them as severally discrete. But those convergent upon the same perikaryon and dendrites, although themselves discrete, reach a surface or synaptic membrane which, since it is that of one and the same cell, is in so far a single entity and is at the same time an arrival place common to the several terminals.

There is no conflict between these statements and the contemporary views on excitatory synaptic action, though on account of technical advances more precise statements are now possible.

SHERRINGTON was fascinated by the problem of central synaptic inhibition right to the end of his scientific life. So much so that his Nobel Oration in 1932 was entitled "Inhibition as a Coordinative Factor." Therein he ventured still further in his attempt to explain inhibition.

It is still early to venture any definite view of the intimate nature of "central inhibition." ... the suggestion is made that it consists in the temporary stabilization of the surface-membrane which excitation would break down. As tested against a standard excitation the inhibitory stabilization is found to present various degrees of stability. The inhibitory stabilization of the membrane might be pictured as a heightening of the "resting" polarization, somewhat on the lines of an electrotonus. Unlike the excitation-depolarization it would not travel; and, in fact, the inhibitory state does not travel.

The recently developed technique of intracellular recording from neurones has shown that inhibition is indeed due to a "heightening of the resting polarization."

However, concurrently with these theoretical developments in which reflex inhibition was explained in accordance with the neurone theory of the structure of the nervous system, there was an alternative view that was based on the reticular theory. It was tentatively proposed first by LUCAS (1917) and later by FORBES (1922) and ADRIAN (1924) that central inhibition might be similar to Wedensky inhibition, and that it could be brought about by the interference of high frequency discharges in the fine anastomosing fibres of the

reticulum. This suggestion had the merit of relating the blockage of excitatory pathways to factors that were known to operate in the peripheral nervous system, i.e. to refractory states, but it required many subsidiary postulates in order both to convert single afferent inhibitory volleys into high frequency discharges and to ensure that these discharges were effectively fed into the reflex excitatory pathways. Moreover, it was inseparably linked with the reticular theory, and interest in it declined as evidence accumulated against the reticular theory.

When it had been shown that a nerve impulse was followed by a prolonged state of hyperpolarization and an accompanying depression of excitability, it was proposed by GASSER (1937) that inhibition was due to depression of interneurones that were common to the central inhibitory and excitatory pathways. Prior activation of some of these interneurones by the inhibitory volley would be followed by the prolonged depression during which they could not be activated by the excitatory volley, and hence the excitatory volley would be less effective in evoking the discharge of motoneurones, i.e. there would be inhibition of its reflex response. This postulated mechanism failed, however, to account for the inhibition of monosynaptic reflexes, which have been employed in investigating many different types of inhibition (RENSHAW 1941, 1942, 1946b; LLOYD 1941, 1946a; LAPORTE and LLOYD 1952; BRADLEY, EASTON and ECCLES 1953). Nevertheless it still could be a contributory factor in the inhibition of polysynaptic reflexes.

Meanwhile evidence had been accumulating that synaptic excitatory action on a neurone caused primarily a depolarization, which if sufficiently intense evoked the discharge of an impulse. Initially this depolarizing potential was recorded after electrotonic transmission from motoneurones along their axons to the ventral root (BARRON and MATTHEWS 1936, 1938; ECCLES and PRITCHARD 1937; BREMER, BONNET and MOLDAVER 1942; BONNET and BREMER 1948; ECCLES 1946a; BERNHARD 1947), but later it was recorded as an extracellular field potential by a microelectrode in close proximity to the motoneurones (BROOKS and ECCLES 1947b; BROOKS, DOWNMAN and ECCLES 1950). It was generally agreed that this synaptically evoked potential of neurones in the central nervous system was analogous to the endplate potential of muscle and the synaptically evoked potential of ganglion cells. However some experimental evidence was interpreted as indicating that, both in ganglion cells and the central nervous system, synaptic excitation could directly evoke the discharge of impulses by a brief early action (called originally "detonator action" [ECCLES 1936] and more recently "transmitter potentiality" [LLOYD and MCINTYRE 1955; HUNT 1955]) in addition to a secondary action via the more prolonged synaptic potential. More precise investigations with intracellular recording (Chapter IV) have shown that this additional postulate is no longer required, because the synaptically evoked depolarization,