THE MAMMALIAN CEREBRAL CORTEX

by

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PREFACE

Of all the biological sciences, perhaps neurophysiology is the most adept at concealing simple concepts behind long names. Moreover, the neurophysiologist frequently needs to employ relatively complex apparatus to obtain his results; I must admit, that in my own work, I have often felt ashamed that so much apparatus should produce so little understanding. The temptation, of course, is to cloak one's thinking in measurements with little relation to hypothesis, to employ ill-defined technical terminology and somehow to side-track the reader with a lengthy description of unessential electronic technique. Perhaps the most eloquent protest against this sort of thing has come from Dr. Walshe (1948). He says, "For too many amongst us, also, the inadequate conception that 'science is measurement' and concerns itself with nothing but the metrical has become a thought-cramping obsession, and the more nearly a scientific paper approximates to a long and bloodless caravan of equations, plodding across the desert pages of some journal between small infrequent oases of words, the more quintessentially scientific it is supposed to be, though not seldom no one can tell—and few are interested to ask—whither in the kingdom of ordered knowledge the caravan is bound."

I have tried therefore to write, for readers who do not operate oscilloscopes, about those aspects of neurophysiology which particularly intrigue me. I have tried to avoid terminology which is particular to neurophysiology alone; where the use of such terminology has seemed essential in the cause of brevity, I have attempted to define the "long words" that are used as precisely as possible. I have intentionally stressed my own opinions and prejudices at the expense of space which might have been devoted to a fuller account of established fact; moreover, I have included some descriptions of unpublished and incomplete work. I have done so in the hope that my interpretations and my unfinished experiments, whether right or wrong, may stimulate further research.

For permission to publish Figure 10, I am indebted to the Editorial Board of the Physiological Society. I would also like to thank Dr. G. B. Frank, Professor J. C. Eccles and Professor F. C. MacIntosh for permission to reproduce Figures 16, 17 and 20.

Those parts of my own work to which this monograph refers were made possible, at first by the British Medical Research Council, and later by the Canadian National Research Council. Both bodies have at all times generously provided me with all that I have needed and I cannot miss this opportunity to thank them for their liberal policy towards my work. Dr. A. S. V. Burgen and Dr. Richard Birks were kind enough to read through the first draft manuscript and made many helpful suggestions. Lastly, I should like to thank the editors of the Physiological Society Monographs for the constructive criticism which led to the final manuscript.

B. DELISLE BURNS

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I

THE GENERAL PROPERTIES OF ISOLATED CEREBRAL CORTEX

A description of the preparation and general properties of neurologically isolated, mammalian, cerebral cortex seems for two reasons a useful introduction to the later chapters of this monograph. First, most of the author's own work has been with this preparation and there will be frequent occasion to refer to it in the later sections. Second, a description of early experiments with isolated cortex will provide opportunity to point out some of those difficulties of design and interpretation which are common to most experiments with the central nervous system; these are difficulties which are not always fully understood by those who are interested, but are not themselves working in this field.

It is probably wise to admit at the outset that less is known about the functions of normal cerebral cortex than any other organ in the body. The great volume of experimental results carefully collected during the last century enables us to make many statements which define the details of cortical reaction to particular forms of excitation. Nevertheless, no one has so far succeeded in constructing from the individual known properties of the cerebral hemispheres an intelligible picture of normal function. The main reason for slow progress in this branch of neurophysiology is undoubtedly the anatomical complexity of connections within the cerebral cortex and its intimate association with the activity of other parts of the nervous system. Beneath one square millimetre of the brain's surface the cortical grey matter contains some 50,000 cell bodies (Thompson, 1899). Moreover, many of these cells in the intact animal are in a state of continual activity.

Only within the last fifteen years have techniques been developed whereby the electrical activity of individual cells can be studied. In general, the extracellular electrical changes which accompany activity are so small that one can only record the response of many cells firing together. What is known of the behaviour of single units has mostly been derived from records of the action of large populations of cells. This type of argument, from the statistical to the particular, requires that the cell populations

studied should be histologically and geometrically homogeneous. For this reason our knowledge of events in peripheral nerve fibre is far greater than it is of nerve processes elsewhere. In a peripheral nerve trunk large numbers of fibres of similar dimensions lie parallel to one another, and side by side. But the body is not everywhere so conveniently arranged. It was not until a few skeletal muscles were discovered in which the muscle fibres ran parallel to one another with the terminals of their motor nerves attached in neat rows at right angles to the lie of the muscle fibres, that end-plate potentials were recorded (Schaefer and Haass, 1939; Eccles and O'Connor, 1939). Even where anatomical arrangement is relatively uniform, the analysis of electrical response can be difficult. The twitch-tension of an unfatigued muscle is a good measure of the number of motor units responding to a motor-nerve volley, but the magnitude of the peak action-potential is not linearly related to the number of active muscle fibres (Bigland, Hutter and Lippold, 1953).

Within the central nervous system, where there is rarely either histological or geometrical homogeneity of cells, our knowledge of function is least. Sherrington's early studies of the central nervous system were necessarily made without the help of electrical recording devices. The activity of motoneurones in the spinal cord was recorded in terms of contractions of the skeletal muscle they innervated. Although the method used was simple in comparison with current electro-physiological techniques, interpretation of results was made difficult by factors of which Sherrington was fully aware. The tetanic tension of a skeletal muscle can change either because of a change in the number of active motor units, or because of a change in the frequency of discharge of an unchanging number of anterior horn cells (Adrian and Bronk, 1929; Brown and Burns, 1949). It was impossible without electrical analysis to decide the relative importance of these two factors where there was an alteration in reflex response. Thus Sherrington and those who worked with him were only able to make statements about the *mean* central excitatory state or inhibitory state of *all* the motoneurones in a given motor nucleus. Changes in the behaviour of any single nerve cell could not be deduced with certainty. Since 1935 attempts have been made to record the activity of small numbers of anterior horn cells within a motonucleus (Brooks and Eccles, 1947, 1948) by the use of a micro-intercellular electrode, but the interpretation is made

difficult and unreliable because of the lack of geometrical homogeneity among neighbouring cells. Records from single axons of cells within the anterior horn have provided some information about the behaviour of individual nerve cells during a reflex response (Granit and Strom, 1951). Far more information is likely to come from the recent technique in which the soma of a motoneurone is pierced with a saline-filled micro-electrode (Brock, Coombs and Eccles, 1952).

Undoubtedly the most irritating feature of the central nervous system from the point of view of the physiologist is the irregular and almost random entanglement of many neurones of dissimilar shape and different function. Even when applied to peripheral sensory nerve twigs or dorsal roots it is hard to arrange that stimulating electrodes do not excite simultaneously many nerve fibres which normally carry different modalities of sensation. When stimulating electrodes are applied to the central nervous system they must always excite together many cells belonging to different functional groups which never normally fire simultaneously. It is possible to excite individual cells by the use of micro-stimulating electrodes but, unfortunately, to imitate normal function it appears that many separate cells of one functional group must be excited before excitation will spread along its normal routes within the central nervous system. Phillips has recently made an attempt to find out how many Betz cells in the motor cortex of the cat must be excited before a just visible movement of one of its limbs takes place (Phillips, 1956*b*). He first arranged the strength of a 10 msec. stimulus given through a surface electrode so that it would just produce movement; then he explored among the underlying pyramidal cells with an intracellular recording electrode in order to estimate the necessary spread of this threshold activity. His results show that movement will only occur provided that Betz cells, 4 mm distant in all directions from the stimulating electrode, fire repetitively. Thus the area of cortex which includes all Betz cells firing in a threshold movement, both those which fire repeatedly and those which fire a single impulse, is probably greater than a circle of 4 mm radius. A circle of brain cortex of 4 mm radius would contain roughly two and a half million cells and the majority of these would not be Betz cells; it becomes obvious that the common habit of exciting cortex by superficially applied electrodes carries little chance of closely imitating physiological events. The same dense and heterogeneous population of cells makes the

interpretation of records of cortical activity obtained with surface electrodes one of extreme difficulty. Micro-electrodes can give detailed information about the distribution in time of events for one cell, but tell us nothing of the spread of excitation in space throughout a population of cells.

These technical difficulties of experimental design and of interpretation are not restricted to studies of the cerebral cortex. It may be said in general, that the neurophysiologist is usually forced to divine the behaviour of individual cells from records of the more or less synchronous discharge of many thousands of units. To excite these units he must use methods of artificial stimulation which seldom emulate the asynchronous and selective forms of excitation that occur naturally. Moreover, he must usually restrict or abolish the continual activity of neurones which is found everywhere within the intact nervous system before controlled responses of any sort can be recorded from the system of nerve cells to be investigated.

There are, on the other hand, two aspects of the anatomy of the cerebral cortex which make experiment with this organ somewhat easier than with many other parts of the central nervous system. First, its neurones appear histologically to be grouped in laminar fashion so that the structural organization of radial sections seems identical for great distances around any chosen point; this fact makes study of the tangential spread of excitation across the cortex comparatively simple. Were the nerve cells grouped into small nuclei of varying connections and function, the interpretation of spreading responses would be far more difficult. Second, the essential arteries and veins serving the cortex lie upon the surface of the brain; this makes it possible to sever all connection of an area of grey matter with the more central parts of the nervous system without cutting off the blood supply of such an isolated region.

Most attempts to isolate living, cerebral cortex from the rest of the nervous system have been undertaken with the intention of proving or disproving the spontaneous activity of cortical neurones. For various reasons, which are discussed at some length in Chapter III, this direct approach to the problem of spontaneous discharge does not produce results which are easy to interpret. Nevertheless, all authors are agreed that, as the connections of the cerebral cortex with the remainder of the brain are progressively severed, there is a progressive attenuation of electrical activity. Thus, Bremer (1935*a* and *b*) found that separation of the whole

brain from the more caudal part of the nervous system produced an electro-corticogram similar to that of sleeping animals. Undercutting of an area of cerebral cortex, without separation from neighbouring cortex, also produces a reduction of electrical activity within the partially isolated area (Bremer, 1938; Dusser de Barenne and McCulloch, 1941; Obrador, 1943). These results made it seem probable that complete neurological isolation of an area of cortex might so reduce the usual "spontaneous" activity as to make possible relatively accurate measurements of responses to controlled stimuli. Consequently, the author undertook some preliminary experiments with "slabs" of neurologically isolated cortex in cats anaesthetized with chloralose (Burns, 1949, 1950). The technique of isolating an area of cerebral cortex without significant interference with its blood supply was also developed independently by Kristiansen and Courtois (1949).

The most convenient preparations of isolated cortex are those cut from isolated brain (Bremer, 1935*a*), since in such preparations no anaesthetic need be used. While the animal is maintained on ether, the parietal skull is clipped away and the dura mater is removed so as to expose the brain. The brain-stem is then transected at the level of the tentorium cerebelli without damage to the vertebral arteries and the cat is taken off ether. The result is a decerebrate animal displaying the usual decerebrate rigidity and spinal reflex responses; the only surviving connections of that part of the brain, rostral to the cut, are the first and second cranial nerves. The remainder of the animal serves merely as a convenient heart-lung system for perfusion of the isolated brain. The most easily accessible part of the cortex for isolation is in the parietal region. We have usually cut slabs from the crest of the suprasylvian gyrus; this gyrus is commonly straight enough in the cat to provide a rectangular slab of isolated cortex about 20 mm long, 5 mm wide and 4 mm deep. It is not difficult to isolate a larger area of cerebral cortex containing within its boundaries one or two sulci; but the inclusion of sulci makes the interpretation of results extremely difficult. This fact is frequently overlooked and therefore, perhaps, the point is worth labouring. All responses of cortical neurones produce a gradient of potential across the thickness of the grey matter, a potential gradient along a line which is radial to the general curvature of the brain's surface. Imagine that such a response is surface-positive when it lies immediately beneath a superficial recording electrode (Fig.1*a*). Should this response

travel across the cortex and dip into a nearby sulcus, it will subsequently cause relative negativity at the recording electrode, since the negative end of this "dipole" will become closer to the recording point than will its positive extremity (Fig.1*b*). When the response has invaded the depths of the sulcus and begins to climb the opposite wall (Fig.1*c*), the recording electrode will again become positive to the remote reference electrode. Fig.1 illustrates how a simple monophasic response, travelling across a sulcus, can

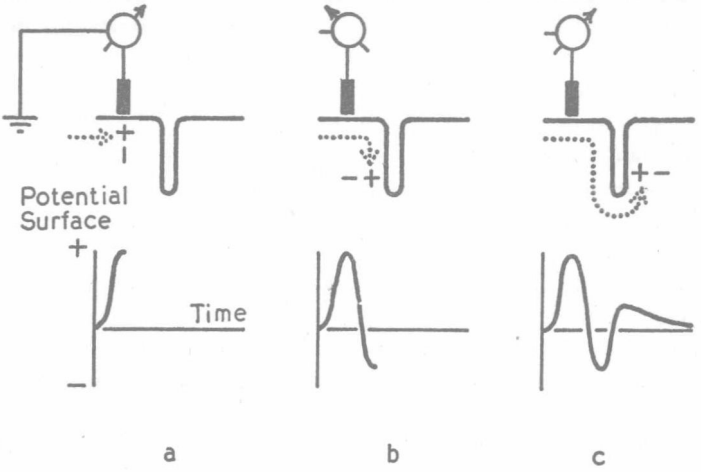

FIG. 1. Illustrating the way in which a surface electrode may record a triphasic response when a simple monophasic wave traverses a sulcus of the cerebral cortex.

The upper row of diagrams shows the progress (*a*, *b* and *c*) of a dipole (+ −) across the brain's surface. The lower row of diagrams illustrates the progressive development of the potential changes recorded from the surface electrode.

cause a triphasic record from a surface electrode; the interpretation of records becomes far easier if responses are prevented from invading sulci by the exclusion of sulci from the isolated area.

In order to isolate a simple rectangular slab of cortex, a knife made from a strip of broken razor-blade, about 20 × 3 mm, is pushed along beneath the crest of the suprasylvian gyrus, some 4 mm below the brain's surface. This procedure undercuts the supra-sylvian cortex. Next a steel wire bent to a right-angle 4 mm from its free tip is inserted into the slit created by the razor-blade. The wire is then rotated so as to bring the tip into view beneath the pia arachnoid and superficial vessels. The only essential in the construction of this wire knife is that the tip be rounded and polished. This makes it possible to work the tip, continually in

view of the operator, around the borders of the cortical area to be isolated, without damage to the overlying blood vessels. The bent wire thus defines the borders of an approximately rectangular slab of neurologically isolated cortex. In fact, subsequent histological examination of such preparations shows that they are invariably isolated, with the exception of occasional strands of nerve tissue in layer I. There is, however, no evidence that these remnants are physiologically active and all tests have demonstrated a perfect functional isolation.

The description given above and the results described below have all been published in detail elsewhere (Burns, 1950; Burns, 1951; Burns and Grafstein, 1952) and no further reference will be made to the original reports.

Cortex isolated in cats under chloralose anaesthesia shows no sign of spontaneous activity. No significant potential changes can be recorded between surface electrodes resting two or three millimetres apart within the isolated area; nor can any spontaneous activity be recorded from individual cell bodies with a deep microelectrode. The isolated cells only discharge in response to artificial stimuli. These statements summarize the author's experience only. Kristiansen and Courtois, for instance (1949), recorded from isolated, anaesthetized cortex a rhythmic activity of low voltage, resembling the normal alpha-rhythm. It is impossible at the moment to say whether these divergent findings are due to differences of technique or of interpretation; since we are here mainly concerned with the responses of isolated cortex to controlled stimuli, it is convenient to postpone further discussion of spontaneous activity to Chapter III. In the author's hands isolated and unanaesthetized cortex is also inactive when no stimuli are provided. Some bursts of spontaneous activity may occur within the isolated area for an hour or two after completion of the operation, but these almost invariably die out to leave the isolated cells in a condition of complete rest. In occasional preparations (about 1 in 10) these spontaneous bursts of activity do not cease; they can be shown to originate from some fixed point within the isolated area and we have always assumed that the focus was caused by accidental local injury.

In the first experiments with isolated cortex it was clearly important to assess the healthiness of the isolated tissue. In this connection it was encouraging that a single weak stimulus delivered to the surface of the brain, within the isolated area, would

elicit the surface negative response first described by Adrian (1936) for intact cortex. Like intact cortex, isolated cortex responds to a series of strong stimuli at 20/sec. with an epileptiform after-discharge (Adrian, 1936). Moreover, spreading depression (Leao, 1944) can be produced in both intact and isolated cortex and spreads across both with approximately the same velocity (Grafstein, 1956). Such experiments with electrical stimulation certainly demonstrate that the isolated area contains living nerve cells, but it seemed that such tests as these might be too crude to demonstrate damage resulting from operation. We have already pointed out that an electrical stimulus applied through surface electrodes must excite many structures simultaneously which, under normal circumstances, never fire together. The fact that some sort of a response to artificial stimulation was obtained from the isolated cells did not necessarily prove that the procedure of isolation had not disturbed their more subtle physiological activities; the absence of spontaneous activity might itself be a sign of injury. As a result of these considerations an attempt was made to drive the cells of an incompletely isolated area of cerebral cortex by activity conducted across a small bridge of cortex, connecting the "isolated" area with intact brain. It was felt that the nature of any excitation which might cross a connecting bridge of this sort would be very similar to that available to the cells of intact cortex. The results of these experiments with partially isolated cortex made it quite clear that the "isolated" neurones were capable of responding to both artificial and physiological stimuli. It was found that the spontaneous activity of intact cortex would spread across a bridge of tissue and drive cells within an isolated area provided that the "entrance gate" was wider than roughly 3 mm. Moreover, a slab of "isolated" cortex (only partially undercut), connected with the remainder of the nervous system only through a strand of centrally running fibres, demonstrated spontaneous activity until isolation was completed.

Responses of isolated cortex to single stimuli

Isolated cerebral cortex appears to give only two types of response to single stimuli of the cortical surface. Surprisingly enough, these responses are as constant and predictable as are those of an isolated nerve-muscle preparation. A weak stimulus of duration 0·5–1·0 msec, given between two electrodes about 0·5 mm apart,

produces the surface negative response first described by Adrian (1936). This response is short-lasting and does not spread far from the excited point; during the response the surface of the cortex beneath, and immediately around the point of stimulation, becomes electronegative to inactive cortex. With progressive increase of the strength of test stimuli the surface negative response grows in magnitude until suddenly an entirely different type of response appears. This is a prolonged burst of neural activity which spreads outward from the stimulating electrodes to invade all parts of the isolated area of cortex; parts of the cortical surface above this activity become electropositive to inactive tissue. This spreading response to a single stimulus has been called the surface positive burst response of isolated cortex. Unlike the surface negative response, it is not seen in intact cortical tissue.

The surface negative response

Usually, in unanaesthetized preparations the surface negative and burst responses are seen together. But the surface negative response is quite unaffected by the presence of anaesthetics, while the burst response (see p. 10) is absent in the anaesthetized preparation. It is convenient, therefore, to describe the properties of negative response as seen in the fully anaesthetized cat.

In practice, the surface negative response may be demonstrated by arranging a pair of stimulating electrodes so that their tips lie 0·5–1·0 mm apart on the brain's surface; the stimuli used need not exceed 1 msec. in duration. (Because the surface negative response can only be recorded relatively close to the stimulating electrodes, the two recording electrodes should lie upon a line at right-angles to the line joining the stimulating electrodes and the stimulus should be "push-pull" or "floating" with respect to ground. These precautions reduce stimulus artefact to a minimum.) In order to obtain a monopolar record of the response the "live" recording electrode can be placed 3–4 mm from the stimulating electrodes, while the reference electrode should rest upon killed brain some 20 mm distant.

The relationship between stimulus strength and magnitude of the surface negative response is shown in Fig.2. It will be seen that as the strength of test stimuli is raised, there is a progressive increase of the response up to a maximal value; a just maximal stimulus is usually reached with a strength some five times threshold. Further increase of the stimulus strength produces no