NEURONAL INTERACTION

IN THE EPILEPTIFORM AFTERDISCHARGE

A Thesis Presented to

The University of Manitoba

In Partial Fulfilment of

the Requirements for the Degree of

Master of Science

by

John Richard Brock

1967

ABSTRACT

The work reported in this thesis represents part of a research program aimed at elucidating the mechanism of the epileptiform afterdischarge. The experimental preparation used was the neuronally isolated slab of cat's cerebral cortex. Stabilisation was by means of a rigidly mounted silver trough arranged so as to wholly contain the slab below the intact pia; surface stabilisation was by a matching lid which also carried stimulating and recording electrodes. Recording from single cells was by extracellular microelectrodes arranged so that recordings from two separate points could be made simultaneously. Intracellular recordings were also made on some occasions.

Epileptiform afterdischarges were induced in the slabs by repetitive electrical stimulation at the pial surface. The afterdischarge activity was recorded by the microelectrodes as typically a series of repetitive slow negative potential 'bursts' each with a superimposed train of single cell spikes. Photographic records of large numbers of these burst-spike episodes were examined from both acutely and chronically isolated slabs. Measurements were made from large numbers of individual burst-spike episodes to determine the values of certain characterising parameters. By investigating the dependences of these on time, distance, and other relevant factors, a number of qualitative and quantitative conclusions are drawn from the data obtained in this study.

The individual burst is identified as a gross integration at any moment of all cellularly developed potentials of all forms over a wide region. The associated spikes represent activity in excitable cells immediately adjacent to the microelectrode tip. The probability of spike

firing is the same for all excitable cells affected simultaneously by a burst.

It appears that the effect of stimulation is the establishment of a shell of activity, ideally spherical with the stimulated point as its centre, in which activity is maintained by continuous recirculation between the shell itself and the central region. The shell thus behaves as a focus, and from it at each recirculation a burst spreads out into the remainder of the slab as a continuous wave of activity which may or may not remain coherent. In the acute slab all activity ceases when the focal shell becomes exhausted; but in the chronic slab, perhaps because of looser interneuronal coupling resulting from degeneration, burst activity becomes independent of any single focus and may continue to reverberate for many hours.

It is recommended that further data should be obtained to supplement those already acquired; and it is suggested that profitable extensions of the work might be made along the lines already established, that complementary studies should be made of the intact cortex, and that relevant histological investigations should be undertaken. It is also stressed throughout that there is much need and scope for a parallel theoretical development, and some attempt is made to provide an elementary basis for this.

ACKNOWLEDGMENTS

I wish to record my thanks to the following; I am glad that in retaining for the future a copy of this thesis I shall retain also these names:

For overall supervision:

Dr. Carl Pinsky

For typing:

Mrs. Sylvia White

For technical assistance:

Mr. Nick Diakiw

Mr. Zenon Gorchynski

Mr. Rome Innes

Mr. Kevin MacLaughlin

Miss Yetta Shatkin

Mr. Harald Strom

And to all the above for advice and encouragement expressed in many ways and forms.

J.R.B.

CONTENTS

TABLE OF FIGURES AND PLATES

TABLE OF SYMBOLS

X, Y, Z	Bipolar surface stimulating electrodes in lid.
A, B, C, D	Surface recording electrodes in lid.
V_S	The surface recording channel; or the potential recorded by it.
V_1	The d.c. microelectrode recording channel in parallel with V_F; or the potential recorded by it.
V_2	The d.c. microelectrode recording channel not in parallel with V_F; or the potential recorded by it.
V_F	The high gain high-pass filtered recording channel in parallel with V_1; or the potential recorded by it.
(x_0, y_0, z_0)	Reference coordinates: vernier readings when microelectrode tip is at corner of slab.
(s_p, y_p, z_p)	Probe coordinates: vernier readings before commencing probing by oil drive.
r_1	Horizontal distance anteriorly from stimulated point to tip of microelectrode recording V_1.
r_2	Horizontal distance anteriorly from stimulated point to tip of microelectrode recording V_2.
r_F	Whichever of r_1 or r_2 has the greater scalar value.
r_N	Whichever of r_1 or r_2 has the lesser scalar value.
r	Used for both r_1 and r_2 when distinction is unnecessary.
(r, θ)	Polar coordinates with pole at stimulated point and initial line extending anteriorly.
$\|r\|$	The scalar (absolute) value of r; similarly for r_F, r_N, and Δr.
$\bar{r}$	The scalar mean of r_1 and r_2; given the sign of r_F.
Δr	The scalar difference of r_1 and r_2; given the sign of r_F.
d	The burst-spike delay; measured from beginning of burst to first associated spike.
$\bar{d}$	The distribution mean of d, either overall or within a specified interval of r.

V_{MAX1}	The maximum negative amplitude of V_1 during an epileptiform burst, measured from the local inter-burst potential.
V_{MAX2}	The maximum negative amplitude of V_2 during an epileptiform burst, measured from the local inter-burst potential.
V_{MAX}	Used for both V_{MAX1} and V_{MAX2} when distinction is unnecessary.
$\bar{V}_{MAX}$	The distribution mean of V_{MAX} within a specified interval of r.
δ	The burst burst-delay; measured from the beginning of the burst recorded at r_N to the beginning of the burst recorded at r_F.
$\bar{\delta}$	The distribution mean of δ , either overall or within a specified interval of r.
Δf	Fraction of d values in interval d, $d + \Delta d$.
N	Number of observations in a specified group of data.
σ	Standard deviation.
σ_N	Standard error of the mean.

I. INTRODUCTION

The present work was undertaken in the Department of Pharmacology and Therapeutics of the University of Manitoba, mainly during the academic year 1964-1965. As part of a research program aimed principally at elucidating the fundamental mechanisms of the epileptiform afterdischarge, it was suggested that it would be of interest to determine what, if any, is the relationship between epileptiform burst-spike delay and the distance of the point of recording from the point of the initiating stimulation. It should be noted now that throughout this thesis the word 'spike' is used to denote pulsatile potential changes of the order of one millisecond duration, presumably to be identified with classical action potentials; and the word 'burst' is used to denote those slower changes in potential, of the order of some tens of milliseconds duration, upon which, in microelectrode recordings from the vicinity of a single neurone, the spikes are apparently superimposed. Such bursts evidently correspond to similar potential changes which may be recorded from the cortical surface, the electroencephalographer's 'spikes'.

The most immediate problem which arises is that the mode of generation of the burst itself has never been clearly defined. The 'burst-spike delay', of course, is the time delay, usually a few milliseconds, between the commencement of the burst and the appearance of the first spike. Since the burst and spikes are apparently in some respect interdependent, it was hoped that, by studying this parameter and identifying the factors upon which it may depend, some progress might be made towards determining the mode of generation of the burst.

It was initially hoped to record these epileptiform events intracellularly; but this was for the most part not achieved. However, extracellular recordings proved in most respects quite adequate, and in

terms of results considerably more of interest was found than had been initially anticipated. A number of parameters in addition to the burst-spike delay were analysed, and it is evident that there are still others which might also usefully be studied. Unfortunately, these potentialities did not become apparent until the experimental acquisition of data was practically completed; and some of the data are not well balanced for statistical analysis of parameters other than the burst-spike delay. Nevertheless, much useful information has emerged, and the indications for further study, particularly if a computer can be used for analysis as is hoped, are extensive. The potential application of this progress to the clinical problem of epilepsy is self-evident; and it seems by no means improbable that some further concept of the workings of the normal cortex may also be forthcoming.

II. REVIEW OF PREVIOUS WORK

1. INTRODUCTION

In a recent review of research into epilepsy, Ajmone-Marsan and Abraham (1963) have estimated that the current rate of publication of papers on this topic is equivalent to one every day; and they have suggested that this rate is really a manifestation of continued frustration in attempting to discover the basic epileptogenic mechanism. The present review is not so much historical as an attempt to outline the current state of knowledge, especially in so far as it pertains to the work recorded in this thesis; no particular attempt has been made to present material in chronological order of publication, for it was felt that in view of the immense volume of published work available, and the consequent unequal development of different aspects of the subject, to do so would inevitably result in loss of clarity. For the same reasons, the published work herein referred to represents what it is hoped may be an adequate sample of the whole, but certainly no attempt has been made to be comprehensive. Furthermore, a comprehensive historical review covering much of the same material has recently been written in this department by Reiffenstein (1964).

Experimental epileptic seizures may be produced (Ajmone-Marsan and Abraham, 1963) by direct stimulation of the cerebral cortex by means of electric current, local freezing, or locally applied drugs (such as strychnine, eserine, alumina, or penicillin), or by systemically applied drugs (convulsants). Evidently all of these means but the latter may be expected to produce that type of seizure known as 'focal'. The designation 'idiopathic', applied to clinical epileptic cases in which no origin can be found for the condition other than presumed genetic, is becoming progressively less applicable as it is realised that a wide range of aetiological

factors, biochemical and metabolic as well as physical, may contribute to the establishment of functional epileptic foci. Ajmone-Marsan and Abraham (1963) have suggested that research into seizure mechanisms may be divided into two categories, firstly studies of intrinsic local factors such as the relation of slow electrical potential changes in the cerebral cortex to the activity of single cortical neurones, and secondly studies of indirect factors such as the influence of other parts of the central nervous system. The present work falls clearly into the first of these two categories.

The particular manifestation of convulsive activity which is studied in the present work is the electrically recorded response of the cerebral cortex to repetitive electrical stimulation, the epileptiform afterdischarge. The basic characteristics and possible modes of generation of this response have been extensively studied by Burns (1958). Burns (1949, 1950) and, independently, Kristiansen and Courtois (1949) have described a method for the neuronal isolation of a slab of cerebral cortex while still retaining its nutritive blood supply intact. This method, a slight modification of which has been used in the present work, allows study of intrinsic cerebral cortical mechanisms without the confusion of effects caused by interaction between the cortex and lower centres. The epileptiform afterdischarges which may be elicited in such a slab shortly following its isolation appear virtually identical to those which may be elicited in normal intact cortex. However, much interest has centred on the finding that, in slabs which have been isolated for several weeks or months, afterdischarges once initiated may last for many minutes or even hours, instead of the 'normal' duration which is only a matter of seconds. Grafstein and Sastry (1957) and Sharpless and Halpern (1962) have followed the progress of this change from 'acute' to 'chronic' response, the latter by means of permanently implated electrodes. Spontaneous electrical

activity may be recorded from the chronic slab as little as two weeks after its isolation, as compared with the acute slab which is generally considered to be electrically silent (Burns, 1958). The slab develops decreased threshold for initiation of epileptiform afterdischarges, and the mean afterdischarge duration increases, over roughly the same period. A number of investigators (Echlin, 1959; Sharpless and Halpern, 1962; and Reiffenstein, 1964) have suggested that these characteristics of chronic isolation may be due to the development of denervation supersensitivity to chemical mediators; Sharpless and Halpern pointed out that the time taken for their appearance is approximately the same as that required for the development of denervation supersensitivity in peripheral structures. Clinical epilepsy might thus be expected to result from functional denervation of some part of the brain due to growth of scar tissue or a tumour.

An important byproduct of work on such evoked responses as the epileptiform afterdischarge is knowledge which may be acquired concerning the structure and function of the normal brain. Indeed, such knowledge must ultimately be of far more significance than that concerned specifically with the origin and treatment of epilepsy; and in fact it seems doubtful whether the latter can ever be fully comprehensive in the absence of the former. It has been frequently pointed out (e.g., Burns, 1958) that procedures such as direct electrical stimulation of the cortex must cause many cells to fire together which would never do so in the course of normal function; nevertheless, from any response it should be possible to draw at least some conclusions regarding the structure producing it, and this should be so even if the structure itself has been interfered with, providing that the nature of the interference is known. However, most published descriptions of experimental investigations of cortical physiology do not concern themselves more than incidentally with the implications of their work in this direction.

There seems, in fact, to be a fairly wide dichotomy between publications dealing primarily with specific investigations of the various evoked responses of the cortex and those dealing with the properties of the cortex as a whole. The former are largely concerned in their interpretations with effects that might be expected to arise at the level of the single cell, or as a result of interaction between large numbers of cells while still reflecting the properties of the single cell; in short, their interpretations are largely within the bounds of classical neurophysiology. Papers of the latter type, on the other hand, are often not written by physiologists at all, but by physical scientists who see in the immense number of interconnecting neurones which comprise the cortex a system which should in some way be amenable to statistical analysis. The remainder of this review is occupied for the most part in summarising knowledge which has been acquired by investigators using one or other of these two separate approaches.

Attempts to integrate these two approaches have been rather few and far between. One of the most notable experimental contributions has been the histological work of Sholl (1956); and a good statement of some of the problems involved, both the direct problems and the underlying 'mind-brain' problem, has been provided by Eccles (1953); these contributions also will be further discussed in the pages that follow. If the present work can lay any real claim to originality, it is perhaps chiefly that, while the initial intention was to remain well within the bounds of the 'single cell' approach, some of the conclusions finally drawn suggest that the 'statistical' approach, and, more important, the truly integrated approach, may also usefully be brought within the bounds of experimental physiology.

2. THE STRUCTURAL BASIS FOR CORTICAL ACTIVITY

The histology of the cerebral cortex has been widely studied, and many of the anatomical features of 'typical' individual neurones are well known. Sholl (1956) has suggested that there are really only two basic neurone types in the cortex, stellate cells and pyramidal cells. The former are most concentrated in regions concerned with the reception of impulses from sensory receptors; and it is the latter which have been most generally implicated in the genesis of evoked responses, including the epileptiform afterdischarge. The most prominent pyramidal cells have large somata in layer V from which their axons and basal dendrites project towards the underlying white matter, and their apical dendrites as branching trees towards the cortical surface.

The so-called 'neurone doctrine', the fundamental thesis of which is that the neurone is the basic structural unit of the nervous system, has, as Bullock (1959) has pointed out, come to be understood as implying that the neurone is also the basic functional unit. The time has come, Bullock suggests, when this implication must be to some extent revised. Thus there is much evidence that the axonal membrane, including probably the membrane of the axon hillock, is specialised in a manner not shared by the somatic and dendritic membranes, and that only in the axonal membrane can a regenerative action potential be established (see also Clare and Bishop, 1955; Eccles, 1957; and Grundfest, 1958). Conduction in the soma and dendrites must therefore be decremental, and this means that labile and integrative processes are not restricted to synapses as would be the case if a possible outcome of all synaptic activity were the immediate establishment in the vicinity of the synapse of a regenerative action potential.

Considerable interest has centred particularly on the role of the apical dendrites both in normal cortical function and in the genesis of

artificially evoked responses. A large area of the membrane of the apical dendritic tree is occupied by the receptor sites of axo-dendritic synapses; indeed, it has been suggested that this may be one reason why the dendritic membrane differs in behaviour from the axonal membrane (Eccles, 1957, 1964). Afferent activity in the axons may thus be expected to establish transient potential differences across the dendritic membrane at one or many sites. The manner in which such potential differences might be expected to spread by decremental conduction throughout the branching tree and to the soma has been worked out mathematically by Rall (1962, 1964), and, using Rall's calculations as a basis, has been studied in a model by Sances and Larson (1965). The basic conclusion is that transient potential differences established in the dendrites, presumably more or less pulsatile in the immediate vicinity of their synaptic origins, will become diffuse and attenuated in the course of conduction to the soma, and will combine with similar potential differences from throughout the dendritic tree to produce only slowly varying modifications of the somatic membrane potential.

Experimentally, this same general conclusion has been reached by a number of investigators. Clare and Bishop (1955) suggested that synaptic activity in the dendritic tree may result in either facilitation or inhibition, depending on the induced polarity, of activity induced in the soma by axo-somatic synapses. Bishop (1958) went on to point out that the output of one neurone is thus influenced by the integrated activity of the many other neurones whose axons form synapses with its soma and dendritic tree; and thus the single neurone is a fundamental unit of cortical integration. Similar conclusions have been reached by Chang (1959), Andersson (1965), and Wall (1965); and the latter two investigators have recorded small spike-like potentials which they have identified as individual excitatory postsynaptic potentials appearing in the dendrites. Gloor *et al.* (1961) have

implicated a similar transmission of activity via dendrites to somata in the genesis of epileptic discharges in the hippocampus. Pinsky (1965) has suggested that decrementally conducted potentials in the dendrites may still be of sufficient amplitude on reaching the soma to themselves initiate action potentials without any need for additional axo-somatic synaptic activity. Fox and O'Brien (1965), with the aid of a computer, have shown that the probability of firing of a single cell following sensory stimulation corresponds closely to the average potential recorded at the same point following the same stimulation but after destruction of the cell which had been firing. They suggest that this average potential, which thus evidently controls the probability of firing, might be a summation of electrotonically conducted somatic and dendritic postsynaptic potentials; though it might also be merely a summation of discharge potentials from other cells in the vicinity.

Investigations of the relationship between single cell activity and the electroencephalogram (EEG) recorded at the cortical surface have produced contradictory results. A number of investigators have reported finding no relationship at all (e.g., Mountcastle et al., 1957). However, Enomoto and Ajmone-Marsan (1959), Verzeano and Negishi (1960), Goldensohn and Purpura (1963), Fromm and Bond (1964), and others have all recorded increased single cell firing during surface positive EEG waves, and vice versa. This suggests that cells fire most readily when their deeper parts are depolarised and their superficial parts polarised, i.e., as might be expected, when their somata are depolarised and their apical dendrites are not. Fromm and Bond suggested that dendritic depolarisation, corresponding to negative EEG waves may actually inhibit cell firing. Brazier (1955) pointed out that the periodicity of EEG waves is of the order that might be expected from dendritic conduction times. Clare and Bishop (1955) and

Schmidt et al. (1959) likewise suggest that the EEG probably results from dendritic activity. Klee et al. (1965) have used cross-correlation analysis to show a close correlation between EEG waves and slow changes in single cell membrane potentials; they suggest that EEG waves reflect integration of membrane potential changes in both dendrites and somata.

A corollary of these concepts centring on decremental dendritic conduction is the possibility that differential depolarisation within a single neurone might result in repetitive firing of that neurone and thus provide a basis for seizure discharges. An action potential induced in the axon hillock region of the soma would be expected to cause antidromic decremental conduction in the dendrites; the resulting dendritic depolarisation would persist after the soma membrane potential had recovered, and under the influence of the dendrites the soma would again be depolarised to generate a further action potential. A mechanism of this type was proposed by Burns (1958), and studies of radial cortical potential gradients have given support to the theory (e.g., Burns, 1958; Pinsky, 1961; and O'Leary and Goldring, 1964). Ward (1961) observed that the same effects would result if the dendrites became permanently depolarised as a result of some abnormality such as deformation by astrocytic gliosis; anatomical evidence is consistent with this possibility. More recently, Atkinson and Ward (1964) have suggested that this depolarisation may extend to the soma as well as the dendrites, with the resulting repetitive activity restricted entirely to the axon. A somewhat different possibility, while still retaining the same capacity for repetitive discharge within a single neurone, is that axonal collaterals may form synapses with the dendritic tree of their own neurone; decremental conduction in the dendrites would provide the time delay necessary for recovery of the axonal membrane following each circulation of activity (Chang, 1959; Beritoff, 1965).