THE PHYSIOLOGY OF THE PAROXYSMAL AFTERDISCHARGE

by Carl Pinsky

A thesis presented to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Master of Science.

Department of Physiology,
McGill University,
Montreal.

August, 1957.

ACKNOWLEDGMENTS

I wish to express my deepest thanks to Prof. B.D. Burns, who directed the research done in this study, and whose invaluable help has been unstintingly given. His keen insight and enlightening critisism have been most stimulating. It has been a privilege to work under his guidance.

I am deeply grateful to Prof. F.C. MacIntosh who helped to make it possible for me to do graduate work in his department.

My sincere thanks go also to Prof. P. Sekelj, under whose watchful care I became familiar with the intricacies of electronic equipment.

Mrs. J. Malin gave unfailing help with the biological preparations, and with the photography. Mr. G. Mandl was efficient in caring for the electronic equipment.

The cooperation of Miss R. Forrer and Mr. D. Cameron in preparing the photographs is greatly appreciated. Dr. W. Black, of the Department of Zoology, provided useful information.

Mr. G. K. Smith deserves sincere thanks for the generous way in which he helped prepare the illustrations. Thanks are due also to Dr. G. Salmoiraghi whose discussions and advice have proved most valuable, and to Dr. G.B. Frank for helpful discussion.

To my wife, who typed this thesis, I am sincerely grateful.

This work was supported by a grant to Prof. B. D. Burns from the United Cerebral Palsy Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

THE PHYSIOLOGY OF THE PAROXYSMAL AFTERDISCHARGE

INTRODUCTION

Since biblical times, physicians have been challenged by the difficulties of finding the proper treatment for the disease of epilepsy. That challenge has not gone unanswered at any time in the recorded history of medical science. The quest for the solution of this problem is being carried out vigorously today on many fronts. The clinician and the research worker are helping each other to understand the nature of the disease, and to provide a rationale for its causes and treatment. Advances in the treatment of all diseases have gone hand in hand with increasing knowledge of fundamental biological phenomena. This study undertakes to investigate a biological

phenomenon known to be associated with epilepsy, namely paroxysmal electrical activity on the cerebral cortex.

The work done in this investigation was designed to serve as a study of certain characteristics of paroxysmal electrical activity on the cortex. Further, experiments were done to ascertain the most pertinent problems to be solved in order to establish the physiological mechanisms whereby a cortical paroxysmal afterdischarge is mediated. It is the hope of the author that this study may serve as a contribution to the work done by others in answer to nature's challenge.

HISTORICAL REVIEW

I. INTRODUCTION.

John Hughlings Jackson was the first to break through the interface between the ancient era of treatment of epilepsy and the modern era of research which continues today. (Jackson, 1870) It was Jackson who first correctly described the convulsions associated with epilepsy as arising from a discharge in the highest centres of the central nervous system. This concept was promptly confirmed by the experiments done by Fritsch and Hitzig, (1870). To these latter two workers belongs the distinction of being the first to stimulate the cortex electrically. This technique, used by numerous workers over the past eight decades, has made possible the precise mapping of the sensory and motor functions of the cerebral cortex. It has helped the neurosurgeon to safely excise injured brain tissue while leaving important cerebral tissue intact, and has enabled the neurophysiologist to produce paroxysmal electrical activity on the cortex, in order to investigate that phenomenon under controlled conditions. (Penfield and Rasmussen, 1950)

It has been known since 1875 that electrical activity exists in the brain. Caton (1875) recorded this activity, using a capillary electrometer. In 1924 Berger used a string galvanometer to record the first electroencephalogram from the human brain. His work was first published in 1929. (Berger, 1929) In subsequent work he clearly established that an epileptic seizure is accompanied by a paroxysmal electrical discharge in the brain. This led other workers to use the paroxysmal afterdischarge as an index of the effects of epileptogenic agents. (Adrian and Matthews, 1934)

II. AFTERDISCHARGES.

The first use of the term "afterdischarge" appears in the work of Sherrington. In his book "The Integrative Action of the Central Nervous System" (Sherrington, 1906) he uses the term to describe the reflex contraction persisting after the stimulus which initiated the reflex had been removed. Since that time studies of similar phenomena have been made on numerous sites in the mammal. Adrian stimulated the cerebral cortex of the anaesthetized rabbit and cat and produced an electrical afterdischarge of cortical neurones which he recognized as being similar to the electrical activity recorded from the cortex during an epileptic seizure. (Adrian, 1936) Burns was able to produce similar afterdischarges within isolated slabs of cat's cortex. (Burns, 1949) Kristiansen and Courtois also reported comparable results from isolated slabs of cortex. (Kristiansen and Courtois, 1949)

The afterdischarge which followed electrical stimulation of the cortex closely resembled the activity present there during an epileptic seizure. This led many workers to employ this means of inducing an experimental epilepsy. The afterdischarge itself became known as the "epileptiform afterdischarge". Attempts were made to relate the pathogenesis of epilepsy and the physiology of the afterdischarge.

Among the first theories put forth to explain the afterdischarge was the concept of reverberating circuits. (Lorente de Nó, 1938) The objections to this theory were considerable, particularly when it was found that the afterdischarge could easily be obtained upon small, neuronally-isolated cortical slabs. As yet, there is no experimental evidence which conclusively proves that reverberatory chains of neurones play any role in

the paroxysmal afterdischarge, and there is much suggestive evidence that it does not. (Penfield and Jasper, 1954)

Other theories presented to explain the epileptiform activity included the theory that a "triad" of cerebral nuclei interacted to produce large cortical potentials. (Lennox and Robinson, 1951) This theory did not take into account the fact that afterdischarges can be produced upon totally isolated slabs of cortex. A theory which does take this into account was proposed by Echlin, Arnett and Zoll.(1952) These workers based their theory on the fact that infrequent bursts of electrical activity can be recorded from both completely and partially isolated slabs of cortex. They proposed that an epileptic seizure was the result of these bursts arising from cortical areas that had become isolated due to neurological or other lesions.

Investigations have been made to establish whether or not epileptogenic lesions in the cortex may be caused by disorders of cerebral circulation. Jasper and Erickson (1941) carried out studies of both blood flow and cortical pH in areas of cortex where paroxysmal afterdischarges had been produced by electrical stimulation and by Metrazol. They found that the afterdischarge was accompanied by vasodilatation and an increased blood flow, but concluded that these effects were secondary to the mechanism which mediates the afterdischarge. Penfield and Jasper (1954) later arrived at identical conclusions.

Various biochemical mechanisms have also been proposed to explain the paroxysmal afterdischarge. It is known that cholinesterase activity is higher in an epileptogenic cortical focus than in normal cerebral cortex.

(Tower and Elliott, 1952) Tower (1956) suggests that this increase of acetylcholine might be the cause of epileptiform activity, either directly, or indirectly by interference with certain enzyme systems. The same author also suggests that the production of acetylcholine in epileptogenic tissue is related to an imbalance of electrolyte metabolism, particularly of the potassium ion.

III. THE THEORY OF DIFFERENTIAL REPOLARIZATION.

The isolated cortical slab proved to be a very useful preparation for investigating the responses peculiar to cortical tissue. (Burns, 1950) In his experiments upon isolated cortex Burns showed that many forms of neural activity produced by electrical stimulation outlasted the stimulation, and that this seemed to be a distinct property of cortical tissue. (Burns, 1950, 1951, 1954) In order to explain this property, Burns proposed his theory of differential repolarization.

In this theory it was postulated that a depolarized type-B neurone would repolarize more quickly at the superficial end than at the somatic end, and that this condition would give rise to afterdischarges. (Burns, 1954) An outline of the theory of differential repolarization, as proposed by Burns, is given in the following.

The mechanism of differential repolarization is dependent upon neurones which do not repolarize uniformly along their length when their membranes recover from depolarization. Thus, should a neurone become depolarized due to driven excitation, one end of the neurone will repolarize before the other end has done so. This will set up a potential gradient between the quickly-repolarizing end and the slowly-repolarizing portion.

The potential gradient will cause current to flow from the recovered portion of the neurone to the still-depolarized region. When the current flow reaches a critical level, the repolarized portion will discharge, due to the catelectrotonus caused by the positive current streaming out from the recovered portion of the neurone. This will give rise to an action potential in much the same fashion, as an action potential in peripheral nerve is propagated. (Brazier, 1951) At the end of the action potential the neurone is once more depolarized, and the cycle is repeated.

In order to test the parameters of the mechanism proposed in the foregoing, Burns designed an electronic analog of a differentially-repolarizing neurone, and constructed several networks composed of these model "neurones". (Burns, 1955) The model neuronal networks gave responses exactly similar to the responses of type-B cells in isolated cortex.

Experimental evidence which substantiated the hypothesized differential repolarization was subsequently reported. Records made inside the Mauthner cells of the catfish showed that certain neurones do indeed repolarize differentially in the manner postulated by Burns. (Tasaki, Hagiwara and Watanabe, 1954) Studies of single neurones in the cortex during a period of epileptiform activity led other workers to postulate a form of differential repolarization as being responsible for the paroxysmal discharge during an epileptic seizure. (Ward, Schmitt and Thomas, 1955)

FORMULATION OF THE PROBLEM

The present study is concerned with determining whether or not the paroxysmal afterdischarge is the result of the differential repolarization of a group or groups of neurones in the cerebral cortex. In order to establish if the phenomenon of differential repolarization is the basis for an afterdischarge it is necessary to examine the properties of other afterdischarges which are believed to be caused by differential repolarization.

It has been conclusively shown that the veratrin afterdischarge in frog's muscle is due to differential repolarization of the muscle cells. (Burns, Frank and Salmoiraghi, 1955; Frank, 1956) Hence there is a known system in which an afterdischarge is due to differential repolarization, and the particular properties of this afterdischarge can be enumerated. Furthermore, from the manner in which differential repolarization is assumed to initiate and maintain an afterdischarge, it is possible to infer the general properties of such an afterdischarge. Since these properties are of paramount importance to this study they will be outlined here.

In a review of the theories proposed to explain various afterdischarges Burns (1957) states that an afterdischarge which is the result of differential repolarization will have the following properties:

(a) An action potential starting from any point and travelling in any direction will initiate the afterdischarge.

(b) The afterdischarge tends to bear an all-or-nothing relationship to the stimulation

which initiates it.

(c) The latent period between the end of the stimulation and the beginning of the afterdischarge is greater than the time interval between the first two action potentials of the afterdischarge.

(d) The maximum frequency of the repetitive firing of the afterdischarge does not occur at the beginning of the afterdischarge but is usually reached after the first few action potentials.

(e) When the afterdischarge comes to an end, its frequency does not decline asymptotically to zero, but after some reduction in the rate of discharge the series of action potentials stops suddenly.

(f) The production of an afterdischarge requires the presence of an adequate gradient of membrane potential along the length of the cell or cells responsible for the afterdischarge.

The investigation of the physiology of the paroxysmal afterdischarge described here has attempted to establish experimentally whether or not the afterdischarge has the properties common to those which are the result of a neurone or system of neurones which repolarizes differentially.

METHODS

I. THE BIOLOGICAL PREPARATION.

(1.) Initial surgery.

Cats weighing between 1.5 and 4.5 kg were used. Most experiments, however, weremade upon cats weighing about 3.2 kg. On some animals the technique of Burns and Grafstein (1952) was used to isolate the forebrain. ("cerveau isolé" of Bremer, 1935) Such animals did not need to be anaesthetized during the experiment. Initial surgery on such preparations was carried out under ethyl ether anaesthesia, induced with ethyl chloride. Where the forebrain was not isolated the anaesthesia was first induced with ethyl chloride and ether. Chloralose was then injected intravenously, the dose given being 80 mgm per kilo body weight.

A tracheal cannula was inserted, and the carotid arteries were exposed and prepared for clamping off by passing loops of thread around them. It was found that the best results were obtained when the carotids were clamped off for as short a time as possible. To this end the carotids were left unclamped until the bleeding in the skull or brain became profound. After the surgery at least one of the carotid clamps was removed.

Following the tracheal cannulation and exposure of the carotids the animal was turned on its ventral side and the head clamped in a Tshermak holder with the top of the skull about 25 cm above the table. This height was adjusted during the operation so that blood would just barely ooze from the sinuses in the skull. This reduced bleeding to a minimum whilst ensuring that air would not be sucked back into the sinuses.

A skin incision was made in the mid-line, the skin reflected, and the left temporal muscle removed. The bone was trephined and cut away. Sagitally, the removal of the skull extended as far forward as the nasal sinuses, and caudally to the upper margin of the tentorium cerebellum. Transversely the opening extended to within 3-2 mm of the mid-line, and included most of the temporal bone. The cut edges of the skull were sealed off with plasticene.

The dura mater was removed and a drainage hole made into the lateral ventricle as described by Burns and Grafstein. (1952)

At the end of the operation 20 cc of saline was injected intraperitoneally. This helped to restore some of the plasma volume lost by bleeding, and animals so treated remained in better condition than those who were not given the saline injection.

(2) Isolation of the fore-brain.

The forebrain was isolated by the technique described by Burns and Grafstein, (1952) with one minor modification. The spatula used to cut the brain-stem was provided with a notch which served to guide the experimenter. Examination of the skulls of several cats led to the design of a spatula which ensured a complete cutting of the nerve-fibres in the brain-stem with less chance of cutting blood vessels.

A photograph of the spatula is shown in Fig. 1a. Fig. 1b shows how the medial projection of the tentorium serves to guide the sectioning. In practise, the experimenter pivots the notch on the projecting bone. The locus of the spatula tip is such that the mid-brain is sectioned while blood-vessels are left intact.

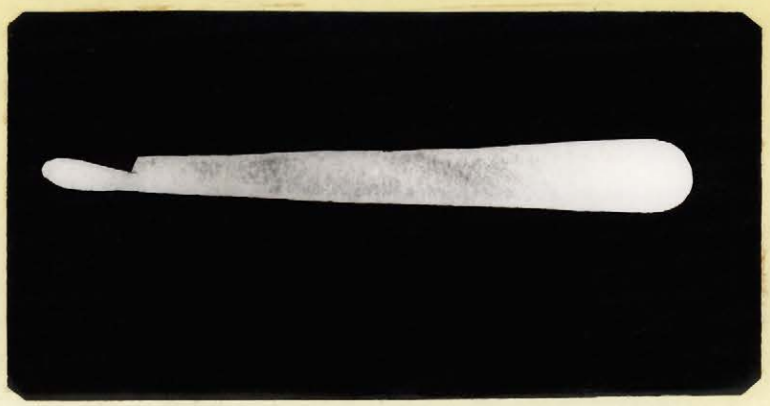


Fig. 1a

1 cm

Spatula used
to isolate forebrain.

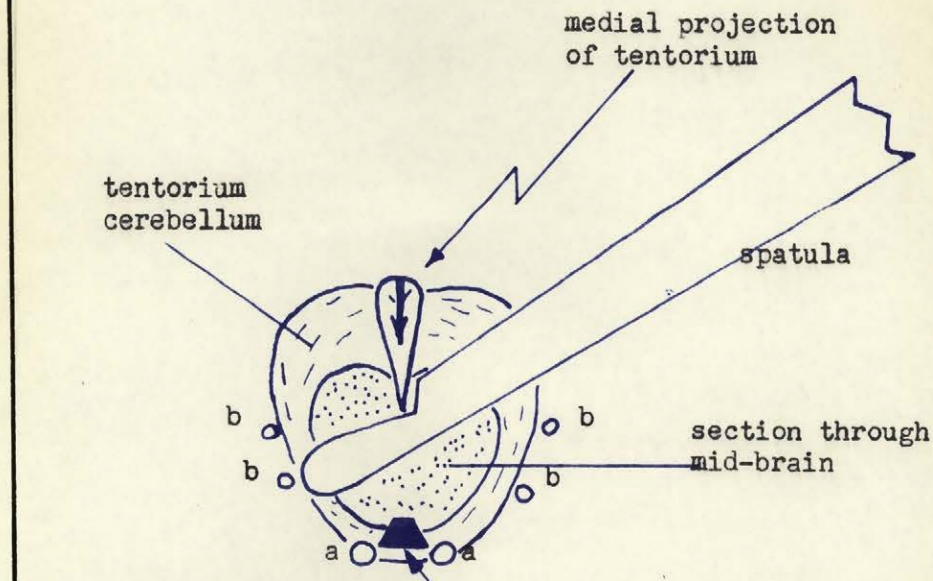


Fig. 1b

(3) Isolated Slabs.

In those experiments where isolated slabs of cortex were prepared, the technique of Burns for undercutting and neurologically isolating the slab was used. (Burns, 1950) All the slabs were made on the suprasylvian gyrus in such a way that no sulci were included. This makes the interpretation of the results much easier. (Burns, 1951) The dimensions of the slab varied with the experiment, and are described in the text.

II. STIMULATION.

Surface stimulation was used in all the experiments. A pair of light platinum electrodes, their ends fused into balls, was placed upon the cortex or slab. The spacing of the electrodes was usually 2 mm. The stimuli were electronically controlled rectangular potential changes, obtained from Tektronix type 161 pulse generators driven by Tektronix type 162 waveform generators. The pulse generators were modified so that the output was controlled by a 10-turn helical potentiometer. (Beckman Helipot type AJ) This enabled the stimulating voltage to be controlled to three significant figures. The number of stimuli was controlled either by the gate circuit of a Tektronix waveform generator, or by a predetermined electronic counter. (Computer Measurement Corporation, Model No. 313A) A low-capacitance isolating transformer (Hammond No. 935) was used to isolate the stimulating pulses from earth, and so reduce the stimulus artefact.

III. RECORDING.

(1) Types of recording.

(i) Bipolar Surface Recording, R.C. Coupled. This type of

recording was used where a record of rapidly-changing potentials on the surface of the cortex was desired. For these measurements platinum electrodes, similar to the ones described for stimulation, were used. Since the spacing of these electrodes was small, the potentials recorded represent the potential gradient at the surface of the cortex measured tangentially to the curvature of the cortex. The potentials recorded in this way may be referred to as "tangential" potential gradients.

(ii) Monopolar Surface Recording. These records were made using non-polarizable silk-wick electrodes. The wicks were imbedded in a gel made of 1% agar in 0.9% NaCl, and projected from the end of a bent tapering glass tube. Connection to the recording system was made by chlorided silver wires dipping in the agar gel. One of the electrodes was placed upon a reference point made on the cortex by killing a small cortical area with electro-cautery. The other electrode is referred to as the "active" electrode and was placed some distance away from the reference point. Potentials measured in this manner are referred to as monopolar potentials.

(iii) Bipolar Depth Recording. Records of potentials at different depths in the cortex were made using microelectrodes as described by Burns. (Burns, 1954) One input terminal of the recording system was connected to the microelectrode by means of a chlorided silver wire dipping into the saline-filled microelectrode. The other input terminal was connected to a silk-wick electrode placed on the surface of the live cortex close to the point where the microelectrode penetrated the surface.

Since such a placement of electrodes records the potential gradient which appears between the surface and some point on the radius of the cortex, the potentials recorded in this way are referred to as "radial potential gradients. It has been shown (Renshaw, Forbes and Morrison, 1940) that potentials recorded in this way are the resultant of the potentials of neurones randomly distributed close to the recording microelectrode. In this study the radial potential gradient is referred to as the "mean" potential of the cortex. This is in keeping with the view that the recorded potential gradient is actually the mean of potentials contributed by neurones in the vicinity of the recording microelectrode.

Since the mean potentials referred to underwent very slow changes in some records, they were recorded direct-coupled.

(iv) Activity of Single Neurones. Records of action potentials from single cells in the cortex were made using saline-filled glass microelectrodes similar to those described by Burns. (1954) The tips of the microelectrodes used for recording unit cell activity were always 10 μ in diameter or less. The action potentials were obtained as a monopolar recording, the reference being a silk-wick electrode in contact with a killed point on the cortex. Connection was made to the amplifiers by means of chlorided-silver wires dipping into the glass electrodes.

Potentials obtained in this manner are referred to as "unit potentials".

(2) Recording Apparatus.

Two channels of amplification were available. Each consisted of an input cathode-follower which fed into a preamplifier driving either

a photographic oscilloscope or a Sanborn two-channel pen-writing recorder. A loudspeaker unit was available which made audible the potentials being recorded.

(3) Presentation of Records.

Wherever applicable an inset diagram has been included with each record or set of records. Where R.C. coupling was used in the amplifier system, a capacitor is drawn in the functional circuit diagram and the effective time-constant is stated in milliseconds. Unless otherwise stated an upward deflection on the record indicates that the active electrode is negative with respect to the reference electrode. In depth measurements an upward deflection signifies that the deep electrode is negative with respect to the surface. Where bipolar surface recording is used an upward deflection signifies that the electrode nearest the stimulating electrodes is negative with respect to the electrode farther away.

All dimensions are in mm unless otherwise stated.

For brevity in labelling some of the graphs and records, the term "afterdischarge" has been abbreviated to the letters "A.D."

RESULTS

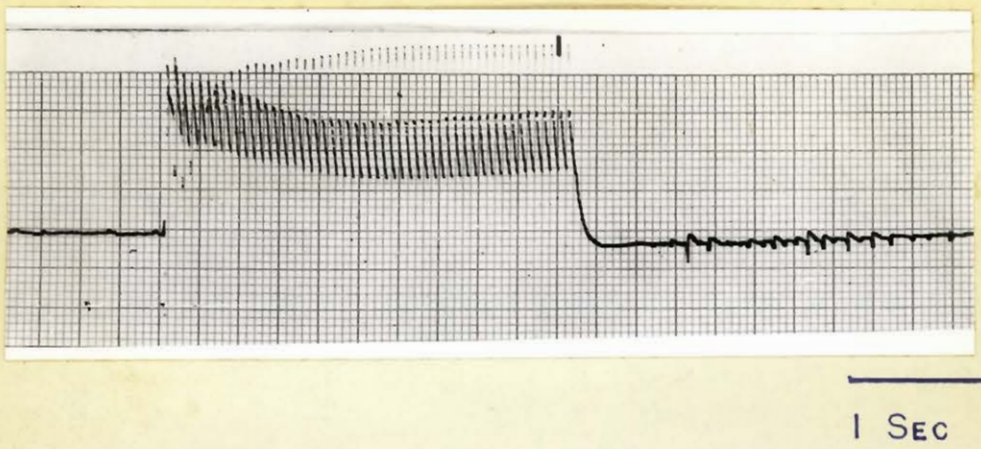
I. THE GROSS FORM OF THE AFTERDISCHARGE.

A general description of the paroxysmal afterdischarge could be given by stating that following a period of repetitive stimulation of the cerebral cortex there is a short interval of inactivity followed by a period of large oscillatory potential discharges of varying form which are repeated in a periodic manner. Although the method and site of recording these repetitive potential discharges will alter the observed waveform to some degree, there are certain patterns which the afterdischarge follows in almost all the records obtained in this study. These patterns can be outlined as follows:

(i) During stimulation there is a change in the mean potential of the cortex. This is seen with all types of recording. Although this change is certainly partly due to a cumulative stimulus artefact, this accounts for only part of the change in mean potential. As can be seen from Fig. 2 reversing the polarity of the stimulus does not necessarily reverse the polarity of the change in mean cortical potential.

(ii) At the end of stimulation there is a latent period in which the mean potential continues to change. This latent period is brought to a halt by the onset of the afterdischarge. The average latent period between the end of stimulation and the beginning of the afterdischarge was >200 milliseconds.

(iii) The afterdischarge begins with a series of repetitive



(a)

2.0 mV

1 SEC

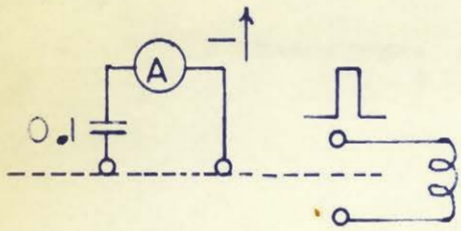
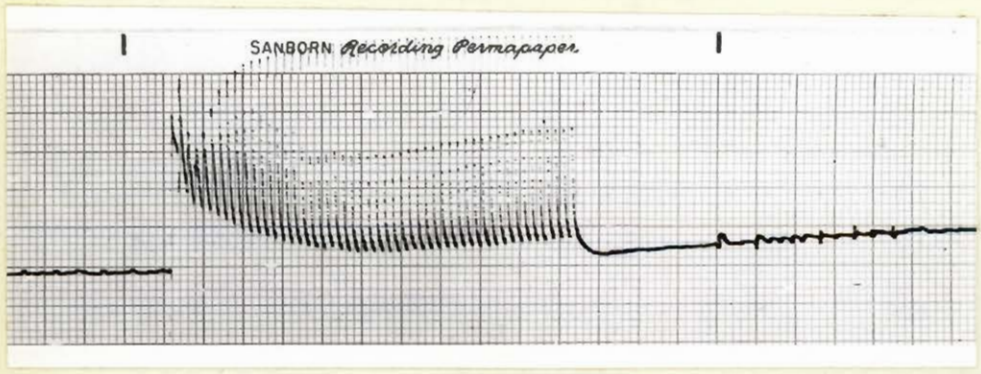


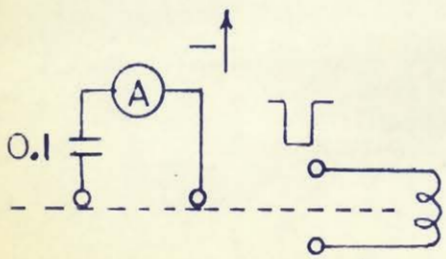
Fig. 2 change in mean potential of cortex



(b)

2.0 mV

1 SEC

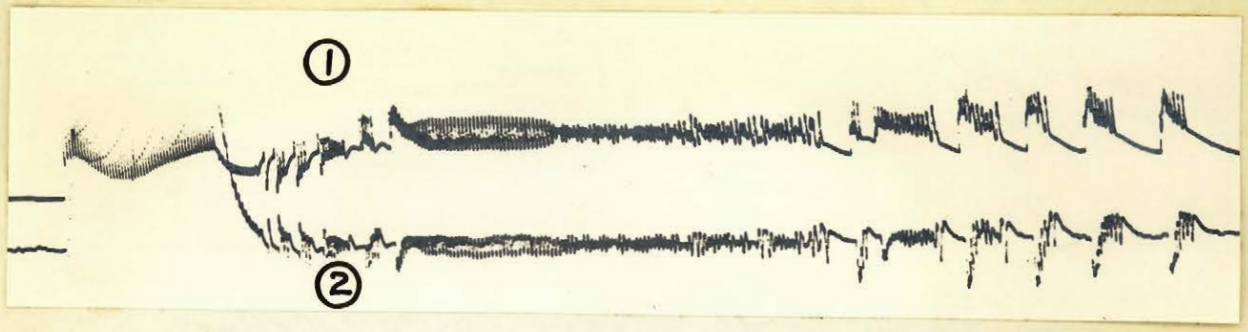


discharges. The time interval between the first two repetitive discharges is always less than the latent period. The average interval between the first two discharges, taken from the same records as for the latent period, was <100 milliseconds. As the afterdischarge proceeds the repetitive discharges change in form and in frequency. At first the discharges are dome-shaped with occasional bursts of higher-frequency activity superimposed on them. As the discharge continues, the shape becomes smoother and the frequency increases. Finally there is a slowing-down of the discharges until they become infrequent bursts of activity. At this point the afterdischarge usually comes to an abrupt end. Fig. 3 shows an afterdischarge which follows this typical pattern.

The results obtained from the experiments done in this study involved a detailed examination of the various components of the afterdischarge. Conditions of preparation, stimulation, locale and recording were varied to provide an insight as to how the afterdischarge begins, how it is maintained, and why it is terminated.

II. PARAMETERS OF STIMULATION.

The stimuli used to initiate the paroxysmal afterdischarge consisted of rectangular pulses of constant potential. These stimuli are of great convenience since they have several independent parameters. Thus it is possible to vary one stimulus parameter while keeping all



2.0 mV

2 sec

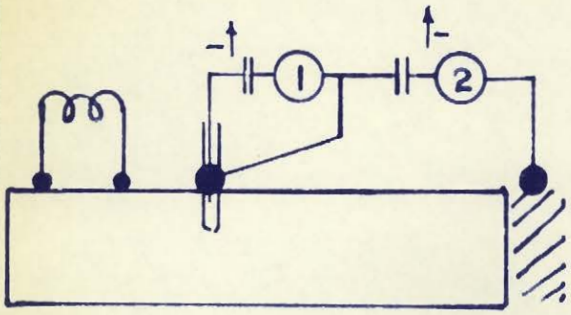


Fig. 3

a typical
afterdischarge

the others constant, and observe the effects upon the afterdischarge.

The parameters of stimulation which were investigated in this study are as follows: (1) pulse amplitude, referred to here as strength; (2) pulse duration; (3) pulse repetition rate, referred to as frequency; (4) total number of stimuli; and (5) polarity.

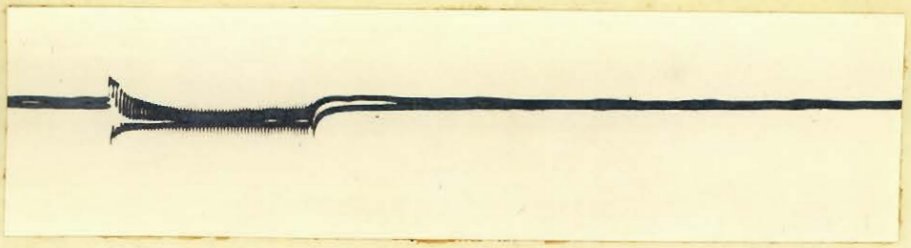
(1) Strength.

In all these experiments bipolar surface recordings were made using platinum electrodes. The paroxysmal activity of the afterdischarge can be recorded with R.C. coupled amplifiers, and this was done to avoid the annoyance of a drifting baseline.

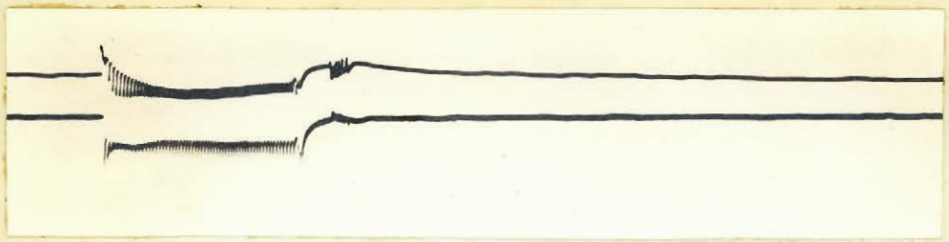
The experiments were intended to determine (a) the threshold of stimulus strength needed to initiate the afterdischarge, and (b) the relationship between the duration of the afterdischarge and the stimulus strength. With a pulse frequency of 20 per second and a pulse duration of 2 milliseconds it was found that a 3 second period of stimulation required a strength of about 5.0 volts to initiate an afterdischarge on most animals. (Fig.4a)

Since the stimulating pulses were obtained from constant-voltage pulse generators, it has been found convenient to state the stimulus strengths in terms of volts. In order, however, to have some estimate of the stimulus current delivered to the cortex, pilot experiments were made in this study to determine the cortical impedance seen by the stimulating electrodes.

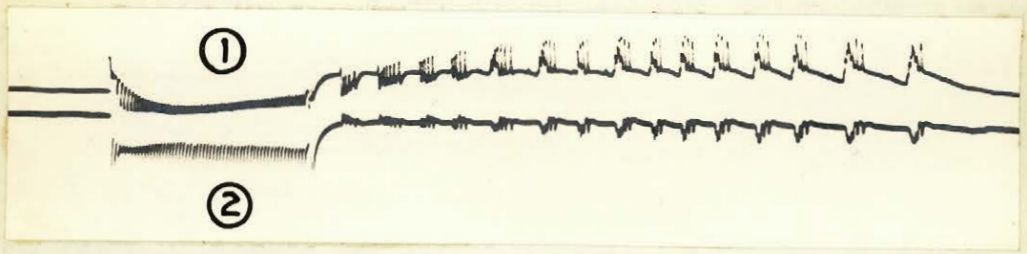
These pilot experiments were done in the fashion indicated in Fig. 5. A 1.0K ohm resistance, "R", was placed in series with the stimulating transformer output and the stimulating electrodes. The peak voltage, E_R of the pulse obtained



(i) stimulus = 20 pps, 60 pulses, 4.5 volts



(ii) same stimulus; 5.25 volts (threshold)



(iii) same stimulus; 5.64 volts

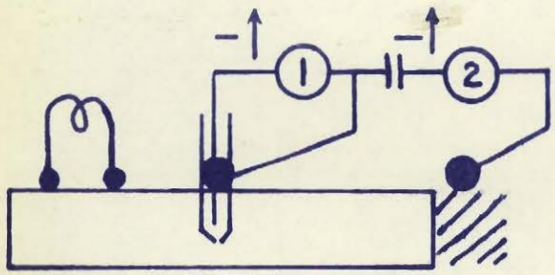
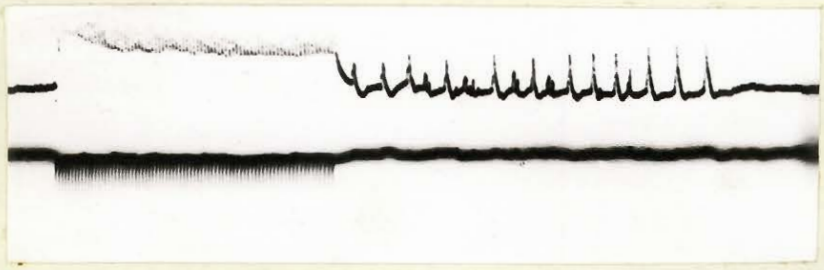


Fig. 4a

establishment of threshold strength



Stimulus at threshold strength

2.0 mV

2.0 sec

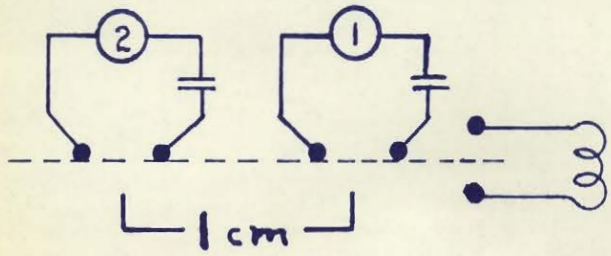


Fig. 4b

lack of spread at low stimulus strength

across R was measured on an oscilloscope. The constant-voltage output pulse E_0 was set to a known value, and measurement of E_R enabled the peak voltage, E_S, of the pulse delivered to the cortex at the stimulating electrodes to be calculated by a simple application of Ohm's Law:

$$E_S = E_0 - E_R$$

The current "I" through R is given by

$$I = \frac{E_R}{R}$$

Since the impedance of the cortex is in series with R, the total current delivered to the cortex is the same as the current through R.

For these determinations measurements were made with E_0 ranging between 275 mVolts and 1.36 volts. At these strengths no visible excitation of the cortex took place. The results obtained with strengths in this range indicate that the impedance seen at the stimulating electrodes is in the order of 10^4 ohms. Such high values of resistance are in agreement with the results of Grafstein (1954) who found the impedance of cortical tissue to be 15X that of normal saline. It is also in agreement with the work of Frank (personal communication, 1957) who measured the specific resistance of cortical tissue using rectangular pulses. Frank finds the specific resistance of cortical tissue to be approximately 1000 ohm-cm, which is close to 10X that of normal saline.

The effects of polarization of the stimulating electrodes

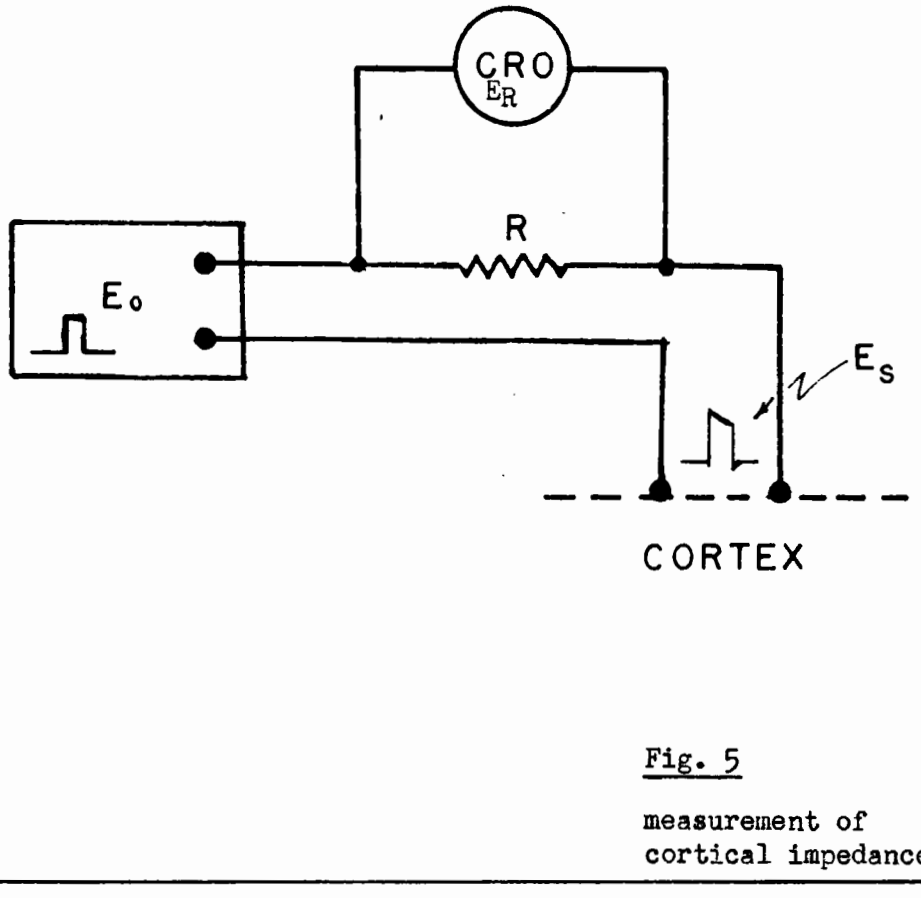


Fig. 5

measurement of
cortical impedance

probably add to the impedance measurements described here. Nevertheless, since these are in series with the cortex it is still reasonable to assume that the current delivered to the cortex during stimulation is equal to the pulse voltage divided by 10^4 ohms. This is only true if the spacing of the stimulating electrodes, as well as the specific resistance of the cortex remains constant during the experiment. For this reason these results are included only to indicate the order of currents used in this study. All further statements regarding stimulus strengths are made in volts to ensure reproducibility when using constant-voltage pulse-generators.

At the threshold strength the afterdischarge is very short and does not always spread far from the stimulated area. (Fig. 4b) The duration of the afterdischarge increases in almost all-or-nothing fashion at any strength above the threshold. Increasing strength beyond the threshold value has practically no effect upon the duration of the afterdischarge. (Fig.6)

The upper limits of the effect of stimulus strength were restricted by the fact that strengths great enough to produce the afterdischarge were also at times sufficient to produce spreading cortical depression. This phenomenon was first described by Leao in 1944. It consists of a spreading front of lessened cortical activity accompanied by a marked decrease in cortical excitability. The advent of spreading depression

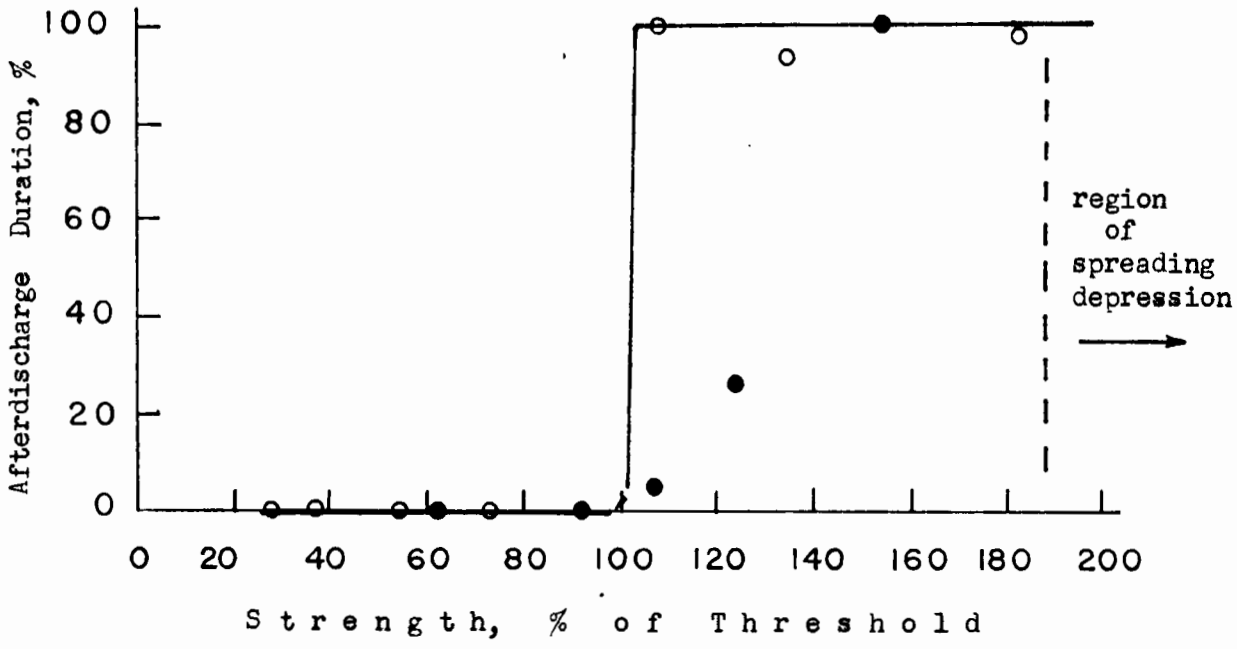

100
80
60
40
20
0
Afterdischarge Duration, %
0 20 40 60 80 100 120 140 160 180 200
Strength, % of Threshold
region
of
spreading
depression

Fig. 6

during an experiment, then, can obfuscate the results seriously. Since other workers have postulated that the phenomena responsible for spreading depression may also be responsible for epileptiform activity, (van Harreveld and Stamm, 1953; Sloan and Jasper, 1950) the experiments described here were designed so that the afterdischarge could be studied without interference from the onset of depression. In order to detect the presence of cortical depression,a switching arrangement was provided so that the stimulating electrodes could be connected either to the repetitive stimulus used to initiate the afterdischarge, or to a single-shock stimulus chosen to elicit the surface-negative response described by Burns. (1950, 1951) It has been shown by Grafstein (1954) that the surface-negative response disappears during the large negative wave which accompanies spreading depression, and reappears at its pre-depression level about 2 minutes after the peak of the negative wave. Thus the surface-negative response serves as a good estimate of the viability of the cortex. The absence of the surface-negative response was taken to mean that the cortex was depressed, and no further tests were made until the surface-negative response returned to normal. Thus there is reasonable assurance that the results obtained are not complicated by the presence of spreading depression.

It was found that strengths greater than 20 volts were particularly liable to cause spreading depression, and so the effects of varying stimulus strength were never tested beyond this limit.

(2) Pulse Duration.

In these experiments the threshold strength for initiating

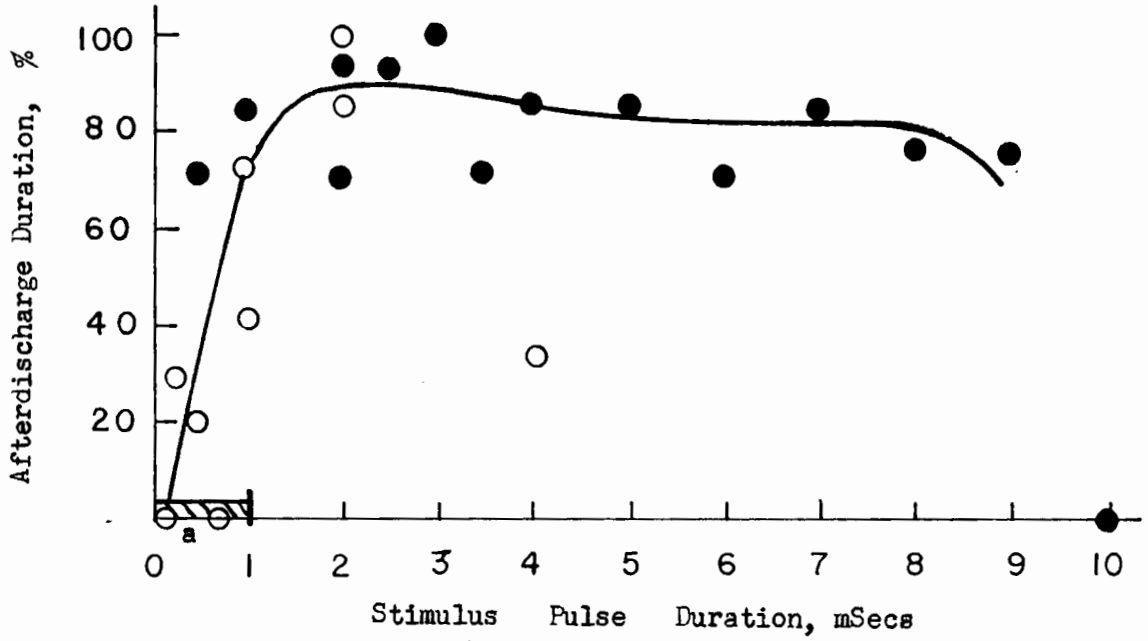
an afterdischarge was determined for the area of cortex to be tested. The strength was then adjusted so that it was well above threshold for the afterdischarge but below the region where spreading depression was likely to occur. (Fig. 6) The pulse duration was then varied while the strength and other stimulus parameters were held constant. Fig. 7a shows the results of plotting the duration of the afterdischarge vs. the pulse duration. This graph shows that pulse durations shorter than 0.3 milliseconds fail to produce any afterdischarge. The duration of the afterdischarge increases steeply with pulse durations from 0.3 to 1.0 milliseconds. This region is shown in an expanded form in Fig. 7b. Increasing the pulse duration beyond 1.0 milliseconds has very little effect upon the length of the afterdischarge until the pulse duration becomes greater than 9.0 milliseconds. Beyond this point the afterdischarge duration falls off very quickly and no afterdischarge whatsoever is produced by stimuli whose pulse duration is 10 milliseconds or greater. This steep fall of afterdischarge duration is explained by the fact that pulse durations of 10 milliseconds or greater were very likely to cause spreading depression of the cortex.

(3) Frequency.

These experiments were designed to test the relationship between the stimulus frequency and the duration of the afterdischarge.

Experiments made with strengths twice threshold show that the afterdischarge could not be elicited by frequencies lower than about 6 pulses per second. Above the threshold frequency the duration of the afterdischarge increases with the stimulus frequency until about 70 pulses per second. After this point the duration of the afterdischarge decreases

Fig. 7a



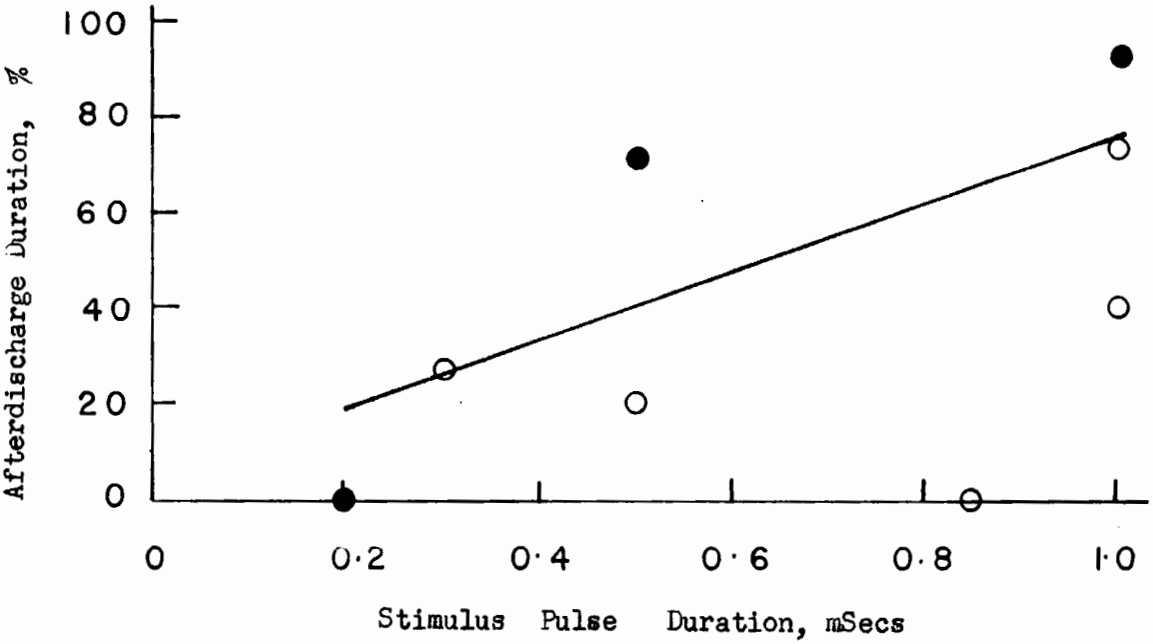


Fig. 7b

expansion of region "a"

with an increase of frequency until finally, at 400 pulses per second, the stimulation fails to produce any afterdischarge. The peak at 70 pulses is not very sharp, the curve being rather broad and flat-topped. (Fig.8)

(4) Number of Stimuli.

The number of stimulating pulses used to initiate an after-discharge has a marked effect upon the duration of the afterdischarge. There is a threshold number of stimulating pulses needed to initiate the afterdischarge. At a frequency of 20 pulses per second this threshold number ranged between 25 to 35 pulses. An increase in the number of stimuli above the threshold number results in an increase of the duration of the afterdischarge. This relationship holds almost linearly until the number of stimulating pulses is about 4X the threshold number. (Fig.9a) After this point the duration of the afterdischarge increases very little with the number of stimulating pulses. If the number of stimuli is increased beyond 6X the threshold number, then the duration of the after-discharge decreases with an increasing number of stimuli. The curve tapers off sharply after the number of stimulating pulses reaches 15X threshold. (Fig.9b)

When the number of stimulating pulses is more than 6X to 8X the threshold number it is at times possible to see paroxysmal activity during the period of stimulation.(Fig.10) Strictly speaking this cannot be called an "after" - discharge, and this period was not considered as part of the duration of the afterdischarge under investigation. The fact that the period of stimulation occupies part of the paroxysmal

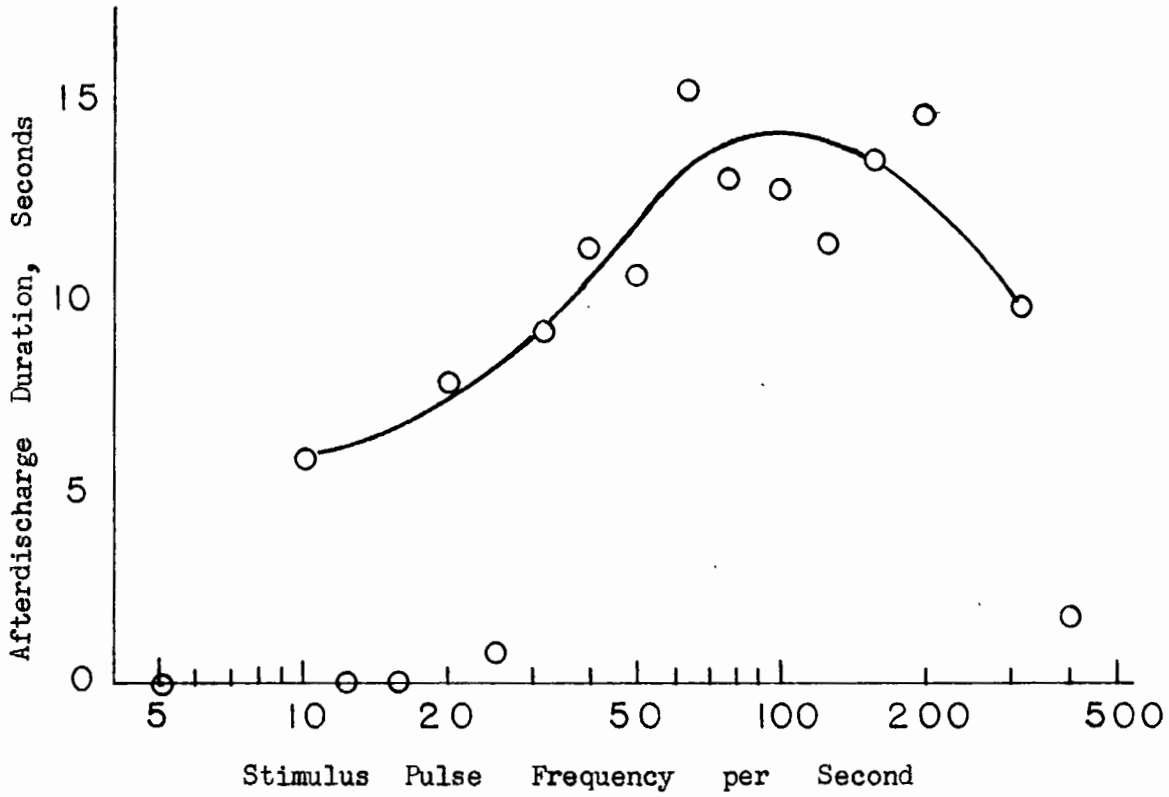

Afterdischarge Duration, Seconds
15
10
5
0
5
10
20
50
100
200
500
Stimulus Pulse Frequency per Second

Fig. 8

activity could account for the declining portion of the curve between 6X and 15X threshold. (Fig.9b)

When a large number of stimulating pulses (e.g. 10X the threshold number) is delivered to the cortex, spreading depression is likely to occur. This accounts for the abrupt termination of the curve in Fig.9b at 15X the threshold number of pulses.

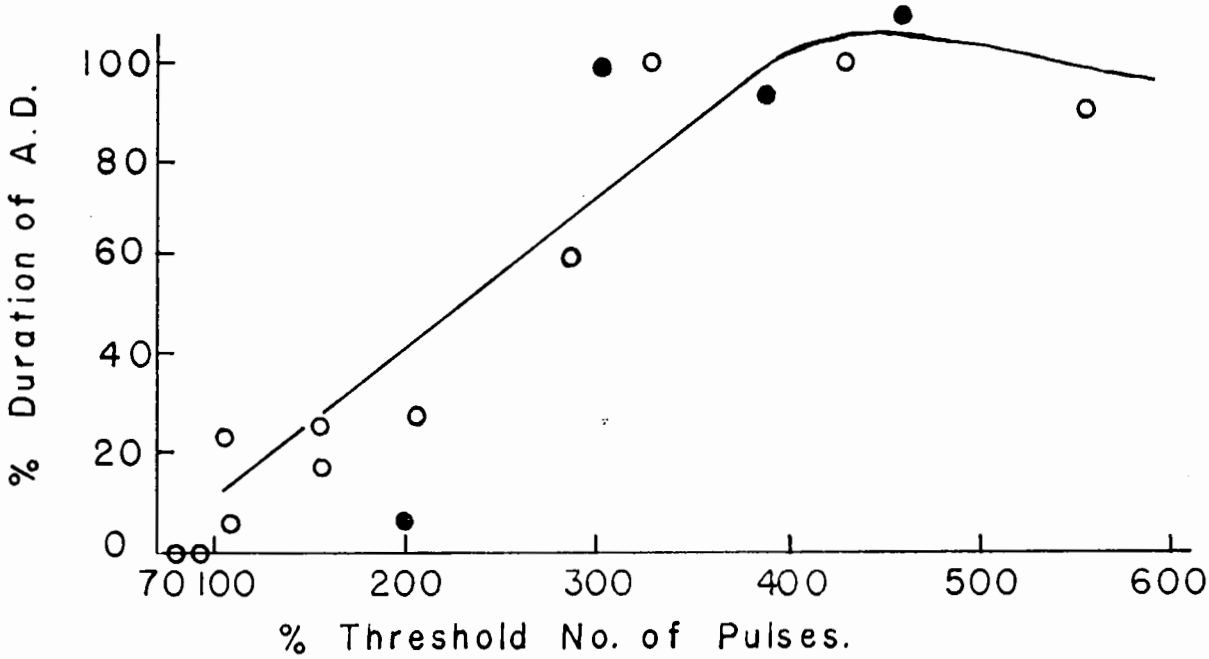
When repeated bursts of very large numbers of stimuli (30X threshold and greater) were delivered to the cortex at intervals of one minute for five minutes, the depression was sometimes replaced by a paroxysmal discharge which lasted for unusually long periods, often two to three minutes. A similar phenomenon has been described by van Harreveld and Stamm (1953).

(5) Polarity.

Since the stimulating waveform is asymmetrical it can be shown that it has a D.C. component. (Moskowitz and Racker, 1951) Such a D.C. component could be effective in influencing a net ion flux in one direction, or in polarizing cortical structures in some particular plane depending upon the placement of the stimulating electrodes.

In order to test whether this D.C. component had any effect upon the afterdischarge an experiment was done in which the stimulus polarity was reversed upon alternate tests. A slab cut in the suprasylvian gyrus of a chloralosed cat was stimulated every ten minutes for a total of 19 tests. The results show that there is no significant difference between either the probability of producing an afterdischarge or the average duration of afterdischarge when comparing the effects of different stimulus polarities.

Fig. 9a


% Duration of A.D.
100
80
60
40
20
0
70 100
200
300
400
500
600
% Threshold No. of Pulses.

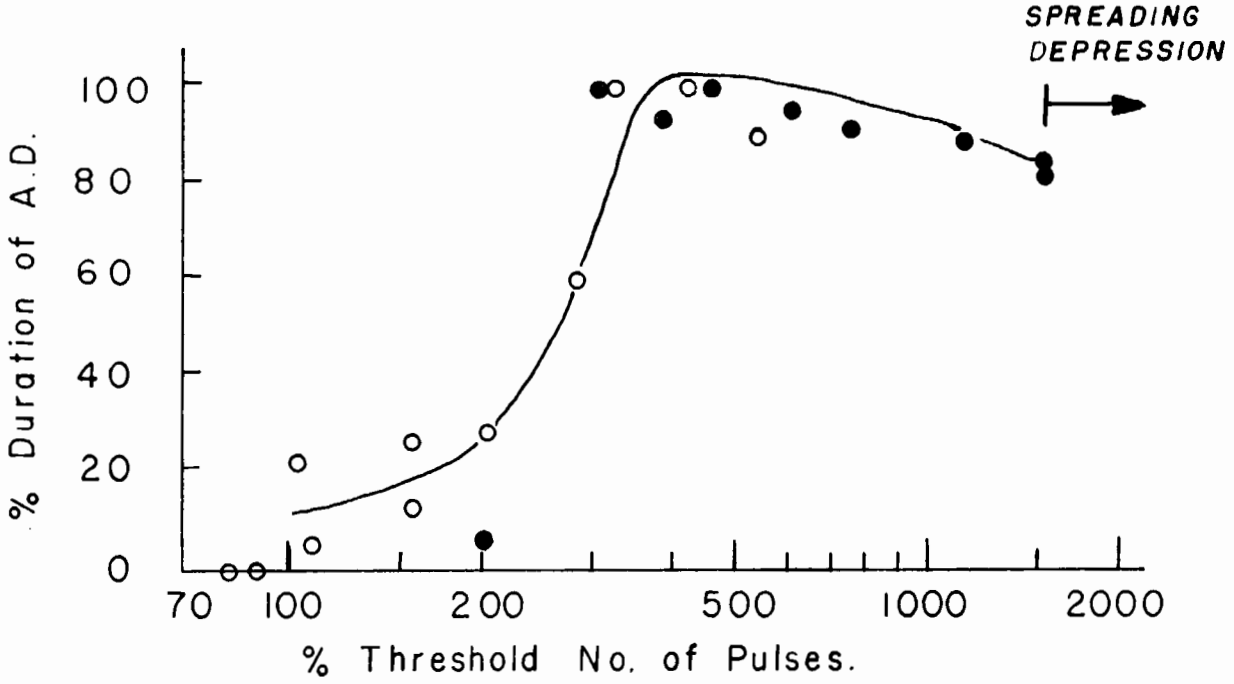
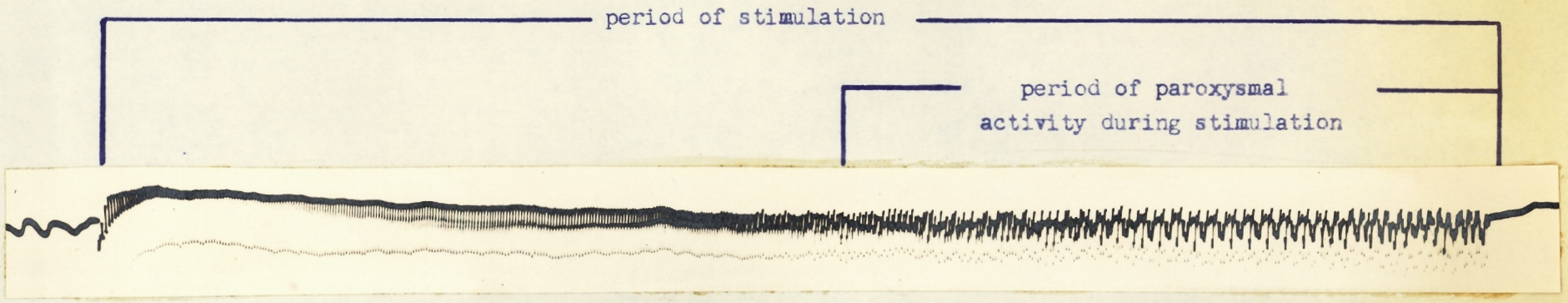
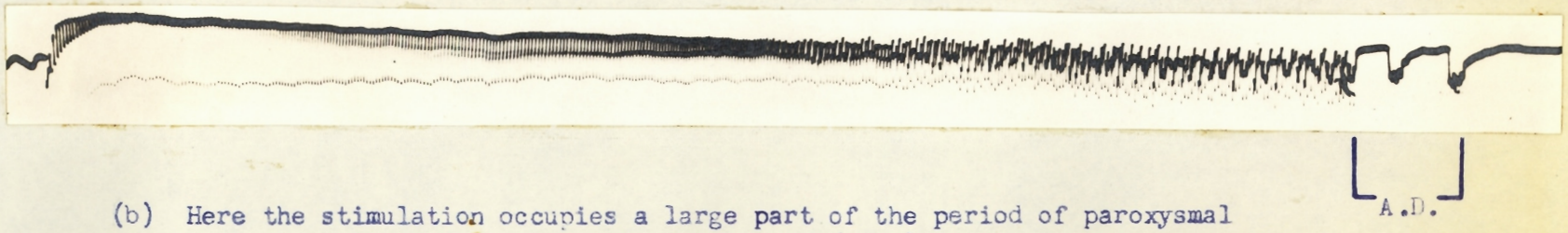

SPREADING
DEPRESSION
% Duration of A.D.
100
80
60
40
20
0
70
100
200
500
1000
2000
% Threshold No. of Pulses.

Fig. 9b



(a) The period of stimulation occupies the total period of paroxysmal activity, hence no "after" - discharge is seen. (see text)

Fig. 10a



(b) Here the stimulation occupies a large part of the period of paroxysmal activity. The duration of afterdischarge, (A.D.) is thus made to appear very short.

Fig. 10b

III. LOCALE OF STIMULUS.

It was found possible to initiate the discharge on any region of the cortex. Most of the experiments were done upon the suprasylvian gyrus for reasons of technical accessibility. To establish the effects of varying the stimulus locale, experiments were done on an isolated slab. The simplified geometry of a slab makes the interpretation of results easier than if the intact cortex had been used.

The frontal and caudal ends of an isolated slab were stimulated alternately. There was no difference between the resulting probabilities of producing an afterdischarge or the durations of the afterdischarges. When both ends of the slab were stimulated simultaneously the resulting afterdischarge was similar to that produced by stimulation at either end of the slab alone.

IV. STIMULATION WITH POTENTIATING PULSES.

For these experiments isolated slabs were prepared upon cat's unanaesthetized cortex. (See page 12) The stimulation consisted of pairs of pulses, the second pulse in each pair being referred to as the potentiating pulse. A pulse duration of 2 milliseconds was used and the interval between the two pulses was varied from 2 milliseconds to 100 milliseconds. The paired stimuli were repeated at rates which ranged from one pair every 400 milliseconds to one pair every 250 milliseconds.

An afterdischarge could be produced by a train of paired stimuli consisting of 27 pairs of pulses, with an interval of 3 milliseconds between the first pulse and the potentiating pulse, delivered to the cortex at the rate of four pairs per second. The strength used was twice the threshold strength for an afterdischarge established with a stimulating

frequency of 20 per second, and pulse width of 2 milliseconds. When the strength was reduced to 20% above the established threshold, a train of paired pulses similar to the one first described failed to produce the afterdischarge. At this same strength a spacing of 15 milliseconds between paired stimuli resulted in an afterdischarge. At strengths 3X the extablished threshold, a potentiating pulse spaced 2 milliseconds from the first was no more effective in producing an afterdischarge than was a train of stimuli without the potentiating pulse.

On one preparation a train of paired stimuli with the potentiating pulse spaced 25 milliseconds from the first was able to produce an afterdischarge while pulses spaced at any other interval in the range stated were unable to initiate the afterdischarge. Similar results observed upon several other preparations suggest that an interval of 25 milliseconds between the first pulse and the potentiating pulse is the most favorable interval for the production of afterdischarges.

Experiments were done in order to test the hypothesis that a particular interval between stimulating pulses could be found which was most favorable for the production of afterdischarges. To this end afterdischarges were produced using stimulus strengths of only 10% above threshold, while varying the pulse frequency. If a most favorable pulse interval for the production of an afterdischarge does exist, a certain frequency and its integral multiples will be found which corresponds to this most favorable spacing of pulses. At low strengths the ability of these "most favorable" frequencies to produce longer afterdischarges than other frequencies will be emphasized.

The results of this experiment is shown in Fig. 11.

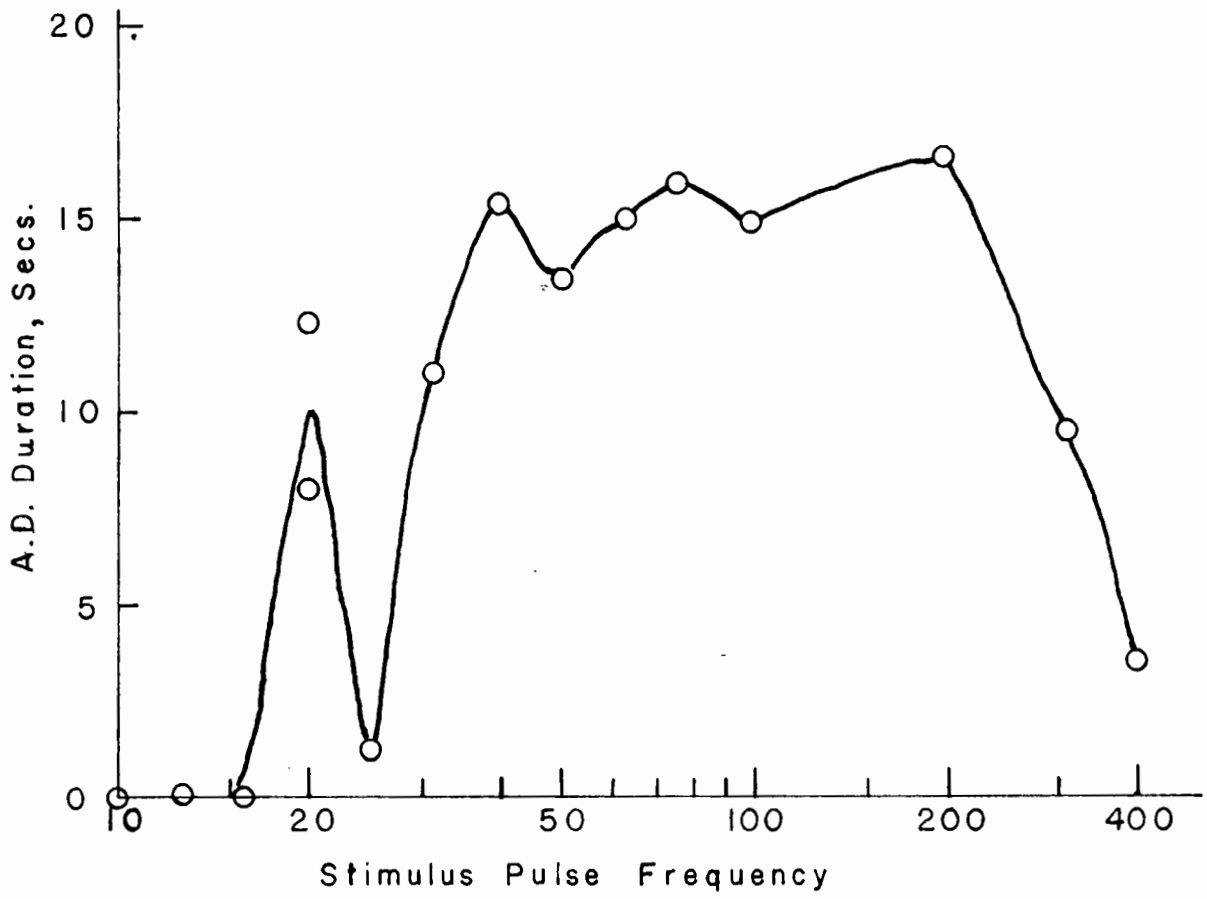


Fig. 11

stimulation at strengths
only 10% above threshold

A series of peaks, occuring at integral multiples of approximately 20 p.p.s., is seen in the graph of frequency vs. duration of afterdischarge.

V. THE AMPLITUDE AND PHASE OF THE PAROXYSMAL AFTERDISCHARGE AT DIFFERENT DEPTHS.

(1) Rationale and Methods.

The experiments described so far were concerned mainly with attempts to quantitate the afterdischarge which results from a particular stimulation, and to see what variations in the afterdischarge are caused by variations in the stimulus. For such purposes the gross form of the afterdischarge, recorded R.C. coupled in bipolar fashion from the surface of the cortex, is an adequate gauge of the effects of stimulation.

In order, however, critically to test the hypothesis that the paroxysmal afterdischarge is dependent upon the differential repolarization of neurones in the cortex, the gross form of the afterdischarge is inadequate. Since the theory of differential repolarization presupposes that following a period of repetitive stimulation there will be a potential gradient radial to the curvature of the cortex, (Burns, 1954) experiments were done where this radial potential gradient was measured.

Measurements were made on isolated cortical slabs using microelectrodes with diameters from 50 μ to 100 μ i.d. A silk-wick reference electrode was placed on the live cortex immediately above the point of insertion of the depth electrode. In this way bipolar records of the radial potential gradient are obtained, as described on page 13. D.C. coupling was used to make possible measurement of the slowly-changing

components of the potentials recorded.

(2) Measurements at the Focus.

The recording electrodes were placed as close together as was technically possible, and the stimulating electrodes were then placed so that the recording electrodes were sure to be in the region of heaviest stimulus current density, i.e. at the stimulated focus. Two channels of recording were used, the first channel being a bipolar record of the radial potential gradient. The second channel was a monopolar surface recording of the activity of the cortex at the focus, with reference to a killed area of cortex some distance away. This second recording served as a control on the consistency of the afterdischarge which appeared at the surface of the focal area.

(i) The Phase of the Repetitive Potential Discharges at Different Depths. Although the repetitive oscillatory potential discharges with which the paroxysmal afterdischarge begins show great variations in form and frequency, the first phase of the discharge is invariably a positive-going change in potential when monopolar surface recordings are made. In records of the radial potentials made with microelectrodes below the surface of the cortex the first phase of the repetitive discharge may sometimes be positive-going if the microelectrode is close to the surface. At all depths below 0.2 mm the first phase of the repetitive discharge is invariably negative-going.

It is now generally accepted that the appearance of surface-positivity in monopolar surface records signifies the depolarization of the lower parts of deep-lying neurones whose processes stretch radially out towards the pia. (Adrian, 1936; Eccles, 1951; Burns and Grafstein, 1952)

Furthermore, Burns and Grafstein (1952) have shown that monopolar recordings of positive bursts at the surface of the cortex can be made simultaneously with recordings of radial potentials directly below the active surface recording electrode. These radial potentials have the same form as the surface record, and until depths of about 0.5 mm are reached, the tip of the deep recording microelectrode goes positive with respect to the surface at the same time as the active recording electrode on the surface goes positive with respect to an area of dead cortex. Burns and Grafstein further show that measurements made deeper than the level mentioned indicate a reversal of phase taking place, the tip of the microelectrode becoming negative when the cortex becomes positive with respect to the killed reference point.

The phase relationships, observed in this study, of the repetitive discharges at different depths in the cortex are consistent with a system of neurones, deep in the cortex, whose population begins to fire spontaneously following repetitive stimulation. In order to determine if such a system could be localized at one particular depth, experiments were done to investigate the distribution with depth of the various components of the paroxysmal afterdischarge.

(ii) The Amplitude of the Repetitive Potential Discharges at Different Depths. The most obvious component among the potentials which appear during the paroxysmal afterdischarge is the repetitive oscillatory discharge whose phase relationships with depth have already been discussed. The amplitude of these discharges was measured as radial potentials at different depths in the cortex, recordings being made in exactly the same manner as for the measurements of their phase. The placement of the

electrodes used to record the radial potentials was such that there was almost no separation between the microelectrode at the surface and the surface electrode. When anypotentials were recorded at the surface it was taken to mean that the potentials so recorded included a tangential component. This tangential component was usually small enough to be disregarded, particularly at depths in the cortex greater than 0.2 mm.

Since the repetitive discharges vary greatly in form and frequency, their magnitude was measured as the mean peak-to-peak value estimated over a period of 1 second, or of at least three discharges, whichever was the longer.

The result of plotting the amplitude of the repetitive discharge against the depth of the recording microelectrode is shown in Fig. 12. The magnitude of the repetitive discharge increases with increasing depth in the cortex, reaching a maximum amplitude at a depth of 1.25 mm. The amplitude of the discharges then decreases with an increase of depth until the recording electrode is about 2 mm below the surface of the cortex. There is little further change with depth beyond this level. Since the slabs cut were usually about 2 mm deep, the flattened portion of the curve coincides closely with the expected region of undercut tissue.

A distribution with depth of the repetitive afterdischarge amplitude such as the one described suggests that a particular group of neurones in the cortex is responsible for the oscillatory phase of the afterdischarge. In order to determine whether this could be correlated with other components of the afterdischarge, further experiments were done to examine the distribution with depth of other potentials present during the paroxysmal afterdischarge.

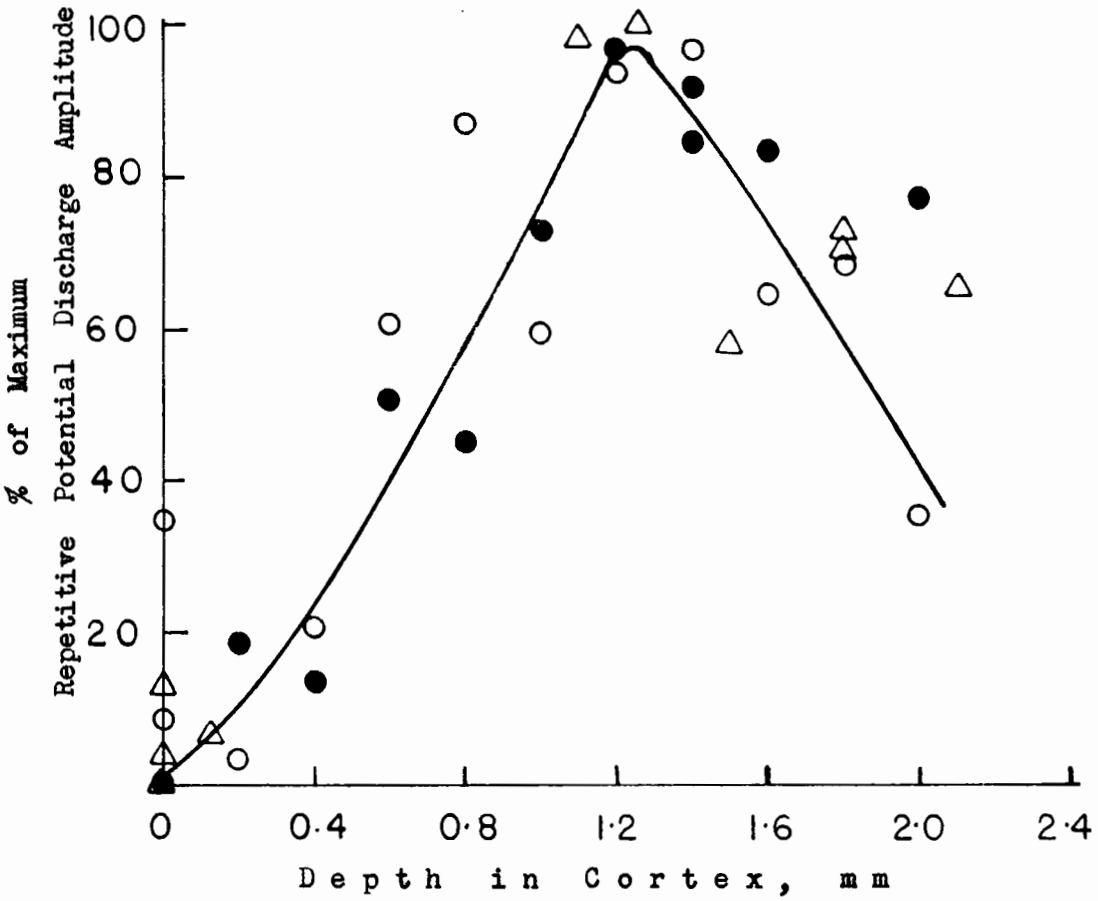
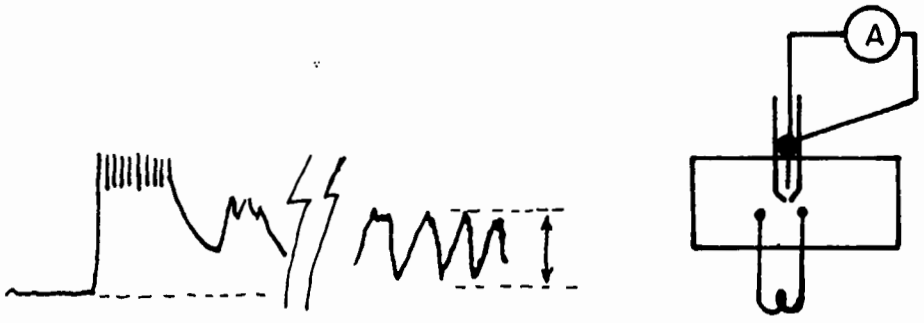

% of Maximum Repetitive Potential Discharge Amplitude
100
80
60
40
20
0
0·4
0·8
1·2
1·6
2·0
2·4
Depth in Cortex, mm

Fig. 12


A

(iii) Potentials at the End of Stimulation. In the description of the gross form of the afterdischarge it has already been mentioned that the stimulation changes the mean potential of the cortex. Measurements of the radial potential gradient of this mean potential were made at various depths at the focus. The results showed that at the end of stimulation the mean radial potential gradient of the cortex, at any depth below the surface, becomes negative with respect to its mean potential before the stimulation.

(iv) Potentials at the Beginning of the Afterdischarge. Between the end of the stimulation and the onset of the paroxysmal afterdischarge there is a latent period during which there are no oscillatory potential discharges. During this latent period the mean potential of the cortex changes. The change is always such that the mean potential tends to return to that recorded before the stimulation. At some point before the mean potential returns to its original value, the first of the repetitive discharges breaks out.

Experiments were done to determine the difference between the mean radial potential of the cortex recorded at a given depth before stimulation and the mean potential at which the repetitive discharge breaks out.

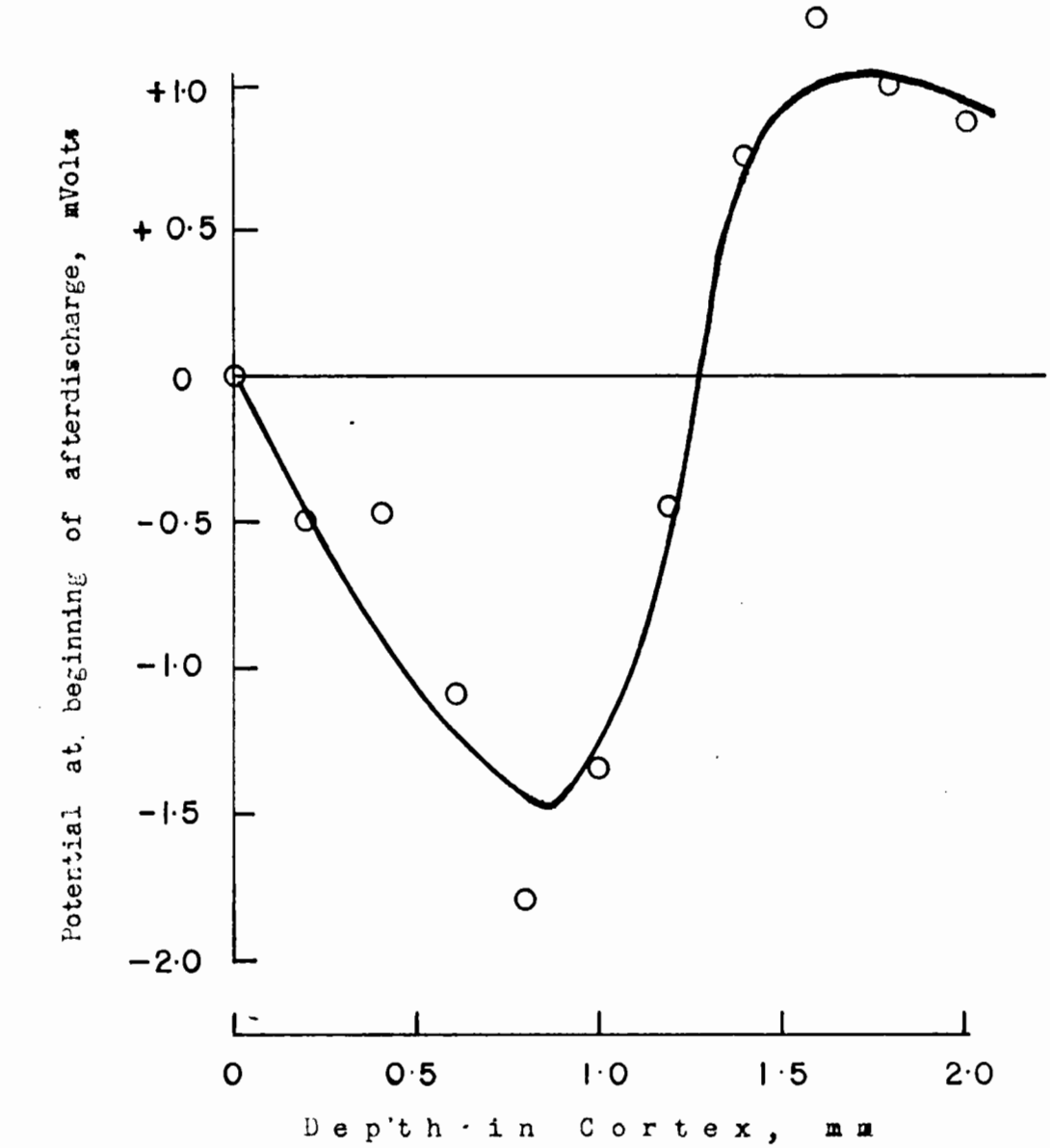
When this difference in potential is plotted against depth in the cortex the results show that there is a negative difference which increases in magnitude with depth until a depth of 0.8 mm is reached. Beyond this the difference decreases with depth and becomes zero at a depth close to 1.25 mm. At further depths the difference is positive, and increases with depth until the bottom of the slab is reached. A rather broad positive peak is seen 1.6 mm deep.

Fig. 13 shows the curve obtained by plotting the difference in potentials against depth in the cortex at the stimulated focus:

(v) Potentials at the End of the Afterdischarge. At the end of the afterdischarge the mean radial potential gradient of the cortex at various depths is negative with respect to the potential recorded before the stimulation. It can thus be seen that stimulation changes the mean potential of the cortex at all depths, and that this change persists throughout the afterdischarge.

Such results are consistent with the view that repetitive stimulation causes a chronic fatigue of neuronal structures in the cortex, and that this state of fatigue persists throughout the afterdischarge.

(vi) Unit Cell Activity. In order to determine the location of the cell bodies of neurones involved in the afterdischarge, records of unit potentials were made at various depths. (See page 14) It must be pointed out here that the experiments were intended to be only a preliminary guide to future work involving more exhaustive studies of unit potentials occurring during an afterdischarge. Even if enough data were available to establish with certainty the region where most cell bodies involved in the afterdischarge are located, many problems would remain. It has not been definitely established whether or not unit potentials from single neurones can be directly correlated with activity on the surface of the cortex. Adrian and Matthews (1934) have postulated a rather direct relationship between unit activity and potential discharges at the cortical surface. Other workers, however, have shown that the relationship between the two phenomena is at best an extremely tenuous one. (Li,


+1·0
+ 0·5
0
-0·5
-1·0
-1·5
-2·0
Potential at beginning of afterdischarge, mVolts
0
0·5
1·0
1·5
2·0
Depth in Cortex, mm

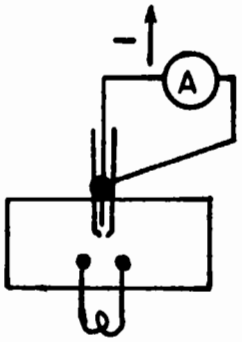

A

Fig. 13

McLennan and Jasper, 1952; Li and Jasper, 1953) For this reason the results of a preliminary and non-exhaustive study of unit potentials, such as has been done here, cannot be drawn upon too heavily when interpreting the connection between unit potentials and the paroxysmal afterdischarge.

It is nevertheless true that some correlation was found in this study between the enormous increase, during an afterdischarge, of the electrical activity upon the surface of the cortex and simultaneous large increases of activity recorded from single cells. The activity of single cells during an afterdischarge was recorded close to the stimulated focus. A record from a cell 1.5 mm below the surface is shown in Fig. 14. Measurements from this record show that the average discharge frequency of the cell increased from 5 per second before the stimulation to 90 per second following the stimulation. Between the end of the stimulation and the onset of the rapid firing of the cell there was a latent period of 89.0 milliseconds during which the cell did not discharge at all. The rapid firing of the cell began before any paroxysmal activity was recorded on the surface of the cortex.

Similar records, and visual observations made during these experiments, indicate that the cell bodies involved in the afterdischarge are found most often at depths between 1.1 mm and 1.5 mm below the surface. Distinction between the cells that actually produce and maintain the afterdischarge and the cells that are driven by the afterdischarge is a problem which will be solved only with far more complete information than is available here.

(3) Measurements Remote from the Focus.

The results of the experiments concerned with the potentials

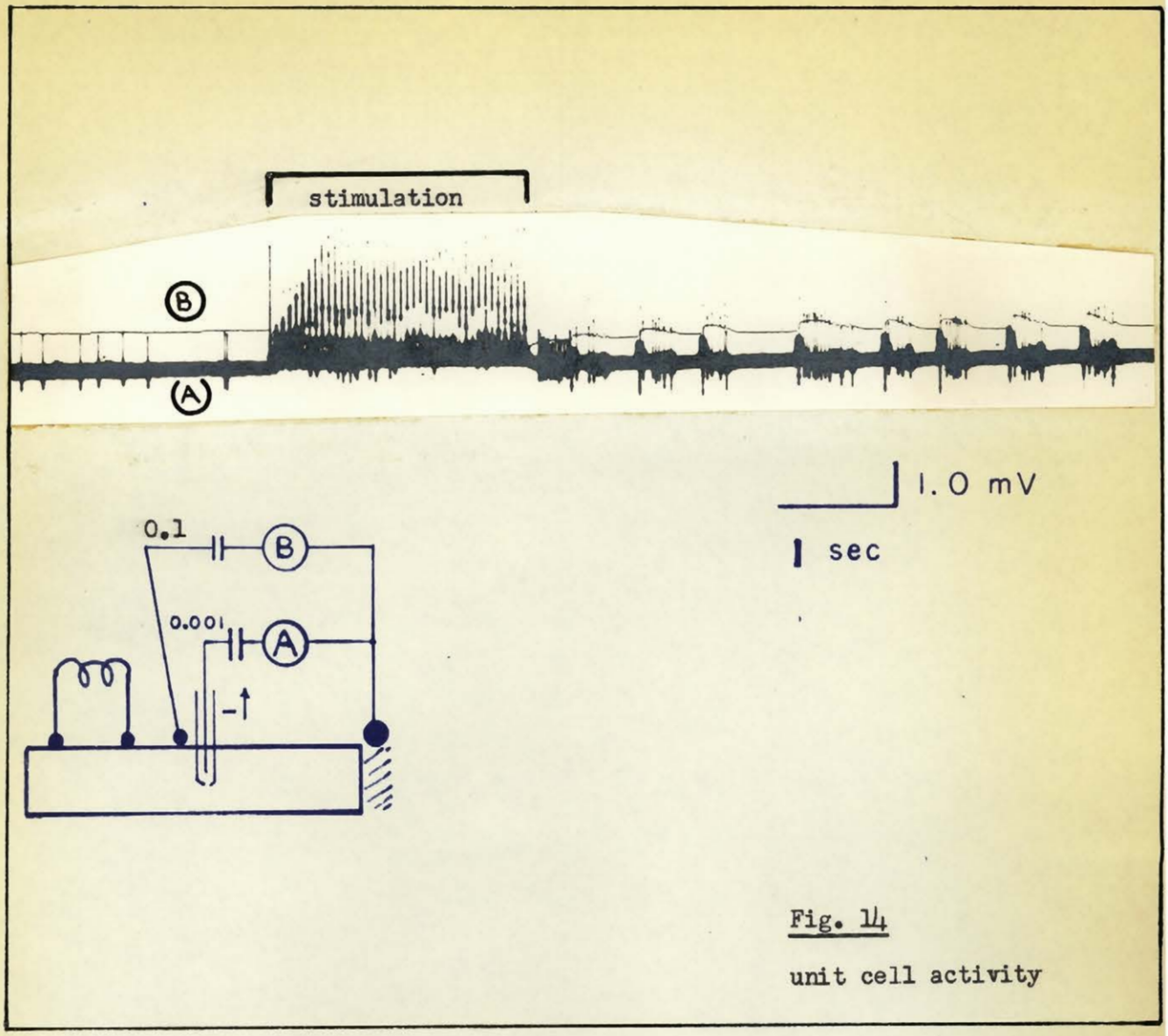


Fig. 14

unit cell activity

at the focus are consistent with a system of neurones responsible for the paroxysmal afterdischarge lying deep in the cortex and oriented tangentially across the curvature of the cortex. In order to determine whether the distribution of potentials found at points distant from the focus was consistent with such a system, measurements of the radial potentials of the cortex were made at points remote from the focus.

(i) The Phase of the Repetitive Potential Discharges at Different Depths. The results of the experiments done at points remote from the focus show that the phase of the afterdischarge changes with depth in a manner identical to that observed at the focus. At depths below 0.2 mm the first phase of the repetitive discharges is invariably such that the microelectrode tip goes negative with respect to the cortex immediately above. This is true for both isolated slabs and intact cortex.

(ii) The Amplitude of the Repetitive Potential Discharges at Different Depths. Measurements were made of the radial potential gradients set up by the repetitive potential discharges which occur during the afterdischarge. The exploring microelectrode was inserted into the cortex at distances of from 5.0 to 9.0 mm from the focus, on both isolated slabs and intact cortex. The distribution with depth of the magnitude of the repetitive discharge was identical on both these preparations. As was the case with measurements near the focus, the amplitude of the repetitive discharge increases with increasing depth in the cortex until an optimal depth is reached. In the case of measurements made remote from the focus this optimum depth was found to be 1.4 mm below the surface. Beyond this the repetitive discharge amplitude decreases with depth. The results of plotting the repetitive discharge amplitude against depth in the cortex at a point

distant from the focus is shown in Fig. 15.

(iii) Potentials which Precede the Afterdischarge. Following stimulation the mean potential of the cortex is different from the potential recorded before the stimulus. Measurement of the radial potential gradient of the cortex remote from the stimulated focus shows that the stimulation causes the mean potential of the cortex at any depth below the surface to become negative with respect to the surface.

When the stimulation has ended, the mean potential of the cortex begins to return to the original value recorded before the stimulation. This continues until the occurrence of the first repetitive discharge of the afterdischarge. Measurements made on isolated slabs show that the potential which immediately follows stimulation is, at any depth, more negative than the potential which immediately precedes the afterdischarge.

(iv) Potentials at the End of the Afterdischarge. As was the case at the focus, the mean radial potential gradient of the cortex remote from the focus is negative at the end of the afterdischarge with respect to the potential recorded before stimulation. Thus it can be seen that the chronic fatigue postulated to persist throughout the afterdischarge is present not only at the focus, but also in cortical regions away from the focus.

The results obtained from measurements remote from the focus can be seen to be quite similar to the results of similar measurements at the focus. The forms of the distributions with depth of the repetitive discharge amplitudes (Fig. 12 at the focus, Fig. 15 remote) are notably similar. Such results suggest that the neurones in the cortex which

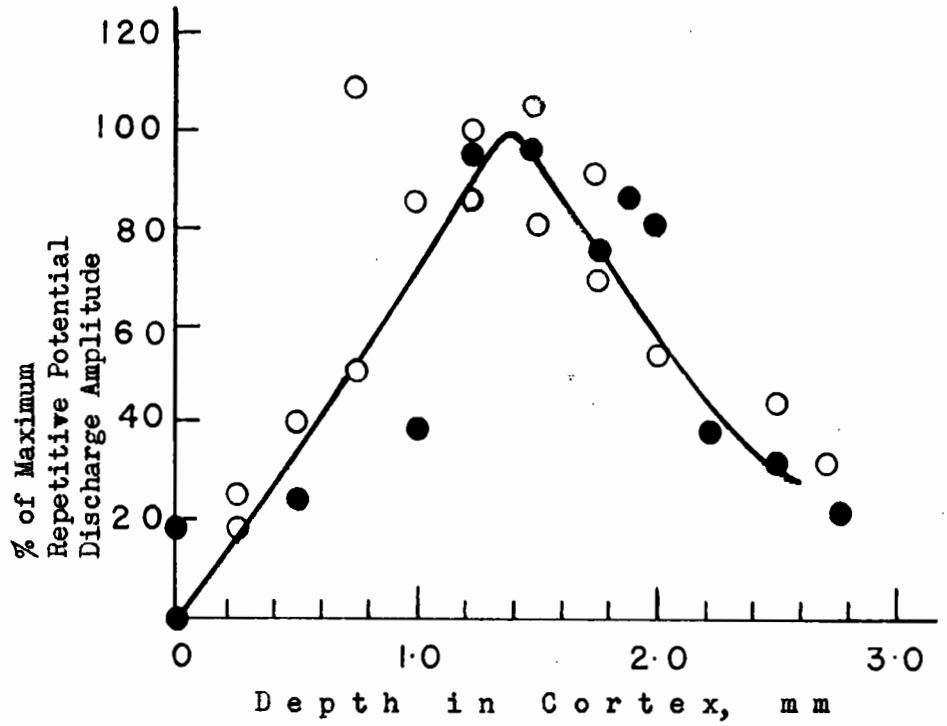
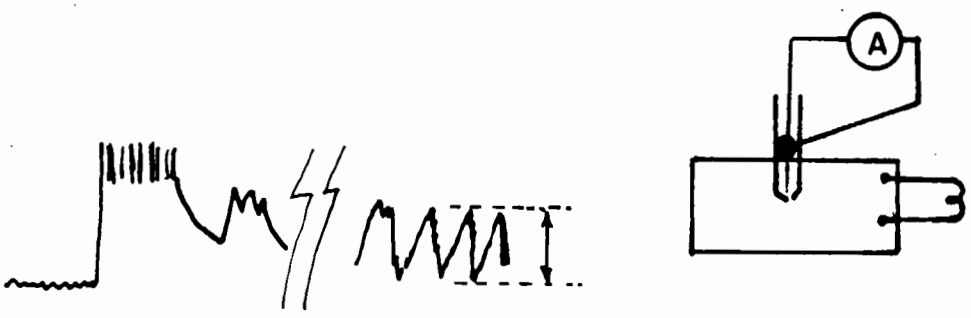

120
100
80
60
40
20
% of Maximum
Repetitive Potential
Discharge Amplitude
0
1·0
2·0
3·0
Depth in Cortex, mm

Fig. 15


A

initiate the afterdischarge at the focus belong to the same population of neurones which carry the spread of the paroxysmal activity across the cortex.

VI. THE PAROXYSMAL AFTERDISCHARGE AND OTHER CORTICAL PHENOMENA.

(1) Spontaneous Activity.

(i) Intact Anaesthetized Cortex. The intact cortex under chloralose anaesthesia exhibits spontaneous activity which can be recorded with surface electrodes. (Brazier, 1951) The probability of producing a paroxysmal afterdischarge upon such a cortex is independent of the amplitude or frequency components of the spontaneous activity. Afterdischarges were readily produced upon preparations where the spontaneous activity of the cortex was barely apparent.

(ii) Intact Unanaesthetized Cortex. The cerebral cortex in an unanaesthetized forebrain shows spontaneous activity consisting of large slow waves. ("cerveau isolé" of Bremer, 1935) Again, the probability of producing an afterdischarge on such a preparation was not related to the spontaneous activity recorded on the surface of the cortex. At times the spontaneous activity was absent while the afterdischarge could be produced without difficulty.

(iii) Isolated Slabs. The isolated slab shows no spontaneous activity of its own, save for an occasional outburst, in about one-fifth of the preparations, of a short-lived oscillatory discharge. This is in close agreement with the work of Burns, (1951) and of Henry and Scoville (1952). There is no relation between the presence or absence of these transient bursts and the probability of producing the afterdischarge.

In general, the results show that there is poor correlation between the amount of spontaneous activity recorded from any preparation and the

probability of producing an afterdischarge.

(2) The Surface-negative Response.

The surface-negative response has already been mentioned as a criterion of the viability of the cortex. (p.21) In preparations where the surface-negative response could not be elicited it was impossible to produce the paroxysmal afterdischarge. The presence of the surface-negative response, however, did not guarantee that an afterdischarge would be produced by electrical stimulation of the cortex.

It would seem that the absence of the surface-negative response indicates that the excitability of the cortex has deteriorated to the extent where all the structures in the cortex, including those responsible for maintaining the paroxysmal afterdischarge, are incapable of responding to stimulation.

(3) The Surface-positive Burst Response.

This response has been described by Burns for both the anaesthetized (1950) and the unanaesthetized isolated cortex of the cat. (Burns, 1951; Burns and Grafstein, 1952) In the preparations investigated in this study it was nearly always possible to produce an afterdischarge upon a cortex where the positive burst could be observed. The absence of the positive-burst response, however, did not preclude the possibility of producing an afterdischarge.

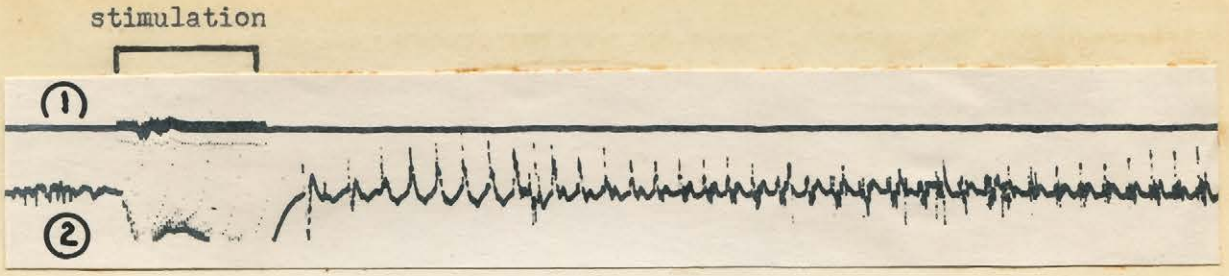
Apparently if the condition of the cortex is viable to the degree that the structures involved in the positive-burst response are able to thrive, then the structures subserving the paroxysmal afterdischarge will certainly be able to survive.

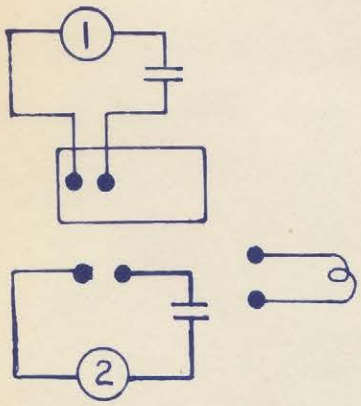
(4) Spreading Depression.

Mention has already been made of the spreading depression of the excitability of the cortex which sometimes follows prolonged repetitive stimulation. (p. 20) The results of the stimulus parameter experiments show that it is possible to produce the epileptiform afterdischarge without causing spreading depression. The onset of spreading depression can be said to preclude the possibility of producing the afterdischarge. Hence it appears that there is no first-order relationship between the two phenomena. Furthermore Sloan and Jasper (1950) have shown that spreading depression can spread through cuts in the gray matter. The paroxysmal afterdischarge cannot do this. Fig. 16 shows the records obtained from an experiment designed to determine whether the epileptiform activity could jump the cut margins of an isolated cortical slab. Stimulation of the cortex outside the slab produced an afterdischarge on the cortex which was not recorded on the slab. The converse was true when the slab was stimulated. The mechanism of propagation of the paroxysmal afterdischarge, therefore, must bear little resemblance to that of spreading depression.

(5) General Relationship of the Paroxysmal Afterdischarge to other Cortical Phenomena.

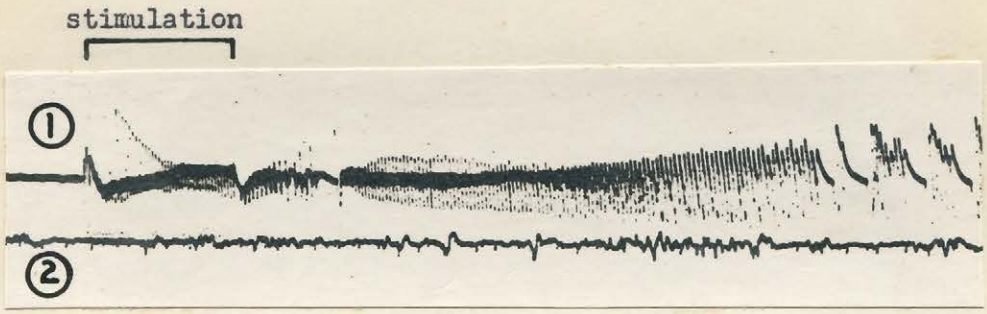
The results of this study indicate that the paroxysmal afterdischarge which follows repetitive stimulation of the cortex is a phenomenon whose mechanisms for production and propagation have no direct counterpart in any of the other phenomena discussed here. The afterdischarge is not an extension of any of these, nor is it initiated by any of them. Inasmuch as the production of the afterdischarge

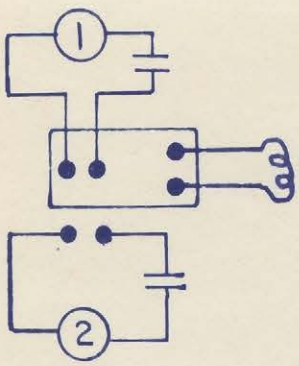




(a) Stimulation of the cortex outside the slab produces an afterdischarge on the cortex, but not on the slab.

Fig. 16 Failure of the paroxysmal afterdischarge to jump the cut margins of an isolated cortical slab.





(b) Stimulation of the slab produces an afterdischarge on the slab, but not on the cortex.

is dependent upon a cortical environment where conditions are suitable for the viability of the structures involved, there is some correlation between the possibility of producing an afterdischarge and the possibility of eliciting some of the other cortical responses. These other responses, particularly the surface-negative response can be used as a control of the suitability of a given preparation for experiments concerned with the epileptiform afterdischarge.

CONCLUSIONS

The results of this study, described in the preceding pages, can now be drawn upon in the effort to answer the questions set at the beginning of the investigation. These questions were, in effect, the following:

(i) How is the afterdischarge initiated?

(ii) How is the afterdischarge maintained?

(iii) Why does the afterdischarge terminate?

(iv) Is differential repolarization responsible for the phenomenon of the paroxysmal afterdischarge?

Consideration of the experimental results has led to the conclusions which follow.

(1) Independence of the afterdischarge from total stimulus current.

The results of varying the stimulus pulse-duration show that both the probability of starting an afterdischarge and the duration of the afterdischarge are independent of the total current delivered to the cortex. In fact, the independence of the afterdischarge from the total amount of current delivered to the cortex during stimulation is borne out by most of the experiments where the nature of stimulation was under investigation. It can be seen from the results obtained by varying the stimulus strength that at any strength greater than threshold the total amount of current delivered to the cortex has no effect upon the afterdischarge. (p. 20) The experiments where the stimulating frequency was varied, and the experiments with potentiating pulses, show that the probability and the duration of an afterdischarge can vary over

a wide range with stimulus conditions, although the total current delivered to the cortex be kept constant. Furthermore, neither the locale nor the polarity of stimulus has been seen to affect the after-discharge. (p. 25,24)

These results confirm the view that the afterdischarge is a biological response to stimulation, and not an artefact due to injury of cells in the cortex as might be inferred from the results of other workers, such as Adrian and Matthews, (1934) or Marshall, Nims and Stone, (1941).

It is therefore concluded that both the probability of producing an afterdischarge, and the duration of the afterdischarge, are independent of the total stimulus current. The results of experiments concerned with stimulus strength (p. 20,Fig. 6) show that the production of the after-discharge is dependent, in an all-or-nothing fashion, upon the excitation of a minimum number of certain neurones in the cortex.

(2) Dependence of the afterdischarge upon the number of stimulating pulses.

The total number of stimulating pulses delivered to the cortex during the period of stimulation is seen to have a marked effect over a wide range upon the duration of the afterdischarge. (Fig. 9) In addition the duration of the afterdischarge increases, over a small range, with an increase of stimulus pulse duration. (Fig.7b) It is reasonable to assume that, over the range shown in Fig.7b, the number of times which the neurones at the focus are excited by the stimulus increases with the stimulus pulse duration. The results, therefore, show that the production of the afterdischarge is dependent upon a threshold number of driven dis-charges at the focus, and that the duration of the afterdischarge increases

with increasing numbers of these driven discharges.

Since an increase in the total number of exciting pulses cannot alter the spread of current, an increase in the total number of pulses cannot excite a larger number of neurones. This must mean that the neurones at the excited focus give rise to longer afterdischarges the more times they are excited. If it be assumed that cumulative excitation increases the state of fatigue or depolarization of the neurones, then a direct relationship is seen between the degree of depolarization of the neurones and the duration of the afterdischarge. Since a threshold number of pulses is necessary to produce an afterdischarge, the production of the afterdischarge must be critically dependent upon a threshold state of depolarization or fatigue. Once this critical state has been reached, an afterdischarge will occur.

It is therefore concluded that an adequate focus for the initiation of an afterdischarge requires that at least two critical conditions be fulfilled. The first is that a minimum critical number of neurones be excited to give all-or-nothing discharges with each stimulus pulse. The second condition is that a critical minimum number of all-or-nothing discharges be driven from the minimum number of neurones. If it be assumed that the number of driven discharges is linked to the degree of depolarization of the neurones, then a system may be postulated where the focus for an afterdischarge consists of a critical minimum number of neurones driven into a critical state of depolarization.

(3) <u>The chronic net depolarization of the neurones involved</u>.

It has been postulated that the paroxysmal afterdischarge is mediated by a system of neurones which undergoes a state of fatigue, or

depolarization, when excited by repetitive stimulation. It has also been suggested that the degree of fatigue increases with an increasing number of driven discharges at the focus. From the results of experiments concerned with stimulus strength, and from those concerned with the number of stimulating pulses, it appears that stimulation forces some neurones in the cortex into what might be called an "epileptogenic state". Furthermore, the results of varying the stimulus pulse frequency (p.22) show that if more than 200 milliseconds is allowed to elapse between the driven discharges of the neurones at the focus the paroxysmal after-discharge will not occur. (i.e. pulse frequencies lower than 5 per second do not produce afterdischarges) This strongly suggests that the postulated "epileptogenic state" of the neurones wears off in 200 milliseconds. It also suggests that the epileptogenic state will accumulate when the neurones are excited more rapidly than five times per second. The results have shown that stimulation changes the mean potential of the cortex in a fashion that indicates the depolarization of structures deep in the cortex. (p.28) It is clear that the greater the number of neurones excited by the stimulation the greater will be the change in mean potential. Since the production of the afterdischarge requires that a minimum number of neurones be excited at the focus, there is apparently a relationship between the degree of depolarization of neurones in the cortex and the production of the afterdischarge. This suggests that the duration of the afterdischarge increases with increasing numbers of stimulating pulses because the degree of depolarization accumulates with each driven discharge of the neurone. The concept of cumulative depolarization is strongly favored by the fact that even large numbers of stimulating pulses fail to produce an afterdischarge,

unless the pulse frequency is above five per second. It would thus appear that the proposed "epileptogenic state" can be equated with the degree of depolarization of the neurones at the focus.

For the reasons given in the foregoing paragraphs of this section, the neurones which mediate the production of the paroxysmal afterdischarge are postulated to undergo a cumulative depolarization with each driven discharge. This depolarization is accompanied by a change in mean potential of the cortex, and this change has been shown to persist from the end of stimulation until after the paroxysmal afterdischarge has terminated. (p. 32) Such results are consistent with the view that the afterdischarge is mediated by a population of neurones which undergo a cumulative depolarization when excited by repetitive stimuli. Should the stimulation result in an afterdischarge, the neurones do not recover completely from their depolarized state until after the paroxysmal activity has ended. (see p .32) Thus the neurones are subjected to a chronic state of net depolarization which begins at the end of the stimulation and ends after the afterdischarge is terminated.

(4) Location of the neuronal structures involved in the afterdischarge.

The distribution with depth of the radial potential gradient of the cortex, measured immediately before the afterdischarge breaks out, has been plotted at the focus and is shown in Fig. 13. The observed distribution exhibits a negative peak at 0.8 mm below the surface, and a rather broad positive peak close to 1.6 mm deep. Since these potentials are the result of the depolarization of structures deep in the cortex, it appears that the parts of the structures closest to the surface are more depolarized by the stimulation than are the deep parts. In fact, a

distribution of the radial potential gradient such as is seen in Fig. 13 would result from the existence in the cortex of a potential dipole. If a dipole were radially oriented in the cortex, with the negative end 0.8 mm below the surface, and the positive end 1.6 mm below the surface, then the distribution of the radial potential gradient would be similar to that seen in the figure. Burns (1957) has mentioned the results of measuring the potential gradients produced by artificial dipoles placed in a conducting medium. When the artificial dipole was oriented in the experimental bath in a fashion which corresponds to the orientation of the dipole postulated here to exist in the cortex, the distribution of the empirically-determined potential gradients was quite similar to the distribution shown in Fig. 13.

Thus it is possible to postulate that the stimulation which initiates the afterdischarge depolarizes a system of neurones in the cortex in such a fashion that a virtual potential dipole is created. This dipole, and hence the neuronal structure, is radially oriented in the cortex, and lies between 0.8 mm and 1.6 mm below the surface. The positive end of the dipole lies deepest in the cortex.

The fact that the deep end of the dipole is positive with respect to its upper end suggests that the neuronal structure recovers from depolarization more rapidly at its deep end than at the end nearer the surface. If this be the case, then there would be a potential gradient across the neuronal structure during the whole of the afterdischarge. This is in keeping with the known result that the neurones involved in the afterdischarge exhibit a degree of depolarization throughout the afterdischarge.(p. 32)

The results of measuring the distribution with depth of the amplitude of the repetitive discharge potentials at the focus show that these discharges are largest at a depth of 1.25 mm in the cortex. This depth, then, must be the region where, at any given instant, the greatest number of neurones involved in the afterdischarge are discharging in such a way as to produce rapid changes in the mean cortical potential. This region, located between the ends of the dipole is just that region where the repetitive discharge would be expected to occur if the paroxysmal afterdischarge were caused by the differential repolarization of the neurones. (see p. 7) The results of recording the activity of single neurones in the cortex show that the cell bodies of the neurones involved in the paroxysmal afterdischarge are located mostly close to the region where the bottom of the dipole is found.

For the foregoing reasons, it is concluded that the paroxysmal afterdischarge is mediated by a system of neurones in the cortex which lies between 0.8 and 1.6 mm below the surface. Repetitive driven excitation of the neurones which comprise the system leaves them in a state of depolarization which builds up with each driven discharge. The lower end of these neurones repolarizes more rapidly than the superficial end. This results in the establishment of a potential dipole in the cortex, with the positive end of the dipole situated at the deep end of the neuronal structure.

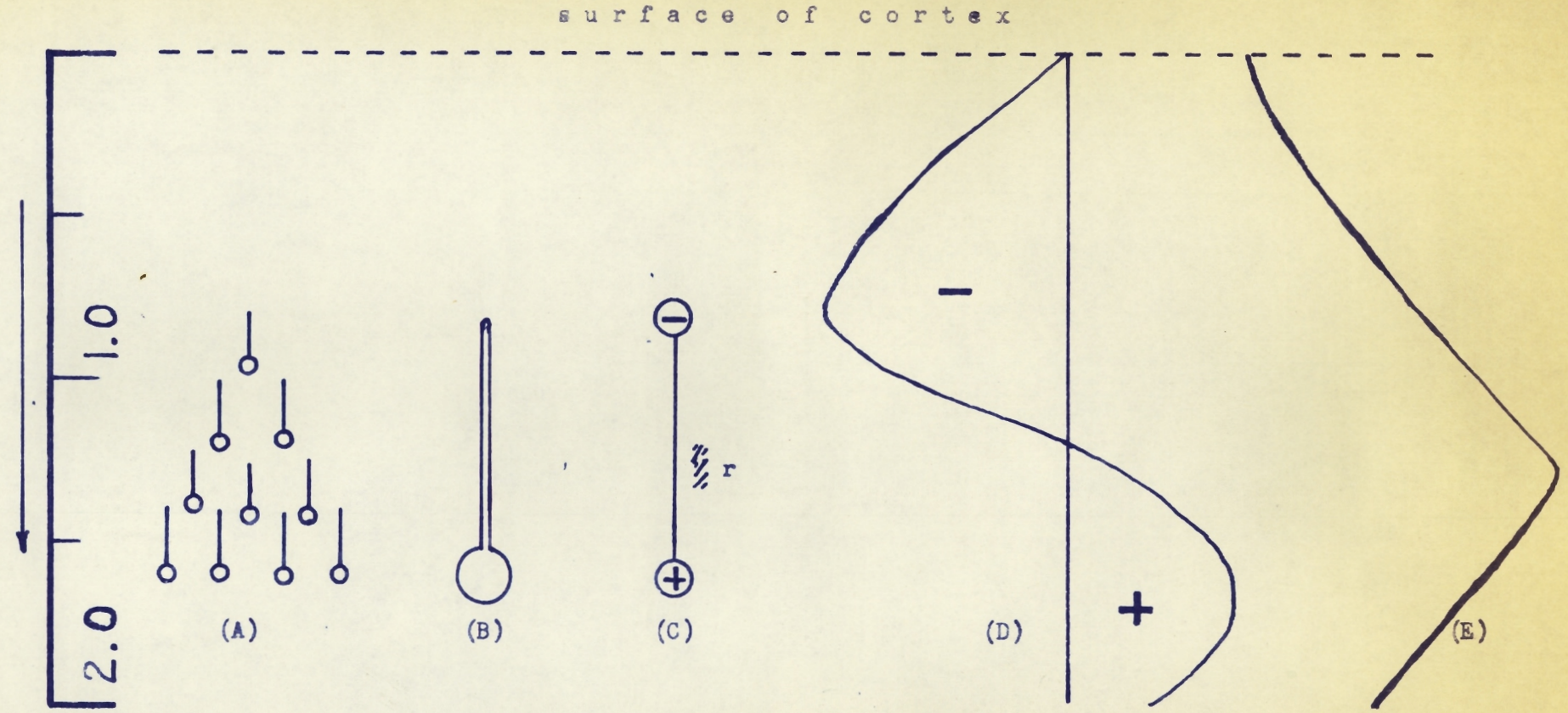
No direct evidence is available as to whether the proposed neuronal system consists of one continuous unit which stretches over the length postulated. One alternate possibility is that the dipole consists of a contiguous system of neurones situated in the region where the dipole is found.

This system would have the smallest percentage of its neurone population at the top of the dipole, and the largest concentration of neurones at the deep end. A system consisting of a single continuous neuronal structure seems to agree more favorably with the evidence available from this study. The fact that unit potentials are found mainly below 1.2 mm deep suggests that only very few cell bodies of neurones involved in the afterdischarge are found above this depth. Furthermore, a non-continuous system of neurones would not be expected to give curves as smooth as those obtained here for the distribution of radial potentials with depth.

Fig. 17 shows a diagrammatic representation of the neuronal schemes suggested here. On the same diagram the postulated dipole and its resultant radial potential gradients have been plotted. The curves can be seen to resemble the distributions with depth of the radial potential gradients in the cortex, as measured in this study.

(5) <u>The mechanism of differential repolarization applied to the neurones involved.</u>

The conclusions arrived at up to this point permit the description of a system of neurones which mediate the paroxysmal afterdischarge through a mechanism consistent with the results of this study. It has already been concluded here that the neurones which mediate the afterdischarge do not repolarize uniformly throughout their structures, but that the deep ends of these neurones repolarize faster than the superficial parts. Since this is the basis for afterdischarges which are due to differential repolarization, (see p. 6) it is therefore pertinent at this point to examine the agreement between the general properties expected for such afterdischarges, and the



(A) = a contiguous system of neurones which could form the dipole (C)

(B) = one continuous neurone, which could form the dipole (C)

(C) = the postulated dipole

(D) = the radial potential gradient expected for a dipole corresponding to (C)

(E) = radial potential gradient expected for repetitive discharges arising in the region "r" in (C)

Fig. 17

actual properties of the paroxysmal cortical discharge investigated in this study.

Inspection of the form of more than 300 recorded afterdischarges, obtained from 32 different animals, reveals certain properties which may be directly compared with the criteria as outlined on pages 8-9. These properties are enumerated here, for comparison, in the same order as were the criteria.

(a) There is no direct evidence as to whether or not the driven discharges produced by the stimulation need be at any particular region of the radially-oriented neuronal structure. However, it has been shown that there is no preferential location, on the cortex or isolated slab, for the stimulation to produce the afterdischarge. (p. 25) This suggests that the production of the afterdischarge is independent of the geometry of the exciting currents in the cortex.

(b) The amplitude of the paroxysmal activity recorded from the surface of the cortex during an afterdischarge is independent of the strength of stimulation used to elicit the afterdischarge. In addition, it is impossible to adjust the conditions of stimulus strength to affect the duration of the afterdischarge in any but an all-or-nothing fashion. (p.20)

(c) The average latent period between the end of stimulation and the beginning of the afterdischarge is greater than the average interval between the first two repetitive discharges. This is true whether the recorded activity is from single cells (p.33) or is a record of the change in mean potential of the cortex. (pp. 16-17)

(d) Examination of the gross form of the afterdischarge has already

shown that the maximum frequency of the repetitive potential discharges occurs when the afterdischarge is about one-third over. (p.17, Fig.3)

(e) Towards the end of the afterdischarge the frequency of the repetitive discharges slows down to a more or less constant rate, and then ends abruptly. (Fig.3)

(f) No direct evidence is available from this study that the afterdischarge is critically dependent upon the presence of an adequate gradient of membrane potential along the radially oriented neuronal structure. However, the results strongly suggest that the production of the afterdischarge is linked to the establishment of a net chronic depolarization of the neurones at the initiating focus. The results further suggest that there is a relationship between the degree of the depolarization and both the probability of producing the afterdischarge and its duration.

Further evidence is available that the paroxysmal afterdischarge requires an adequate gradient of membrane potential along the length of the neurones involved. If the afterdischarge be recorded as a radial potential gradient, using direct-coupling as in Fig. 4a,iii, then the repetitive potential discharges are seen to be superimposed upon a dome-shaped envelope of slowly changing potential. The peak of this slow potential is in coincidence with the occurrence of the largest potential discharges of the afterdischarge. The afterdischarge ends abruptly, but the slow potential envelope continues to decline. (Fig. 4a,iii) This is exactly what would be expected to occur if the production of the afterdischarge depended upon an adequate membrane potential gradient. Furthermore, this offers a clue as to why the afterdischarge ends so abruptly. The termination of the afterdischarge coincides

with the declining portion of the slow potential envelope. This suggests that the termination of the afterdischarge coincides with the time when the membrane potential gradient has declined to the extent where it can no longer maintain the afterdischarge.

The resemblance, outlined in paragraphs (a) to (f) between the general properties of a differentially repolarizing afterdischarge and the properties of the afterdischarges observed in this study are in good agreement with the hypothesis that the paroxysmal afterdischarge on the cortex is mediated by a population of differentially repolarizing neurones in the cortex.

A system, consistent with the results obtained in this study, which explains the paroxysmal afterdischarge in terms of the theory of differential repolarization can now be offered here. This system consists of neurones situated between 0.8 mm and 1.6 mm deep in the cortex. Repetitive stimulation of these neurones results in a cumulative net chronic depolarization of their membranes. When the stimulation has ended, recovery of the membrane potential begins at the deep end of the neuronal structure, and is complete there before the membrane potential of the superficial end has recovered. This sets up a potential gradient between the deep end of the neurone and its superficial end, thus causing current to flow from the recovered portion of the neurone to the depolarized portion above. Should the current flow reach a critical level, the lower end of the neurone will discharge, and begin to repolarize once more, thus re-setting the conditions for further discharges. These discharges, occuring simultaneously

in a population of neurones, give rise to the electrical activity recorded on the cortex during a paroxysmal afterdischarge. (see p.14)

The peak amplitude of the repetitive discharges at the focus has been shown to occur at a depth of 1.25 mm. It has already been mentioned (p. 46) that the region where the largest repetitive discharge potentials are recorded is expected to correspond to that region where the largest number of discharges from the individual neurones originate. This places the origin of the discharges somewhere between the differentially-repolarizing regions of the neuronal structure, rather than at either extreme. This is in agreement with the work of Frank, (1956) who found the same for frog's veratrinized muscle cells. The fact that the first potential discharge occurs deep in the neuronal structure accounts for the fact that the first phase of the repetitive discharge, recorded monopolarly at the surface is always positive. (p.28)

(6) Discussion of the results of some other investigators.

Not only is there agreement between the conclusions regarding differential repolarization and the results obtained in this study, but there is agreement with the results of other workers. Goldring and O'Leary (1950) have shown that a surface-positive polarization of the cortex could initiate the paroxysmal afterdischarge. The paroxysmal afterdischarge took place only after the polarizing current had been removed. A polarizing current in the direction described would result in the depolarization of structures near the bottom of the gray matter in the cortex, and hence could be expected to initiate the differential repolarization cycle of the neurones. Such a cycle would begin only after the polarizing current had been removed, as was the case.

The same workers found that the paroxysmal afterdischarge could be stopped by a period of surface-negative depolarization. A current in this direction could hyperpolarize the neuronal structures near the bottom of the cortical gray matter, and thus prevent the depolarization of the lower end of the neurone from causing an action potential when excited by the radial currents arising due to differential repolarization.

It is, of course, possible that the surface-negative depolarization caused an unintentional period of spreading cortical depression. This would terminate the afterdischarge whatever the mechanism of its mediation. Hence the work of Goldring and O'Leary is not presented here as corroborative proof of the postulated mechanism of differential repolarization, but rather as evidence that the hypothesis is consistent with the results of other workers.

Further evidence of work that is consistent with the conclusions arrived at in this study has been published by Burns (1955) in a description of his model neurone. The results of varying the stimulus parameters for the model neurone are very similar to the results obtained here for the same experiments on the cat's cortex. The model neurone was designed so that stimulation would cause a chronic "depolarization" of potentials in the model which corresponds to membrane potentials of actual neurones. Chronic depolarization of the "membrane potential" of the model neurone sets up repetitive discharges by a mechanism analogous to the mechanism postulated here for differential repolarization. The similarity between the results for the electronic model and the actual cortex again suggests that the experimental results, as well as the conclusions arrived at here

are consistent with a system consisting of neurones which repolarize differentially.

(7) Some alternate theories to explain the paroxysmal afterdischarge.

Certain theories, other than that of differential repolarization, may be presented to explain the paroxysmal afterdischarge. Among these, the theories of reverberating chains of neurones, and of a persistent humoral transmitter are prominent. However, certain negative evidence is available which strongly suggests that the two latter phenomena cannot be responsible for the afterdischarge investigated in this study.

Recordings of unit cell activity show that, during an afterdischarge, cells close to the focus discharge paroxysmally for some time before repetitive potential discharges are seen on the cortex. (Fig.14) This would be distinctly characteristic of a differentially repolarizing afterdischarge, since a reverberatory chain of neurones is not capable of producing repetitive discharges at one link in the chain without exhibiting repetitive discharges throughout the reverberating system. In the case of a persistent humoral transmitter, the activity would again be expected to break out simultaneously over large areas of the focus, and hence give rise to an afterdischarge which has no latent period following the stimulation, and whose frequency of repetitive discharge is greatest at the beginning of the afterdischarge. This is the case, for example, with the afterdischarge produced from stimulation of the interneurones of the anterior horn of the spinal cord, an afterdischarge which has been proven to be the result of a persistent humoral transmitter. (Renshaw, 1941 and 1946; Eccles, Fatt and Koketsu, 1954; Burns, 1957). The afterdischarge recorded in this study does not have these characteristics.

Thus it is very unlikely that the cortical paroxysmal afterdischarge

is the result of either reverberating chains of neurones, or of a persistent humoral transmitter.

It has been shown that the paroxysmal afterdischarge can be explained by the theory of differential repolarization. This theory is consistent with the results obtained in this study, and is consistent with the results obtained by other workers. There is good agreement between the properties of the afterdischarges studied here and the general properties expected from afterdischarges which result from differential repolarization. At the same time, other theories which might explain the afterdischarge are not consistent with the results. It is therefore concluded that the paroxysmal afterdischarge on the cerebral cortex is due to the differential repolarization of neurones in the cortex.

(8) Refractory period of the neurones involved.

The results of experiments done to investigate the stimulus frequency show that the duration of the afterdischarge decreases with frequency when frequencies higher than 180 pulses per second are used. (Fig.8) In the range of strength and numbers of pulses used in this study, the afterdischarge was not produced by frequencies higher than 400 pulses per second. When these results are compared with the results of the experiments using potentiated pulses,we find a noticeable agreement. A train of pulses with a potentiating pulse placed 2 milliseconds from the first was no more effective in producing an afterdischarge than a train of non-potentiated pulses. When the spacing was increased to 3 milliseconds,an afterdischarge could be produced if high stimulus strength was used. (p.25) Since the

production and duration of an afterdischarge has been linked to the number of times which neurones at the focus are stimulated, it is not difficult to conclude that pulses which fall closer together than 3.0 milliseconds fail to excite the neurones every time a pulse is delivered, but rather do excite them every second or third pulse. Furthermore, a pulse which is delivered 2.0 milliseconds or less after the neurones have been excited will fail to re-excite the neurones at any strength.

This leads to the conclusion that the neurones responsible for the paroxysmal afterdischarge have a relative refractory period of about 3.0 milliseconds and an absolute refractory period of 2.0 milliseconds or less.

(9) The hyper-excitable phase of the neurones involved.

The results suggest that stimulation of the cortex with a train of potentiated pulses results in an afterdischarge most readily when the potentiating pulse is spaced about 25 milliseconds from the first pulse. The curve of frequency vs. duration of afterdischarge (Fig.8) shows that a pulse frequency of 70 per second (which corresponds to a pulse interval of 14.3 milliseconds) produces the longest afterdischarges. The curve of frequency vs. duration of afterdischarge, made at very low stimulus strengths, shows peaks at multiples of 20 p.p.s. (Fig.11) There is, of course, an interval of 50 milliseconds between pulses at this frequency. It has already been concluded that the production and duration of the afterdischarge is dependent upon the number of times which the neurones at the focus are excited. It appears that pulses which are spaced from 14.0 to 50 milliseconds apart are most likely to excite the neurones each time a pulse is delivered.

This implies that the neurones responsible for the afterdischarge

exhibit a phase of hyper-excitability which persists for about 50 milliseconds after excitation. Such a phenomenon has been described for other known neurones. (Adrian and Lucas, 1912; Morgan, 1943)

(10) The spread of the afterdischarge away from the focus.

No experiments directly concerned with the spread of the afterdischarge were made in this study. However, some of the results obtained do make it possible to suggest some elements of a mechanism whereby the activity of the afterdischarge spreads out from the focus.

Fig. 15 shows that the distribution with depth of the repetitive discharge potentials has much the same form remote from and at the focus. (p.34) The peak amplitude of these potentials remote from the focus is found only 0.15 mm below the depth where the peak amplitude is found at the focal area. The phase relationships of the afterdischarge change identically with depth both remote from and at the focus. (p. 34) Such results suggest that the neurones which carry the activity of the afterdischarge across the cortex belong to the same population as the neurones which comprise the initiating focus.

If the spread of the afterdischarge is mediated by the same type of neurone responsible for the initiation at the focus then these neurones will repolarize differentially, and maintain their own afterdischarge, once they are excited by impulses arising from the focus. Thus an afterdischarge which spreads is much more likely to be prolonged than an afterdischarge which does not spread far from the focus. This is consistent with the results obtained at threshold strengths. (p. 20, Fig. 4b)

It is therefore suggested that the neurones which carry the spread of the afterdischarge belong to the same population of neurones which initiate the afterdischarge at the focus. There is not enough evidence, however, to firmly conclude this. More direct experiments upon the mechanism of spread should be done in order to clarify this point. The possible nature of such experiments will be discussed in the following section.

INDICATIONS FOR FUTURE WORK

There is strong evidence in favor of the theory of differential repolarization to explain the paroxysmal afterdischarge on the cortex. However, a critical experiment which would conclusively establish that the afterdischarge is dependent upon differential repolarization has yet to be done. Whether or not such an experiment can be devised remains to be seen. A useful approach would be to establish whether or not the production of the afterdischarge is dependent upon a critical radial potential gradient in the cortex. To this end, an experiment could be done in order to plot a family of curves, measured at different depths in the cortex, of the relationship between stimulus strength and the mean radial potential gradient of the cortex. This would require a preparation which remains in good condition for several hours, a matter of some difficulty. Rapid and skillful preparative surgery would be mandatory, and certain aids to the animal's biological condition might be necessary. This could include blood-transfusion and automatic heat control.

The records of unit cell activity made in this study are only preliminary. It will be important to extend the work done with this technique. Further investigation will demand a classification of the cells in the cortex which become involved in the afterdischarge, determination of their distribution with depth, and a description of the relationship between the discharges of single cells and cortical potentials recorded during an afterdischarge.

It has been concluded from the results that the paroxysmal afterdischarge is mediated by neurones situated between 0.8 mm and 1.6 mm below the surface of the cortex. It would be useful to find the region in the

cortex where the excitability of such neurones is greatest. Stimulation of the cortex at different depths could be done with miniature stimulating electrodes. These could be either semi-micro metal electrodes insulated down to the tip, or saline-filled microelectrodes similar to the ones used in this study for recording. Such experiments would not only determine the depth of the cortex at which it is easiest to produce a paroxysmal afterdischarge, but would also provide an answer to the question of whether a single cell, of the type postulated here to mediate the afterdischarge, is capable of responding to repetitive stimulation with an afterdischarge. If single cells are found to be capable of maintaining an afterdischarge this would be strong evidence for the theory of differential repolarization.

Parallel with experiments where deep stimulation is done, a study should be made of the effects upon the spread of the afterdischarge when graded cuts are made to different depths in the cortex. Burns and Grafstein (1952) have shown that such cuts are useful in determining the geometry of the structures which transmit electrical activity across the cortex. It has been postulated in this study that the spread of the electrical activity during an afterdischarge is mediated by neurones belonging to the same population as the neurones which comprise the initiating focus. Experiments with graded cuts, coupled with the deep stimulation experiments, might shed further light upon the mechanism of spread.

It is felt that future work, along the lines suggested in this section, will make a definite contribution to the elucidation of the physiology of the paroxysmal afterdischarge.

SUMMARY

1. A study has been made of the paroxysmal afterdischarge on the cerebral cortex of the cat. Experiments were made upon the intact and neuronally-isolated cortex, anaesthetized and unanaesthetized. A comprehensive description is given of the biological preparation and of techniques used for stimulating and recording.

2. The paroxysmal afterdischarge is not mediated by the same mechanisms which underlie either the surface-negative or surface-positive responses of the cortex. Furthermore, there is no first-order relationship between the afterdischarge and spreading cortical depression.

3. Both the probability of producing the afterdischarge and the duration of the afterdischarge are independent of the total current delivered to the cortex during the period of stimulation. This confirms the view that the afterdischarge is a biological response to stimulation, and not an artefact due to current flowing in the cortex during the stimulation.

4. Two critical conditions must be fulfilled before an afterdischarge can occur. The first is that a minimum critical number of neurones be excited to give all-or-nothing discharges with each stimulus pulse. The second condition is that a critical minimum number of all-or-nothing discharges be driven from the minimum number of neurones.

5. The duration of the afterdischarge depends over a wide range upon the number of driven discharges at the initiating focus. This supports the view that the duration of the afterdischarge depends upon the degree to which the neurones responsible for the paroxysmal afterdischarge are

fatigued or depolarized.

6. Repetitive stimulation, of the type which produces an afterdischarge, results in a cumulative fatigue of the neurones responsible for the afterdischarge. The fatigue persists from the end of the stimulation until after the afterdischarge has ended. This chronic fatigue is recorded as a change in the mean radial potential gradient of the cortex.

7. The chronic fatigue of the neurones responsible for the afterdischarge creates a virtual potential dipole in the cortex. This dipole establishes the location in the cortex of the neuronal structures which belong to the population of neurones comprising the initiating focus. This population of neurones is found to lie between 0.8 and 1.6 mm below the surface of the cortex. Recordings of single-cell activity suggest that the majority of the cell bodies lie below 1.2 mm deep in the cortex.

8. The repetitive discharge potentials which occur during an afterdischarge originate from a region 1.25 mm below the surface of the cortex. This is close to mid-way between the boundaries of the region where the initiating focus is located.

9. There is evidence to support the view that the neurones responsible for the paroxysmal afterdischarge do not repolarize uniformly along their length, and that a potential gradient is created between the ends of the neurones when they have been depolarized by repetitive stimulation. It is postulated that this potential gradient causes current to flow between the differentially-repolarized ends of the neurones, and initiates the paroxysmal afterdischarge.

10. There is close similarity between the properties of the after-discharges recorded in this study and the properties expected for afterdischarges which are due to differential repolarization. Other theories which might explain the afterdischarge are not consistent with the results obtained here.

11. The neurones responsible for the paroxysmal afterdischarge have well-defined parameters of excitability. They have an absolute refractory period of 2 milliseconds or less, and a relative refractory period of about 3.0 milliseconds. The same neurones exhibit a phase of hyper-excitability which persists for about 50 milliseconds after excitation.

12. The results suggest that the neurones which carry the activity of the afterdischarge across the cortex belong to the same population as the neurones which initiate the afterdischarge at the focus.

13. Proposals have been made for future work which could clarify certain aspects of the mechanisms which underlie the production and propagation of the paroxysmal afterdischarge.

BIBLIOGRAPHY

Adrian, E.D. (1936)
The spread of activity in the cerebral cortex.
J. Physiol. 88, 127-161.

Adrian, E.D. & Lucas, K. (1912)
On the summation of propagated disturbances in nerve and muscle.
J. Physiol. 44, 68.

Adrian, E.D. & Matthews, B.H.C. (1934)
The interpretation of potential waves in the cortex.
J. Physiol. 81, 440-471.

Berger, H. (1929)
Über das Elektrekephalogram des Menschen.
Arch. f. Psychiat. 1929. 87, 527.
(cited by Brazier, 1951)

Brazier, Mary A.B. (1951)
The electrical activity of the nervous system.
Sir Isaac Pitman & Sons, Ltd. London, England.

Bremer, F. (1935)
Quelques propriétés de l'activité électrique du cortex cérébrale"isolé".
C.R. Soc. Biol., Paris., 118, 1241-1244.

Burns, B. Delisle (1949)
Some properties of the cat's isolated cerebral cortex.
J. Physiol. 110, 9P.

Burns, B. Delisle (1950)
Some properties of the cat's isolated cerebral cortex.
J. Physiol. 111, 50.

Burns, B. Delisle (1951)
Some properties of isolated cerebral cortex in the unanaesthetized cat.
J. Physiol. 112, 156-175.

Burns, B. Delisle (1954)
The production of after-bursts in isolated unanaesthetized cerebral cortex.
J. Physiol. 125, 427-446.

Burns, B. Delisle (1955)
The mechanism of after-bursts in cerebral cortex.
J. Physiol. 127, 168-188.

Burns, B. D. (1957)
The mammalian cerebral cortex.
Edward Arnold & Co.,
London, England.
(in press)

Burns, B.D., Frank, G.B., and Salmoiraghi, G. (1955)
The mechanism of after-discharges caused by veratrine in frog's skeletal muscles.
Brit. J. Pharmacol. and Chemotherapy, 10, No. 3, 363.

Burns, D., and Grafstein, B. (1952)
The function and structure of some neurones in the cat's cerebral cortex.
J. Physiol. 118, 412.

Caton, R. (1875)
The electric currents of the brain.
Brit. M.J. 2, 278.
(cited by Penfield & Jasper, 1954)

Eccles, J.C. (1951)
Interpretation of action potentials evoked in the cerebral cortex.
E.E.G. Clin. Neurophysiol. 3, 449-464.

Eccles, J.C., Katz, B. & Koketsu, K. (1954)
Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones.
J. Physiol., 126, 524-562.

Echlin, F.A., Arnett, V., and Zoll, J. (1952)
Paroxysmal high voltage discharge from isolated and partially isolated human and animal cortex.
E.E.G. Clin. Neurophysiol. 4, 147-164.

Frank, G.B. (1956)
The mode of action of veratrine on skeletal muscle.
Ph.D. thesis,
Dept. of Physiology,
McGill University,
Montreal.

Frank, G.B. (1957)
personal communication.

Fritsch, R., & Hitzig, E. (1870)
Über die elektrische Erregbarkeit des Grosshirns.
Arch. f. Anat., Physiol. u. wissensch. Med. 37.
(cited by Penfield & Rasmussen, 1950)

Goldring, S. & O'Leary, J.L. (1950)
Experimentally derived correlates between E.C.G. and steady cortical potential.
J. Neurophysiol. 14, 275-288.

Grafstein, B. (1954)
Spreading depression in isolated cerebral cortex.
Ph.D. thesis,
Dept. of Physiology,
McGill University,
Montreal.

Henry, C.E. & Scoville, W.B. (1952)
Suppression-burst activity from isolated cerebral cortex in man.
E.E.G. Clin. Neurophysiol. 4, 1-22

Jackson, J.H. (1870)
A study of convulsions.
Reprinted in "Selected writings of John Hughlings Jackson". Ed. by J. Taylor, London,
Hodder & Stoughton, 1931.
(cited by Penfield & Jasper, 1954)

Jasper, H.H., & Erickson, T.C. (1941)
Cerebral blood-flow and pH in excessive cortical discharge induced by metrazol and electrical stimulation.
J. Neurophysiol. 5, 333-347.

Kristiansen & Courtois (1949)
Rythmic activity from isolated cerebral cortex.
E.E.G. Clin. Neurophysiol. 1, 265.

Leao, A.A.P. (1944)
Spreading depression of activity in the cerebral cortex.
J. Neurophysiol. 7, 359-390.

Lennox, M.A. & Robinson, F. (1951)
Cingulate-cerebellar mechanisms in the physiological pathogenesis of epilepsy.
E.E.G. Clin. Neurophysiol. 3, 197.

Li, C., & Jasper, H.H. (1953)
Microelectrode studies of the electrical activity of the cerebral cortex in the cat.
J. Physiol. 121, 117-140.

Li, C., McLennan, H., & Jasper, H.H. (1952)
Brain waves and unit discharges in cerebral cortex.
Science 116,656-657.

Lorente de Nó, R. (1938)
Analysis of the activity of the chains of internuncial neurons.
J. Neurophysiol.1, 207-244.

Marshall, C., Nims, L.F. & Stone, W.E. (1941)
Chemical changes in cerebral cortex following local thermocoagulation and local freezing.
Yale J. Biol. & Med., 13, 485-488.

Morgan, C.T. (1943)
Physiological psychology.
McGraw-Hill,
New York.

Moskowitz, S., & Racker, J. (1951)
Pulse techniques,
Prentice-Hall, Inc.,
New York.

Penfield, W., and Jasper, H.H. (1954)
Epilepsy and the functional anatomy of the human brain.
Little, Brown & Company,
Boston.

Penfield, W., and Rasmussen, T. (1950)
The cerebral cortex of man.
The MacMillan Co.,
New York.

Renshaw, B. (1941)
Influence of discharge of motoneurones upon excitation of neighbouring motoneurones.
J. Neurophysiol. 4, 167-183.

Renshaw, B. (1946)
Central effects of centripetal impulses in axons of spinal ventral roots.
Ibid. 9, 191-204.

Renshaw, B., Forbes, A., and Morison, B.R. (1940)
Activity of isocortex and hippocampus: electrical studies with micro-electrodes.
E.E.G. Clin. Neurophysiol. 3, 74-103.

Sherrington, Sir Charles S. (1906)
The integrative action of the nervous system.
Yale University Press,
New Haven & London.

Sloan, N., & Jasper, H. (1950)
The identity of spreading depression and "suppression".
E.E.G. Clin. Neurophysiol. 2, 59-78.

Tasaki, I., Hagiwara,S., and Watanabe, A. (1954)
Action potentials recorded from inside a Mauthner cell of the catfish.
Japanese J. Physiol. 4, 79-90.

Tower, D.B. (1956)
The neurochemistry of seizures.
Chapter XIII in "Neurochemistry"
ed. by Korey, S.F. & Nurnberger, J.I.
Cassell & Co.
London, England.

Tower, D.B., & Elliott, K.A.C. (1952)
Activity of acetylcholine system in human epileptogenic focus.
J. Appl. Physiol. 5, 375.

van Harreveld, A., & Stamm, J.S. (1953)
Spreading cortical convulsions and depressions.
J. Neurophysiol. 16, 353-366.

Ward, A.A., Schmitt, R.P., & Thomas, L.B. (1955)
Some properties of single epileptic neurones.
E.E.G. Clin. Neurophysiol. 8, 167.