# TRANSMISSION CHARACTERISTICS OF THE SURFACE-POSITIVE BURST RESPONSE

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by

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TO

UNNUR ANNA,

MY WIFE

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## ABSTRACT

Transmission characteristics of the surface-positive burst response were studied in isolated slabs of cat cerebral cortex as a means of inquiry into the functional organization of the cerebral cortex.

The principal data collected were measurements of transmission delays of the response at several recording electrodes arranged in a linear array down the length of the slab of cortex. Stimulation was carried out at either end of the slab. Delays in both direction were compared between the several recording points and over the whole length of the slab.

It was found that there is a preferential direction of transmission for the response in any given slab and that the velocity from point to point along the length of a slab is not uniform. Responses recorded serially at a point distant from the stimulating electrode were similar to each other in shape, amplitude and duration. Transverse cuts into the slab only temporarily abolished transmission of the response provided that the underlying white matter, or a small part of the superficial layers of the cortex, remained intact. From this and other data it has been concluded that the surface-positive burst response spreads as a wave of excitation through a highly organized network of functional groups of neurones, but that alternate pathways for spread become available when the local network is interrupted. The significance of such a network is discussed.

RESUME

Cette étude a porté sur les caractéristiques de la transmission corticale cérébrale qui sont engendrées à la surface durant la phase positive (surface-positive burst response) suivant une stimulation électrique. Les expériences ont été réalisées "in situ" ches des chats, sur une portion de cerveau rectangulairement découpée. Ces expériences ont eu pour but d'investiguer l'organisation fonctionnelle du cortex cérébral.

Les résultat recueillis consistèrent principalement dans l'évaluation des délais de transmission qui furent enregistrés par plusieurs électrodes disposées le long de cette portion du cortex. Les stimulations furent appliquées successivement aux deux extrémités de cette portion.

Les délais enregistrés dans une direction et dans l'autre entre les différentes electrodes ainsi que sur toute la longueur de cette portion isolée furent comparees entre eux.

Les expériences ont démontré que la transmission de la réponse jouissait d'une direction préférentielle et que la vélocité d'un point à un autre n'était pas uniforme. Les séries de réponses obtenues à la même distance de l'électrode stimulatrice démontrèrent une similitude marquée quant à la forme, l'amplitude et la durée. Une section transverse exécuté dans le même tissue abolirent temporairement la transmission des réponses à la condition que la matière blanche sous-jacente ou qu'une petite partie des couches superficielles demeurent intacts.

Ces résultats et d'autres faits expérimentaux portent à

travers un réseau de groupes fonctionnels de neurones hautement organisés; mais lorsque ce réseau local est interrompu une solution de rechange s'offre, permettant la transmission de l'excitation. Une discussion commente la signification d'un tel réseau.

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# INTRODUCTION

# INTRODUCTION

This thesis presents some of the transmission characteristics of a unique response to direct electrical stimulation in isolated slabs of cats' cerebral cortex. This response was first described in the intact anaesthetized cortex of the rabbit and cat by Adrian in 1936 which he called the "deep" or "surface-positive" response. The same or a similar response was described in the isolated cerebral cortex of the unanaesthetized cat by Burns in 1951 which he called the "burst response to direct cortical stimulation" or the "surface-positive burst response".

This project was originally undertaken to study the relationship between the passage of the positive wave at the surface of the cerebral cortex and the unit activity displayed by neurones deep within the cortex. These neurones were believed to be mediators of the response in question (Adrian, 1936; Burns, 1949, 1950, 1951, 1958; Burns, Grafstein and Olszewski, 1957). Initially, experiments were conducted to determine the threshold parameters of stimulation for the response in preparations used in this laboratory. Unexpectedly first consideration of the results of these early experiments suggested two important findings not in agreement with the observations of previous investigators. Firstly, it became apparent that there was a preferred direction of transmission of the response in the slab of cerebral cortex, and secondly, the transmission velocity of the response down the length of the slab in either direction was not constant from point to point. Further experiments were done to determine transmission pathways and again the results were in conflict with currently-held views. The results also suggested that the transmission of the surface-positive

burst response might normally best be served by a horizontally layered organization of neuronal elements, but is not necessarily dependent upon such an organization in all situations. Moreover, the results suggested that the passage of the response was not by means of random interaction between the constituent neurones. Rather it resulted from interactions within an ordered system of neurones and consequently gave rise to the consistent pattern of transmission observed. Finally, experiments were conducted to study the original question of the relationship between unit activity and the passage of the surface-positive wave.

In this thesis will be presented the methods used to obtain and analyze the experimental results which led to the above conclusions. A system of interacting neurones possibly mediating transmission of the surface-positive burst response under the conditions imposed by the experimental design will be outlined. This system will be discussed with reference to the findings presented in this thesis and to the work of previous investigators. From the results of the experimental work and from discussion of the model suggested in this thesis, certain inferences will be made concerning the functional organization of the cerebral cortex. These are presented in the final section of this thesis.

# HISTORICAL REVIEW

## HISTORICAL REVIEW

The main purpose of this review is to bring together the pertinent work describing those experimental observations which have defined the surface-positive burst response to direct cortical stimulation as a distinct entity. The interpretation and significance of previous observations will be considered, for the most part, in relation to the discussion of findings presented in the RESULTS section of this thesis. Some early studies will be presented briefly to underline their historical value and to give at least some small recognition to the ingenuity, experimental foresight and intuitive capabilities of the early workers whose contributions have laid the foundation of modern experimental neurophysiology. It is probably just as true in Science, as in Politics that, "those who cannot remember the past are condemned to repeat it" (Santayana) and for this reason careful consideration of previous work is a productive and useful labour on the part of any investigator.

The nervous system is probably the most complex and yet least understood physiological organization known. Much progress has been made in the elaboration of the mysteries of its peripheral divisions but it has resisted, on the whole man's inquiries into its central domains. The most accessible and most studied central area, besides the spinal cord, has been the cerebral cortex.

Among the Ancient Greeks, Herophilos and Erasistratos (Brazier, 1959; Head, 1963) suspected the central function of the brain in the nervous system and the role of nerves in sensation and movement. Nevertheless, these notions were lost among the anatomical and functional

doctrines of Aristotle and Galen that held scientific sway for so many centuries (Brazier, 1959). By the 18th century, many of these latter beliefs were being critically examined. In 1760, Haller concluded from the results of mechanical and chemical irritation of the brain that the cortex was insensitive to stimulation, movement and sensation being focused in the white matter (Brazier, 1959). Convulsive movements in frogs after brain stimulation was reported by Fontana in 1757 and by Caldani in 1784 (Brazier, 1959). By 1890 Rolando had recorded similar results in pigs, goats, sheep, dogs and birds. Flourens (1824) and Magendie (1825) supported Haller's views on the inexcitability of the cerebral cortex, after having failed in their attempts to reproduce Rolando's results (Brazier, 1959). Cabanis (1830) provided positive evidence for the excitability of the cerebral cortex when he provoked convulsive movements in muscle groups the location of which depended on the cortical region irritated (Brazier, 1959). Decisive proof of cortical excitability was derived from the work of Fritsch and Hitzig (1870) who were able to map out the motor cortex of the dog and monkey by electrical excitation (Adrian, 1939; Brazier, 1959; Pinsky, 1961). This evidence, along with Hughlings Jackson's and Broca's correlations between clinical and pathological findings in epilepsy and aphasia (Jackson, 1958; Head, 1963) greatly helped to dispel the older ideas that the cerebral cortex was inexcitable and too highly organized to admit regional differences (Adrian, 1939). Jackson's work led Ferrier (1873) to develop more fully the concept of cortical excitability (Adrian, 1939; Brazier, 1959; Head, 1963). Much credit must go to Caton who in 1875, using Thompson's reflecting galvanometer was the first to record slow potential fluctuations from the exposed

cortex of the rabbit (Caton, 1875; Adrian, 1939; Brazier, 1959). Caton also demonstrated that potential swings related to sensory stimulation could be superimposed on the slow potential fluctuations of the cortex and realized the significance of this for cerebral localization studies. Unfortunately, both Jackson's and Caton's work received little attention at the time despite their demonstration and discussion in front of prominent audiences (Brazier, 1959). Jackson was accustomed to say, "It generally takes a truth 25 years to become known in medicine" (Head, 1963) and it was probably no more true than in this instance. Prawdicz-Neminski in 1925 (Adrian, 1939; Brazier, 1959) used a string galvanometer to record current changes from the exposed cortex in dogs and found a regular fluctuation which persisted in the absence of movement or sensory stimulation. He was able to recognize two wave patterns similar to those described later by Berger in man. He concluded from his observations of spontaneous activity and response to stimuli that cortical neurones have a "special tendency towards periodic activity." Later, Berger in 1929 was able to demonstrate this same tendency through the unopened human skull (Adrian and Matthews, 1934; Brazier, 1959). Berger established that the electrical activity originated from neuronal tissue and that it changed with age, sensory stimulus and physicochemical alterations of the body. He was the first to record from the exposed human cortex and the first to record during a major epileptic seizure in man. The period which began with Berger's work was especially important to the development of neurophysiology as a discipline. Concepts had changed radically - the idea of the inexcitability of the brain had given way to recognition of its excitability by a variety of means; electrical activity was now regarded as arising in the cortex and not

in the white matter; supposedly simple wave forms recorded from the cortical surface were now realized to be highly complex; the effects of a variety of pharmacological agents were recognized to have a modifying influence on cortical activity.

New developments in equipment at that time, such as the cathode-ray tube and increasingly sophisticated electronic amplifiers, greatly aided development in this field. The contributions of many workers put forth during that period were to define the direction of research in this discipline for many years to come. Among those who deserve special mention for their creative and thorough scientific endeavours and for the relevance of their work to the investigations presented in this thesis, are Bartley, (Bartley and Bishop, 1933a, 1933b) Bishop (Bartley and Bishop, loc.cit.; Bishop and O'Leary, 1936) and Adrian (Adrian and Matthews, 1934; Adrian, 1936, 1939). It was they who earnestly attacked the challenging questions which had arisen about cerebral cortical function. Typical of these questions were: (1) what kind of activity is characterized by the wave-forms recorded during spontaneous activity and those recorded after sensory and direct stimulation?; (2) where does this activity originate, and what function does the cortical cytoarchitecture play in the characteristics observed?; and (3) how do action potentials of individual neurones contribute to the form of a particular response?

Bartley and Bishop (1933a, 1933b) recorded a specific series of electrical waves from the optic cortex of lightly anaesthetized rabbits after stimulation of the contralateral optic nerve. They reasoned that the series of waves following a single stimulus reflected the activity of successive neurones along the visual pathway, perhaps

even of successive layers of cortical cells. Later Bishop and O'Leary, (1936) elaborated on the significance of the final, long-lasting wave recorded as positive at the cortical surface. This wave often had superimposed upon it a series of monophasic or diphasic spikes. They suggested that this main surface-positive wave resulted from the discharge of both superficial and deep pyramidal cells. The work of Bartley and Bishop (1933a, 1933b) is notable in several respects: first, this positive-going wave, evoked sensorily has been considered by Burns (Burns, 1951) as being similar to the positive response to direct cortical stimulation seen by both Adrian (Adrian, 1936) and by himself (Burns, 1949, 1950); secondly, they introduced a variety of techniques, many of which were somewhat unsuccessful in their own experiments, but which have been used with modifications subsequently by many investigators (Rosenblueth and Cannon, 1942; Burns, 1950, 1951; Burns and Grafstein, 1952; Grafstein and Sastry, 1957) in studying electrical phenomena in the cerebral cortex. Bartley and Bishop attempted for example, to record the surface response after direct cortical electrical stimulation but were generally unsuccessful in demonstrating any specific activity. They were probably the first to attempt to isolate, both partially and wholly, portions of the cerebral cortex in an effort to localize the origin of spontaneous cortical activity. They introduced the technique of superficial transverse cortical cuts and, in doing so, showed that the spread or conduction of an evoked response across the cut region could be abolished. These two workers recognized several experimental problems which were to plague investigators for years. They realized the problem of interpreting evoked responses superimposed on spontaneous activity, noting that if the responses were

small, they might be lost in the background activity. The variable effect of anaesthesia on portions of the evoked response was also mentioned by them as a complicating factor in analysis of results.

Probably the most definitive work on the response of the cerebral cortex to direct stimulation was that of Adrian in 1936. Earlier, Adrian and Matthews (1934) had studied the stimulating effect of local injury to the rabbit and cat cerebral cortex. The characteristic sequence of potential waves seen enabled them to infer that there were certain time relationships in neuronal activity, but this was at the expense of using a rather drastic form of stimulation. The results of Adrian's experiments with direct electrical stimulation of the rabbit, cat and monkey cerebral cortex were published in 1936 and defined several specific responses to which all subsequent authors refer. Until that time, most information concerning the reaction of the cerebral cortex had come from observations on the peripheral effects of motor area stimulation. Notable exceptions to this generality were the experiments by Dusser de Barenne in 1936, (Adrian, 1939; Burns, 1958) on the effects of thermocoagulation of different layers of the cortex and the previously described work by Bartley, Bishop and O'Leary. The effects of sensory stimulation and convulsant drugs on the cortical potentials had also been investigated in a variety of ways (Adrian, 1936).

Working from the premise that electrical changes in the cerebral cortex provide the most direct index of its activities, Adrian (1936) stimulated the rabbit brain electrically and recorded the resulting responses at the cortical surface. He found that there were

characteristically two types of response namely superficial and deep depending on stimulus strength. The strength of a single shock required for the development of the deep response (positive-going with respect to the surface) was usually two to three times the threshold strength for the superficial (or negative-going) response. The deep response was resistant to the repeated stimulation or superficial thermocoagulation that abolished the superficial response. From the results obtained he concluded that the deep or surface-positive response was due to the formation of dipoles orientated perpendicularly to the cortical surface with their positive ends more superficially situated. He assumed that on stimulation the bodies of the deeper pyramidal cells were activated directly while the slender apical part of the cells remained inactive or positive to the active or negative basal portion, thus forming a relatively positive region on the cortical surface.

By using several recording electrodes, Adrian was able to show that with each stimulus a wave of potential change spread out from the stimulated point. The wave spread as a widening circle, and was approximately uniform except in regions of local injury or where there had been prolonged stimulation. This observation was the first indication that local injury or fatigue may alter transmission of a propagated response. Later, he stated that the wave front was seldom a perfect circle, and in doing so, gave the first hint that the velocity of transmission of such a propagated response was not constant from point to point. The conduction velocity of this response over a 5 cm portion of cortex was found to be around 25 cm/sec with a range of 5 to 60 cm/sec. Adrian suggested also that the velocity of the response may initially have

been faster as it spread out from the stimulated point; that is, it travelled over the cortex with a nonuniform velocity. Another pertinent observation by Adrian which was to be verified in subsequent investigations was that a short pause in stimulation produced an immediate rise in velocity, and a failure of stimulus allowed the next wave to travel much faster. In the same context, the velocity of transmission declined with higher stimulus frequencies. He explained these observations by saying that because the waves travelled slowly, the pathway did not have sufficient time to recover, and that the rate of recovery was diminished as stimulation continued. Rosenblueth and Cannon (1942) also found that the spread of the deep response slowed with repetitive stimulation.

Anaesthetized animals were favoured initially because of the reduction by the anaesthetic of background activity however, it was found that many responses were adversely affected by the presence of a depressant agent. Anaesthetic-free preparations more closely approached the physiological state, but spontaneous activity with its attendant problems in interpretation also increased. In 1935, Bremer devised his now famous cerveau isolée preparation in cats. Transection of the brainstem at the midcollicular level served two purposes: firstly, it eliminated a large amount of sensory input as a source of potential variations in cortical structures, and secondly, with the cutting of the second afferent neurone fibres mediating the reception and appreciation of somesthetic sensation, the animal could be used ethically without continuing anaesthesia. After completion of this procedure the only interpretive complications were those arising from the visual and olfactory activity and the widespread cortical, subcortical and thalamic

interactions. This problem was solved to a large extent when Burns (1949) and Kristiansen and Courtois (1949) working independently developed a technique for isolating a slab of cerebral cortex from all neuronal connections while leaving the blood supply intact through the pial vessels. The complete neuronal isolation of a small portion of cerebral cortex was the logical outcome of numerous experiments to determine the contribution of various brain structures to surface cortical activity.

There was considerable controversy over the question whether the spontaneous rhythmic activity of the cerebral cortex was an inherent property of that tissue or whether it depended upon the interaction of the cortex and subcortical structures. Bremer (1949) favoured the idea that the cortex had an inherent rhythmic activity, and the results gained by Kristiansen and Courtois (1949) in isolated cortical slabs in lightly anaesthetized cats supported this view. The later results of Ingvar (1955a) in isolated cortical slabs of unanaesthetized curarized animals also supported Bremer's hypothesis. Henry and Scoville (1952) and Echlin, Arnett and Zoll (1952) recorded burst activity, or "paroxysmal high-voltage" discharges, from partially and completely isolated portions of cerebral cortex in man. Burns found that isolation of a section of cortex in chloralose-anaesthetized cats produced an essentially "silent" slab (Burns, 1949, 1950, 1951). In some instances however, transient spontaneous activity did occur, and this, he suggested, was due to injury foci within the slab or else, was the result of the initial electrical stimulation. The spontaneous activity in these cases disappeared with time or could be abolished by thermocoagulation or the application of procaine to the offending area if it

could be found with exploring electrodes. Burns suggested that the electrical silence was a reflection of the completeness of isolation (Burns, 1950, 1951) whereas Kristiansen and Courtois (1949) proposed that it was the result of adverse conditions arising during surgery. Further comment on this aspect of cortical excitability will be found in the RESULTS section.

Burns, in the years between 1949 and 1958 (Burns, 1949, 1950, 1951; Burns and Grafstein, 1952; Burns, Grafstein and Olszewski, 1957; Burns, 1958) exploited the usefulness of the isolated cortical slab preparation in characterizing several responses to direct cortical stimulation. In his earliest studies using chloralose-anaesthetized cats, Burns observed that when single stimuli of gradually increasing strength reached a level greater than 60 to 70% of that required to elicit the maximal surface-negative response, a second response appeared. During this response, the surface area involved became positive with respect to the surrounding tissue (Burns, 1949, 1950). He termed this event the surface-positive response and felt that it was comparable to the deep response of Adrian (1936). The response was characterized as a single wave of short duration, spreading from the stimulated point, to involve the whole isolated area. This wave spread in all directions without decrement and with a velocity of about 15 cm/sec. At low frequencies of stimulation, the response seemed independent of frequency and showed neither fatigue nor facilitation. The response appeared to be all-or-none in character, and could be initiated by the summation of two subthreshold stimuli. Recognizing the role that anaesthesia played in modifying cortical responses, Burns extended his earlier observations on the surface-

positive response in chloralose-anaesthetized cats to unanaesthetized cats whose brainstems had been transected (Burns, 1951). Burns also found that these unanaesthetized preparations were electrically inactive without direct stimulation, and that the stimulus strength required to elicit the surface-positive response was only 30 to 35% greater than that required for the maximal surface-negative response. Many parameters were similar to those of the chloralose-anaesthetized slabs; for instance, the response was all-or-none and had a conduction velocity of 10 to 20 cm/sec. One important difference, however, was that in the anaesthetized preparation the surface-positive response was a single spreading wave of short duration while in the unanaesthetized preparation the positive wave lasted for 0.5 to 5.0 sec and was invariably accompanied by a complex discharge which ended with the end of the positive wave. The form of this discharge suggested that it was due to the repetitive, and also at times, synchronous discharge of many cells. This synchronization was presumed to have produced the short periods of almost sinusoidal potentials sometimes observed. Burns termed the composite response, consisting of this oscillatory phenomenon superimposed upon the general positivity, the surface-positive burst response. He observed that, with increasing levels of anaesthesia the stimulus threshold required to elicit the surface-positive burst response was raised and the duration and amplitude of the response reduced, but the velocity of spread was unchanged. Similarly, with increasing anaesthesia the amplitude and magnitude of the asynchronous discharge was reduced but the frequency of the repetitive discharge was not affected. Full surgical anaesthesia abolished response completely. The results relating to the use of anaesthetics

were mimicked by reducing the oxygen supply to the brain.

Burns concluded from his results that the surface-positive burst response was transmitted in a single, deep lying, interconnected layer of cells. Burns held that excitation of this layer resulted in a prolonged, asynchronous discharge of the constituent cells at a frequency of 60 to 75/sec. This discharge, he postulated, was maintained by self re-excitation of these cells within a closed chain.

In 1952 Burns and Grafstein suggested that the surface-negative response seemed to be very artificial and difficult to interpret; however, they considered the surface-positive burst response to be a component of normal physiological activity, since its magnitude and duration were more dependent upon local cortical conditions than upon the nature of the evoking stimulus. That a positive wave originating deep in the cortex might be a more physiological response had been alluded to by other authors (Bartley and Bishop, 1933b; Adrian, 1936). Spontaneous activity similar to the burst response has been recorded from isolated cortex in cat, monkey, dog and man (Burns, 1951; Echlin, Arnett and Zoll, 1952; Henry and Scoville, 1952). The investigations of Burns and Grafstein were designed to provide more information about the location and shape of neurones giving the two responses. They divided the surface-positive burst response into a "steady" component and an oscillatory potential which have been described above. Using an electrode to probe beneath the surface of the cortex and a reference electrode at an indifferent point, they measured the change in potential with depth, and concluded that the active region producing the steady component of the surface-positive response was between 0.8 and 1.3 mm beneath the surface. They felt that these results tended

to confirm Adrian's dipole hypothesis (Adrian, 1936). By plotting the mean peak-to-peak values of the oscillatory potentials over a fixed arbitrary time during the response, they found two maxima, one at or very near the surface and one at a depth of 1.1 mm. At an intermediate depth where the magnitude of the oscillatory potential was minimum, Burns and Grafstein found that the steady potential changed polarity.

When the response was recorded with the reference electrode on the brain's surface directly above the probing electrode, the potential distributions of the two components were the same. They reasoned from these observations that both components of the surface-positive burst response were due to activity in the same population of neurones.

In other experiments, in which they made cuts of varying depth into the cortex of the slab Burns and Grafstein found that the surface-positive burst response passed all cuts made to a depth of 1.0 mm but would not pass a final cut of 1.25 mm. Very superficial cuts decreased the transmission velocity of the surface-positive burst response and cuts exceeding 0.5 mm often prevented spread of the response for a minute or two. Cuts made from the underside of the slab, and which left a small portion of superficial cortex intact, abolished transmission of the response into the region beyond the cut. These observations led them to conclude that the superficial axonal connections of the deep cells which they believed to be carrying the response were not sufficient to mediate transcortical spread.

The superficial application of procaine did not alter the transmission of the response to remote areas of the slab, but at the point of application both the surface-negative response and surface-

positive burst response decayed somewhat. The oscillatory portion of the surface-positive burst response was more affected by the application of the local anaesthetic.

All this evidence enabled them to develop an idea of the morphology of the neurones giving the surface-positive burst response. They were conceived as having deep-lying cell bodies with dentritic trees making synaptic connections laterally with similar cells. Burns and Grafstein supposed them to have in addition radially running processes almost reaching the cortical surface. This morphological picture was supported by observations of unit activity. By stimulating at different depths the region with the lowest threshold to stimulation was found to be slightly below that where the peak in the potential distribution of the steady component of the response was found, or about 1.7 mm beneath the surface. The cells so identified in these experiments were named "type-B" neurones (Burns and Grafstein, 1952). Burns (1951) had earlier assumed that the surface-positive burst response occurred only when superficial cells, believed to mediate the surface-negative response, transmitted excitatory impulses to the type-B neurones just described. Burns and Grafstein ruled out the necessity of this intermediate step and postulated, that instead, the radially orientated processes of type-B neurones could be excited directly by surface stimulation.

Burns, Grafstein and Olszewski (1957) attempted to identify histologically the so-called type-B neurones which had been proposed as mediators of the surface-positive burst response. They were unsuccessful in this endeavour but concluded, from the results of unit activity, that the previously proposed picture of type-B neurones was too simple. Their results suggested to them that the surface-positive

burst response excited the largest neurones whose cell bodies lay in all cortical layers except layer 1 and the lower part of layer 6. They classified the neurones identified into two functional groups: (1) "primary" type-B neurones mediating the tangential spread of the surface-positive burst response; and (2) "secondary" type-B neurones which discharged during the burst response, but did not contribute to the spread of activity. The evidence for primary type-B neurones had been previously presented by Burns and Grafstein (1952). Evidence gained from chronic isolation experiments (Burns, Grafstein and Olszewski, 1957; Grafstein and Sastry, 1957) indicated that the largest pyramidal cells of layer 5 are not part of the primary type-B neurone population. The surface-positive burst response could, however, still be elicited from the chronic preparations. Burns et al (1957) believed that the local surface-positive burst phenomenon seen in chronic preparations was mediated by secondary type-B neurones which were normally excited by the primary type-B neurones. They concluded that there was a net of primary type-B neurones essential for tangential transmission of excitation and that the primary neurones could activate secondary type-B neurones the number of which depended on the state of excitability of the latter.

In view of the important physiological mechanisms suggested by Burns and his colleagues, it is surprising that most subsequent studies involving the surface-positive burst response have tended to use this response as an indication of cortical viability and have not considered it for its own particular physiological significance (Frank and Sanders, 1963; Pinsky and Gabel, 1964; Sanders and Pinsky, 1964, 1967; Frank and Pinsky, 1966).

At various times in history, certain areas of study have been emphasized at the expense of what are probably equally deserving fields of endeavour. The study of the individual responses to cortical stimulation have not received their share of investigation. Examination of such responses was almost technically impossible prior to 1930. During the 1930's they received intense consideration from a select group of investigators only to be put aside for a period while more general fields such as behavioural psychology received greater attention. At this same time, neurohistology and peripheral nerve physiology were providing food for the investigative appetites of talented workers. Recognition in the 1950's of the significance of these comparatively simple responses as a basis of the more complex brain functions led to the present day situation where there now exists a better balance between fields of interest.

In conclusion, an attempt has been made to present chronologically investigations leading to the development of several concepts, the consideration of which initiated this study. Common to these investigations are the recognition and characterization of a specific, directly evoked response from the cerebral cortex, namely the surface-positive burst response. Some hypotheses regarding their possible functional significance have been briefly included. Interpretation of these investigations in greater detail has been left more appropriately to the DISCUSSION section of this thesis. The development of various experimental techniques has also been presented.

# EXPERIMENTAL METHODS

# EXPERIMENTAL METHODS

## (1) PREPARATION OF NEURONALLY ISOLATED SLABS OF CEREBRAL CORTEX

### a.) ACUTE PREPARATIONS

Anaesthesia:

Cats of either sex, weighing between 1.5 and 4.5 kg were used. The animal was placed in a closed wooden box which had a glass observation door. Anaesthesia was induced by placing a cotton wool pad soaked with ether (diethylether, Squibb) on a wire screen which connected a separate upper compartment to the rest of the box. Surgical anaesthesia was evidenced in the animal by unconsciousness, moderate skeletal muscle relaxation, and sluggishness or absence of the corneal reflex. The anaesthetized cat was transferred to the operating table and secured on its back. A cone-shaped mask, covered with linen and containing an ether-soaked pad, was held over the cat's nose and mouth to maintain anaesthesia.

Tracheostomy and Venous Cannulation:

The trachea was exposed by a vertical midline incision and then cannulated via an oblique anterior slit through the second tracheal ring. The brass cannula was secured by tying a piece of heavy string around this portion of the trachea and then was connected by a ten-inch length of rubber tube to a variable bypass ether bottle. An air intake was provided at the cannula in addition to that at the ether bottle to reduce the dead space contributed by the tube. The level of anaesthesia could be maintained by adjustment of the ether bottle bypass valve.

The right or left femoral vein was cannulated to provide a route for fluid and drug administration as required. In some early experiments, a femoral arterial cannula was also inserted to monitor blood pressure during the course of the preparation and the experiment. This procedure was discontinued in later experiments because of circulatory complications arising from the use of the anticoagulant. After these initial procedures, the cat was turned over and its head clamped in a Czermak holder (Palmer); the holder being adjusted to maintain the vertex of the skull about 20 cm above the surface of the table.

Superficial Skull Exposure:

A long midline scalp incision was made and the scalp freed from the temporalis, levator auris longis, and intermedius scutulorum muscles on both sides by blunt dissection of the interposing fascia. The levator auris longis, the intermedius scutulorum and the scalp and ear blood vessels contained in their bundle were then clamped, cut and ligated, freeing the scalp completely from the lateral aspects of the temporalis muscle down to the level of the medial aspect of the external auditory canal. A raspatory was then used to scrape the temporalis muscles from their origins to the point where they pass between the zygomatic arch and the temporal bone. The branches of the external carotid arteries supplying these muscles were found, clamped, cut and ligated. The temporalis muscles were then clamped and cut at the superior border of the zygomatic arch. Care in tying major arteries early in the preparation considerably reduced the incidence of bleeding once the animal had been moved to the recording table. One piece of temporalis muscle was saved and kept moist with physiological saline between several layers of gauze for future hemostatic use during the experiment.

Decerebration:

A #8 dental burr was used to make a rectangular hole, 12 mm by 4 mm in the left lateral wall of the cerebellar fossa. Its posterior margin was 3 mm anterior to the lambdoid ridge, its anterior margin was immediately posterior to the junction of the bony tentorium cerebelli and its superior margin 10 mm inferior to the midline prominence formed by the sagittal crest (Figure 1). Bleeding from the bone at this point and during the major craniectomies which followed was controlled by pressing bone wax (beeswax with 1% phenol) into the bony vascular sinuses. A slit was made into the dura overlying the left cerebellar hemisphere down the long axis of the cerebellar fossa craniectomy, allowing a quantity of cerebrospinal fluid to escape. A wire-loop electrocautery knife (Figure 2) was then passed into the space between the dura and cerebellum, and while being pressed tightly against the bony tentorium cerebelli, was pushed through the superior portion of the brainstem as it traverses the tentorial opening. The knife was then pressed inferiorly and withdrawn, care being taken not to drag the knife forcibly across the base of the skull and so not to compromise the basilar artery. Subsequent autopsies of cats whose brainstems had been transected in this manner showed the cut to be pointed somewhat obliquely through the midbrain at the level of the superior colliculi and to be essentially complete. On occasion, the tip of the knife passed into the medial substance of the contralateral cerebral hemisphere. It was found that this method gave as complete transection of the brainstem as other methods previously employed and did not compromise the blood supply at the base of the brain. Most importantly, this method allowed anaesthesia to be discontinued at an early stage of pre-

paration. Another desirable outcome of this procedure was that the animal usually stopped respiring at this point. This provided an opportunity to control its respiration rate and volume by means of a Palmer respiration pump attached to the tracheal cannula by a short length of rubber tubing.

Craniectomies:

A piece of temporoparietal bone, one cm square, was removed just anterior to its junction with the interparietal bone by means of a dental burr. The area was continually irrigated with warmed physiological saline to prevent air from entering the vascular sinuses before they could be packed with bone wax and to clear the operative area of bone chips.

Most of the parietal bone over both hemispheres was removed with bone rongeurs, as far posterior as the tentorium cerebelli. Large sections of the frontal bones were removed as far forward as the frontal sinuses; the superior portions of the temporal bones were taken away almost to the level of the temporal zygomatic processes. The parietal bones were usually removed across the midline. Any bleeding from the sagittal sinus due to injury or severance of communicating veins was controlled by direct ligature, by oversewing of a piece of muscle after durectomy, or by electrocautery. The dura was removed extensively with scissors after a small slit had been opened in it over the posteroinferior portion of the lateral surface of the cerebral hemisphere. Any vascular attachments, particularly those along the sulcus forming the inferior border of the suprasylvian gyrus, were electrocauterized. The exposed pia was kept moist by continuous irrigation with warmed physiological saline. The wide exposure of the cerebral hemispheres served

two useful purposes. It allowed more manoeuvrability to cut larger and longer slabs in the suprasylvian gyrus and reduced the problem of cerebral swelling sometimes encountered during the experiments. In this way there were no bony edges, most importantly along the superior margins, to compromise the pial vasculature if the brain did swell.

Neuronal Isolation:

A slab of cerebral cortex was isolated in both suprasylvian gyri by a slight modification of Burns' method (Burns, 1951). A bloodless area of the pial surface, approximately 5 mm square, was made by electrocauterization of the posterior end, or sometimes both the anterior and posterior ends, of both suprasylvian gyri. Care was taken to ensure that the electrocautery did not infringe on the central straight part of the gyrus. The brain substance under the cauterized areas was then removed by suction through a tapered glass tube until a connection with the lateral ventricle was effected. The connections to the ventricles allowed cerebrospinal fluid and blood accumulated after the decerebration to drain out and further reduce the possibility of brain swelling.

A composite knife (Figure 3) was then introduced into the posterior sink hole and gently pressed anteriorly, the prong being kept just visible beneath the pial surface, and the flat blade kept as parallel as possible to the pial surface. When the anterior ventricular opening had been reached, or when sufficient distance had been traversed down the length of the slab, the knife was removed along its original path and another knife (Figure 4) was passed along the remaining uncut margin in the long axis. In those cases where no anterior sink hole was made the anterior end of the slab was completed by the passage of the knife down

the original medial cut, then by the movement of the prong across the uncut end portion and finally by the withdrawl of the knife along the original lateral track. This procedure left the slab essentially neuronally isolated while leaving its blood supply, derived wholly from the descending pial vessels, intact. The amount of viable tissue gained in this manner was never less than 20 mm in length and 4 mm in width and depth. Histological preparations of slabs showed the isolation to be essentially complete in most slabs cut, with a cortex: slab depth ratio of seldom less than 1:2. Following isolation of slabs in both hemispheres the exposed cortex was temporarily covered with saline-soaked cotton wool and the animal transferred to the recording table.

Over the period in which results were collected for this thesis, about 25% of the cats for use in acute experiments died, either on the operating table or before supplying useful records. Brock (1967) suggested that the chances of survival were much increased if the total time of anaesthesia was reduced and it was felt that an early decerebration minimized this time in our experiments. A total time of approximately fifteen minutes elapsed from induction of anaesthesia to transection of the brainstem and hence termination of anaesthesia. Almost all those animals which survived the procedures prior to electrical recording remained viable preparations for up to 24 hours.

b.) CHRONIC PREPARATIONS

Cats of either sex weighing between 2 and 5.5 kg were used. Anaesthesia was induced by the injection of pentobarbitone sodium (British Drug House) 35 mg/kg intraperitoneally. The cat's head was loosely held in a Czermak holder to avoid compromising the respiratory tract and to prevent injury to the head. The hair over the scalp was

closely clipped and a moderately long midline incision made. The left lateral temporalis muscle was reflected laterally with a raspatory but not dissected from the scalp. An area of parietal bone over and around the suprasylvian gyrus was removed with a dental burr and bone rongeurs. The dura was reflected from above downwards; its blood supply, via the middle meningeal artery, was left essentially intact. A posterior drain hole was made and the slab was then cut in a manner similar to that described in the acute preparation. The dura was closed with a single continuous 6-0 polyester fibre suture (Mersiline, Ethicon). The temporalis muscle was returned to its original position and its superior fascia sutured across the midline to the fascia of the right temporalis muscle. Neosporin(R) (Burroughs-Wellcome) was sprayed into the open wound at this time. The skin edges were approximated and the incision closed with several Michel wound clips.

These chronic isolation procedures were done with clean but not sterile technique. The animals were kept in separate cages on the laboratory floor until well recovered, usually a period of 3-4 days. If it was felt necessary, that is, if there were signs of infection, tetracycline HCl (Tetrex, Bristol) 250 mg intramuscularly, or penicillin G (Duracillin, Lilly), 400,000 units intramuscularly, was administered daily for 3-5 days. Upon returning to the preoperative level of activity, the cats were admitted to a separate chronic colony in the main animal house. One week after operation, the Michel wound clips were removed and the animal given feline distemper vaccine (Allotab, Haver-Lockhart). These animals were kept for a period of 3-18 months before use. Generally their weights increased by 10-20% during that time.

Reexposure of Chronically Isolated Slabs for Experiments:

Reexposure of the chronically isolated slabs were initiated in a manner similar to that described for acute experiments. Ether was used as an anaesthetic, tracheostomy was performed, and decerebration accomplished before the major craniectomies. The intact side was first stripped of its temporalis muscle and a wide craniectomy performed on that side. The temporalis muscle over the chronic slab was then dissected from the bone and dura with which it had formed adhesions. A postero-inferior triangle of bone was removed from the parietal area on the chronic side and a wide craniectomy performed with rongeurs to encircle the area of the slab. The dura was removed first from the chronic side and then from the opposite hemisphere. The adhesions which had formed between pia and dura along the suture line on the chronic side demanded slow and careful dissection around the area. An acute slab was then cut in the suprasylvian gyrus of the intact side. The drain hole of the chronic slab was not reopened nor was the slab recut.

The dimensions of the chronically isolated slabs were generally somewhat smaller than the acutely isolated slabs due initially to less manoeuvrability during cutting and eventually, to shrinkage associated with gliosis (Weisman, Gorchynski and Pinsky, 1967). The overall survival rate of chronic animals was about 60%.

## (2) PREPARATION FOR RECORDING

After the cat was transferred to the recording table, the edges of the scalp incision were tied to a steel rod bent in a circle of 12 cm diameter, supported in the longitudinal plane by the same stand as that supporting the Czermak holder clamped to the cat's head.

This formed a well over the exposed cortical substance and skull into which was placed warmed mineral oil. The mineral oil pool was kept at 35°C by means of a resistance wire coil connected to a 12-volt DC source, or by directing a heating lamp towards the oil surface and switching it on intermittently as necessary. In some experiments, the oil temperature was varied from 31°C to 37°C; this variation did not seem to affect the parameters of the response being measured.

The cat lay on a perforated metal box into which warmed air was blown through a duct from a heater located outside the recording cage which surrounded the recording table. The heater operation was regulated by body temperature variations detected by a thermistor probe inserted in the cat's rectum. Rectal temperature was maintained at 36.5°C throughout the experiment.

## (3) STIMULATION AND RECORDING

### a.) STIMULATION

In all experiments the stimulation necessary to elicit a surface-positive burst response was obtained from Tektronix 160-series pulse and waveform generators. A 1:1 transformer (Hammond 835) was used to isolate stimulus current from ground. The stimulus was applied to the cortex through bipolar platinum-10% iridium electrodes placed at one or both ends of the slab. These electrodes and the recording electrodes were connected to a switching panel so that a particular directional array could be selected (Figure 5). The measured responses were produced with stimulus duration and amplitude set at 2 to 5X threshold for the response as determined initially in the experiment. The interstimulus interval was kept so that there was little or no tendency to in-

crease any spontaneous activity that might have been present; this was usually 10 to 15 seconds. (For further comments on stimulus parameters, see RESULTS). Stimulation of the isolated cortex was performed first from one end of the slab and then from the other in groups of 20-100 repetitions.

b.) RECORDING ELECTRODES

Surface Electrodes:

Electrodes used to record surface potential changes were either of the following types: (1) Monopolar nonpolarizable silk wicks, embedded in 1% agar gel in 0.9% saline, contained within but projecting from the tip of distally bent tapered glass tubes. Chlorided silver wire leads were thrust into the saline-agar gel at the wide end of the glass tube to make electrical connection between these electrodes and the recording apparatus; or (2) Chlorided silver wire electrodes, 0.4 mm diameter, mounted in a glass shaft and beaded at the end which rested on the cortical surface. Each electrode had a fine wire lead connecting it to the recording apparatus.

Deep Recording Electrodes and Electrodes for Recording Unit Activity:

Glass micropipettes of 50μ barrel diameter, 1μ to 5μ tip diameter, and 150-900 kilohm tip resistance, were used to record extracellular potentials from single cells and from the mass activity of many neurones. These electrodes were filled with 9/10-saturated sodium chloride. A chlorided silver wire lead was inserted in the end which was sealed with bone wax to prevent evaporation and salt crystallization. Contact with the recording apparatus was made via a lead from the chlorided silver wire.

## c.) TYPES OF RECORDING

Surface Potentials in Acute Experiments:

To minimize slight movements of the brain due to respiration, an unilateral or bilateral pneumothorax was produced by an incision through the 4th, 5th, or 6th intercostal space, the ribs being spread by means of a self-retaining rib retractor. To minimize decerebrate spasms, and since respiration was always maintained artifically once decerebration had been completed, gallamine triethiodide (Flaxedil, Poulenc) 2 to 5 mg/kg intravenously was routinely administered every 1 to 1½ hours.

Surface potentials were recorded using either of the two types of electrodes previously described. In the early experiments only two recording electrodes were used. One was left between the poles of the stimulating electrode and the second was moved to different distances from the stimulating electrode. The stimulating electrode was moved to either end of the slab so that recordings in both directions at the different distances could be taken. In all later experiments four recording electrodes were used, arranged in a regularly spaced linear array down the length of the slab, with a pair of stimulating electrodes at each end of the slab. There was a recording electrode placed between the poles of each stimulating electrode with the remaining two recording electrodes spaced equally down the length of the slab. This placement of electrodes effectively divided the slab into three interelectrode zones (Figure 5). Fine positioning was achieved by mounting the recording electrodes in a flexible arm attached to a Prior manipulator. A silk wick electrode similar to that described for recording surface potentials was placed on a piece of periosteum-free bone and served as

the indifferent or reference electrode for the recording system.

The cat, the electrodes and the amplifier probes were supported in a shielded enclosure on a grounded recording table. The cat was grounded by way of a saline-soaked cotton pledget inserted between its tongue and the tooth bar of the Czermak head holder. All experiments were performed in a specially constructed shielded room to minimize electrical interference from the 60-cycle power line and other stray electromagnetic fields.

Signals from all surface recording electrodes were led into a switching panel and from there into the differential cathode follower inputs of direct-coupled preamplifiers (Grass P6). Each preamplifier output was direct-coupled to an operational amplifier (Philbrick P35AU or P65AU). This permitted the amplified signal level to be adjusted for proper operation of a 4-channel frequency-modulated magnetic tape recorder. The ouput of each channel of the tape recorder was monitored during the experiment on a 4-beam oscilloscope system (Tektronix Type CA dual-trace units connected to a Tektronix 502A oscilloscope).

A 4-channel inkwriting polygraph (Grass 5B) was direct-coupled in parellel with the inputs of the tape recorder. The pen-writer record served as a means to evaluate rapidly trends in the response parameters being examined during the experiment and to monitor some aspects of the physiological state of the cortical tissue. The same record also served as a convenient experimental log (Figure 6).

Surface Potentials in Chronic Experiments:

The placement of recording and stimulating electrodes was essentially the same as in the acute experiments except that the recording electrodes often were placed closer together and moved down the

length of the slab almost as a unit. The recording system was identical with that used in the acute studies.

Unit Activity in Acute Experiments:

Glass micropipettes were mounted in a specially constructed variable hydraulic bipiston micromanipulator (Burns, 1961) attached to a Prior manipulator. Gross positioning of the micropipette, including the approach of the tip to the pial surface of the slab was achieved with the Prior manipulator. The glass micropipette was driven perpendicularly through the pia by the hydraulic micromanipulator. The point at which the tip of the glass micropipette was seen just to touch the pial surface on entry was taken as one zero point and compared with the point at which electrical ccntact was broken between electrode and brain on withdrawal. These two points were found to differ on occasion because the micropipette had pushed the pia somewhat downward during penetration and because at times there were some small fluctuations in brain volume. It was decided to accept the withdrawal figure as the most accurate in cases showing large differences. Occasionally a small amount of subpial bleeding acccmpanied the penetration of the glass micropipette but it was not a complicating factor. The micropipette was moved up and down in the cortex by means of the hydraulic micromanipulator, and recordings of unit and mass activity taken at a variety of depths. A silk wick or chlorided silver wire electrode was placed on the pia just to one side of the point of penetration of the glass micropipette to record surface potential changes over the deep area being investigated. A silk wick electrode placed on a piece of periosteum-free bone served as an indifferent electrode. Signals from the surface electrode were handled in the manner previously described for

surface recording. Signals from the micropipettes were led into the differential cathode follower input of a direct coupled preamplifier. Parallel outputs from the operational amplifier were connected to the second input of the frequency-modulated tape recorder and to a channel of the inkwriting polygraph. The operational amplifier output was coupled also through a variable time constant (0.5-2.0 msec) to a second operational amplifier. The output of the second operational amplifier was connected to the input of a gated loudspeaker for auditory monitoring and to the input of one recording channel on the tape recorder. This tape channel recorded the firing of cortical cells. Amplified signals from the surface electrode were stored on another channel of the tape recorder and on the second channel of the inkwriting polygraph. Unit activity on the output of the first channel of the tape recorder was monitored on the upper beam of an oscilloscope (Tektronix 502). The output of the second and third tape channels were monitored on the lower beam of the same oscilloscope after being passed through a dual trace preamplifier (Tektronix Type CA) (Figure 7).

(4) TRANSVERSE CUTS

Transverse cuts were made to variable depths of cortical tissue and white matter in several slabs to determine the effect, if any, on the transmission characteristics of the surface-positive burst response. These cuts were made through superficial or deep approaches.

Deep Approach:

A specially-constructed knife similar to that shown in Figure 4 but with shorter prongs of variable length was inserted through the posterior sink hole and advanced along one of the isolation cuts in the

long axis to the point where the transverse cut was to be made. The instrument was then drawn transversely across the under surface of the slab until its prong reached the opposite longitudinal cut; it was then withdrawn along that cut. Cuts through the white matter and through variable depths of cortical substance were later confirmed histologically.

Superficial Approach:

Cuts were made directly into the cortical substance by either one of two procedures. In the first of these, a specially sharpened #11 scalpel blade with a depth gauge attached was dragged transversely across the surface of the slab. This procedure produced considerable pial bleeding at times but in most instances the bleeding stopped soon after cutting. With the alternative technique, a small sink hole or stab wound was made outside the slab area but in the same gyrus at the point where the transverse cut was to be made. A flattened piece of stainless steel wire of an appropriate depth was then slid through the stab wound, beneath the pial surface of the slab but in contact with it, and advanced across the slab and into the intact tissue on the opposite side of the gyrus. The knife was then withdrawn. The second method considerably reduced the incidence of bleeding and left the pial surface intact. Depth and extent of the cuts were verified histologically in most instances. Responses were recorded before and at various times after the transverse cuts were made.

## (5) APPLICATION OF TETRODOTOXIN

Saline-soaked filter paper strips, 1 mm by 5 mm, were placed gently at right angles to the long axis of the slab between the middle two recording electrodes of a four-electrode array. Responses were

recorded before and after this procedure to test for any effect. The saline-soaked filter paper strip was then removed and replaced with a similar filter paper strip soaked in a solution of tetrodotoxin 5μg/ml (Sankyo, Tokyo). Responses to stimulation were then recorded at various intervals to determine the effect of the drug upon the transmission characteristics of the surface-positive burst response. After a variable interval the tetrodotoxin-soaked filter paper strip was removed and more responses recorded to determine the post-drug effect.

### (6) TERMINATION OF THE EXPERIMENT

At the end of the experiment, the cats were killed by disconnecting them from the respiration pump and injecting intravenously a large dose of sodium pentobarbitone.

### (7) HISTOLOGICAL STUDIES

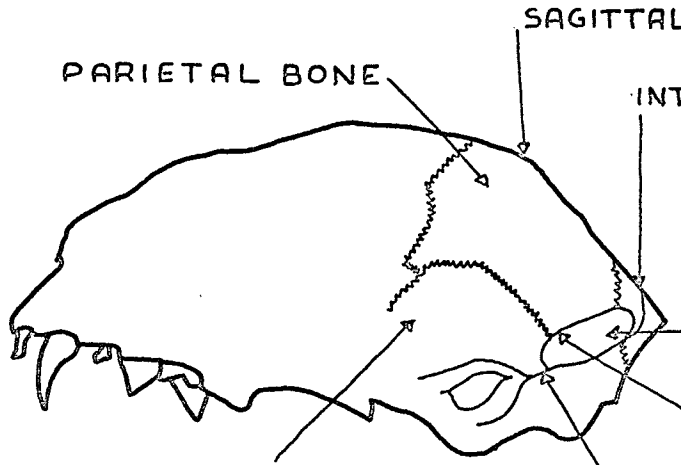
As soon as the cats were dead, large blocks of brain tissue wholly encompassing the slabs previously cut were removed and immediately placed in Osmic acid-Potassium permanganate fixative prior to being stained by a modified Golgi method (Weisman, Gorchynski, and Pinsky, 1967). The blocks were later sectioned, mounted, and studied to determine the thoroughness of isolation and the extent of the transverse cuts.

# FIGURE 1

LATERAL VIEW OF THE SKULL

ACTUAL SIZE

VIEW SHOWS THE DECEREBRATION CRANIECTOMY CUT IN THE LEFT LATERAL WALL OF THE CEREBELLAR FOSSA



SAGITTAL CREST

PARIETAL BONE

INTERPARIETAL BONE

DECEREBRATION CRANIECTOMY

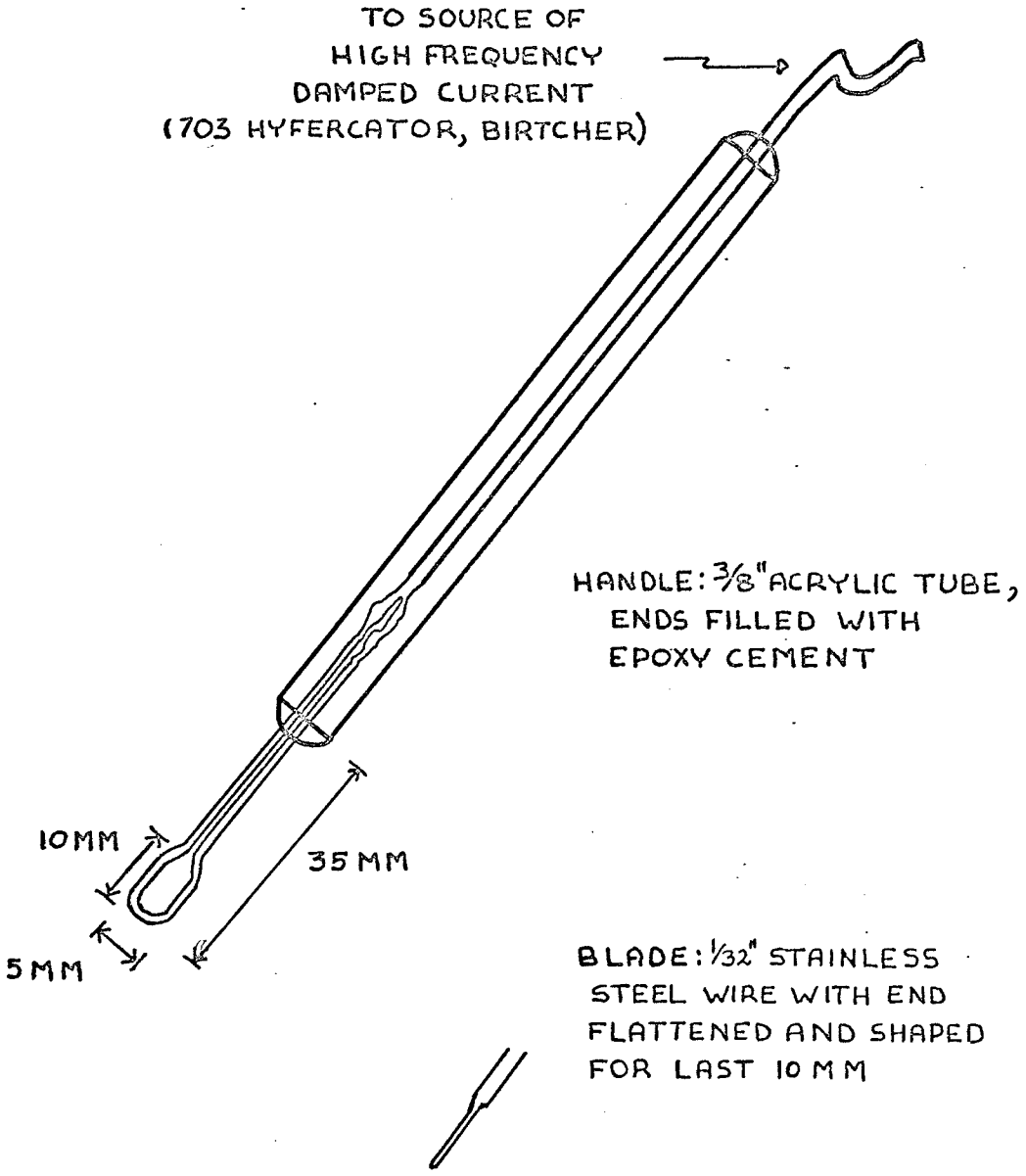
EDGE OF TENTORIUM CEREBELLI

TEMPORAL BONE

LAMBDOID RIDGE

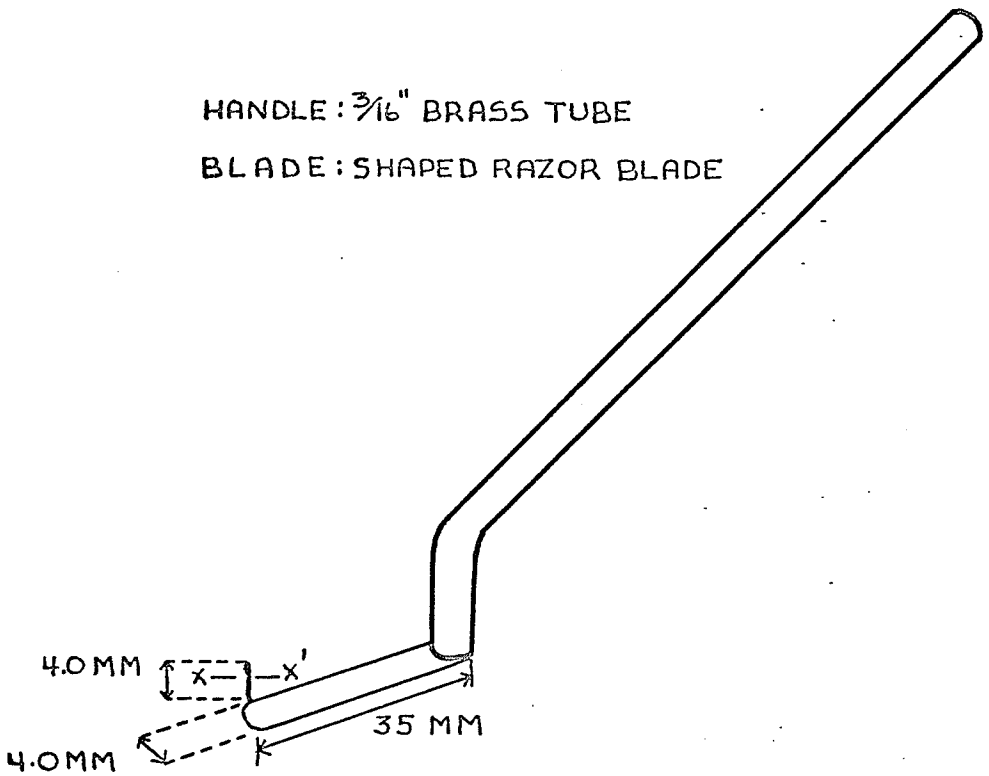
# FIGURE 2

CAUTERIZING DECEREBRATION KNIFE



# FIGURE 3

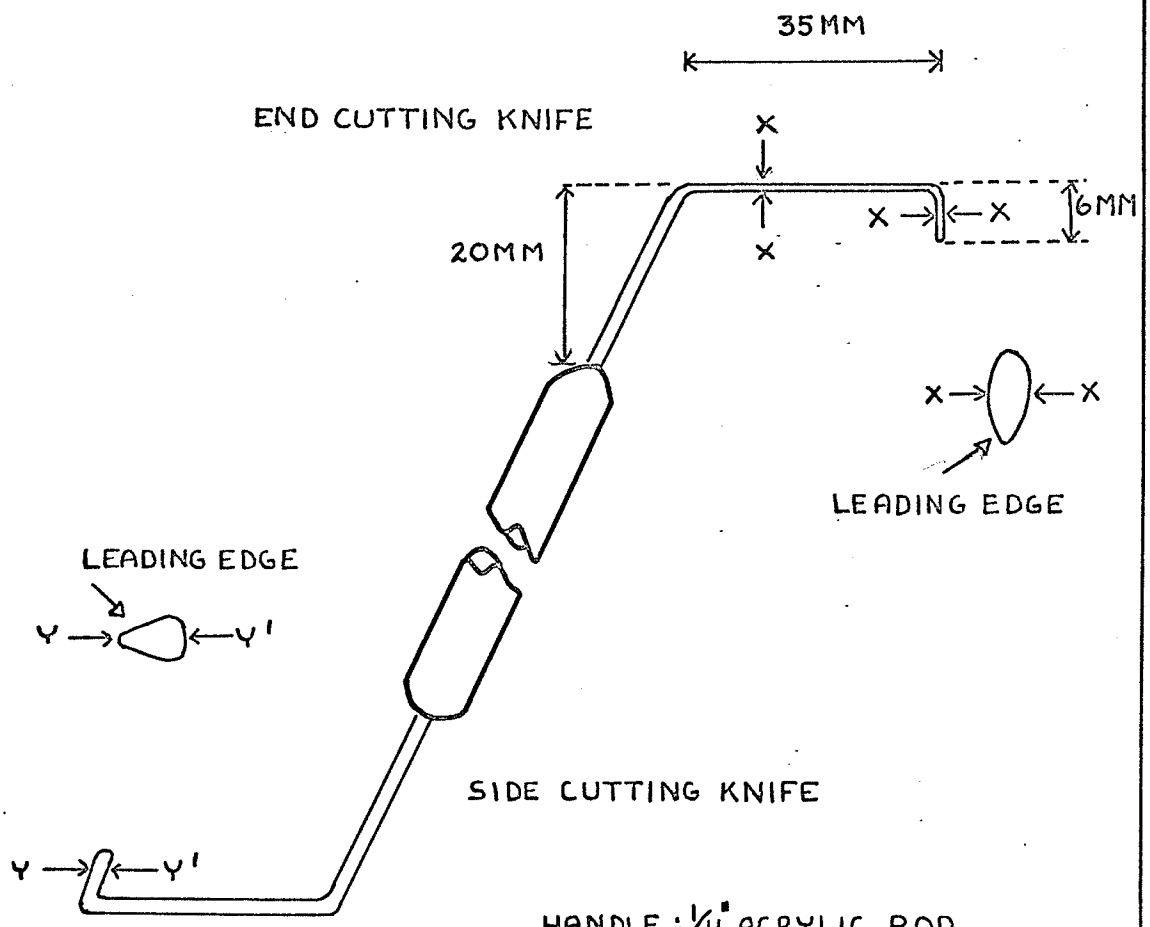
## SLAB UNDERCUTTING KNIFE





SIDE DEPTH GAUGE: 1/32" STAINLESS STEEL WIRE, LEADING EDGE CONTOURED

# FIGURE 4


35MM
END CUTTING KNIFE
X
X
X
X
6MM
20MM
X
X
LEADING EDGE
LEADING EDGE
Y
Y'
SIDE CUTTING KNIFE
Y
Y'
HANDLE: 1/4" ACRYLIC ROD
BLADES: 1/32" STAINLESS STEEL WIRE, LEADING EDGES CONTOURED
MAJOR DIMENSIONS SAME FOR BOTH KNIVES

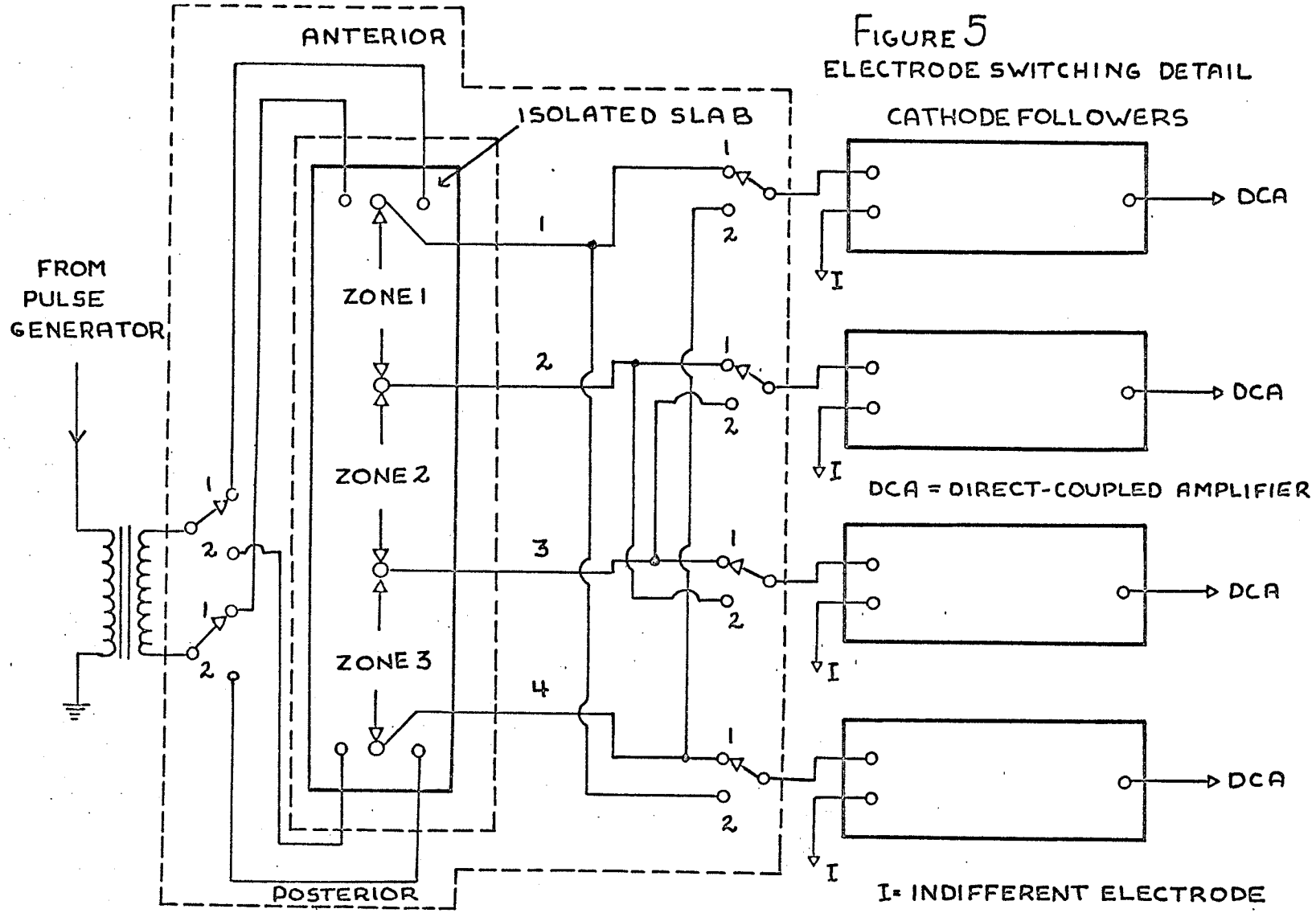



FIGURE 5
ELECTRODE SWITCHING DETAIL

DCA = DIRECT-COUPLED AMPLIFIER

I= INDIFFERENT ELECTRODE

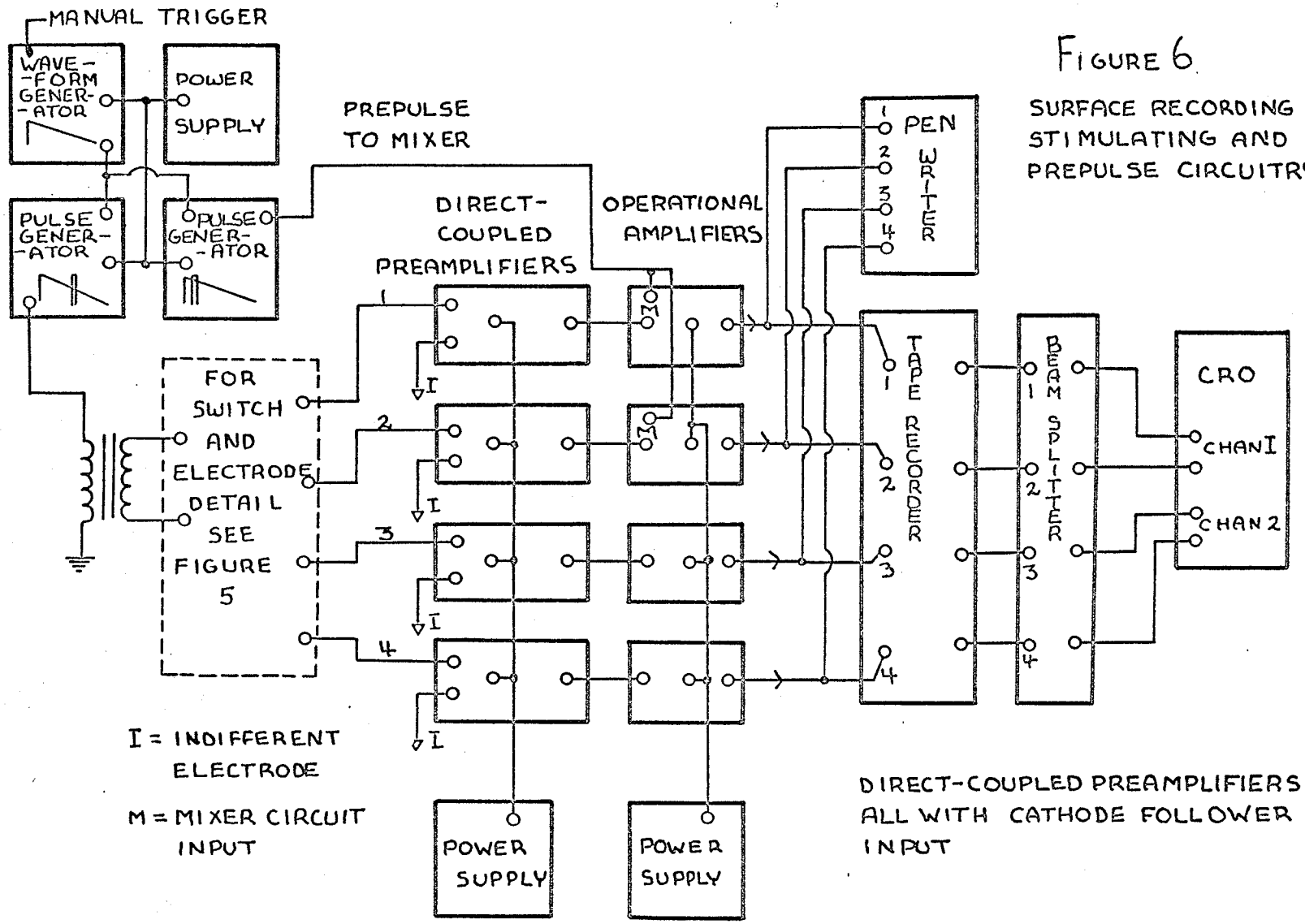
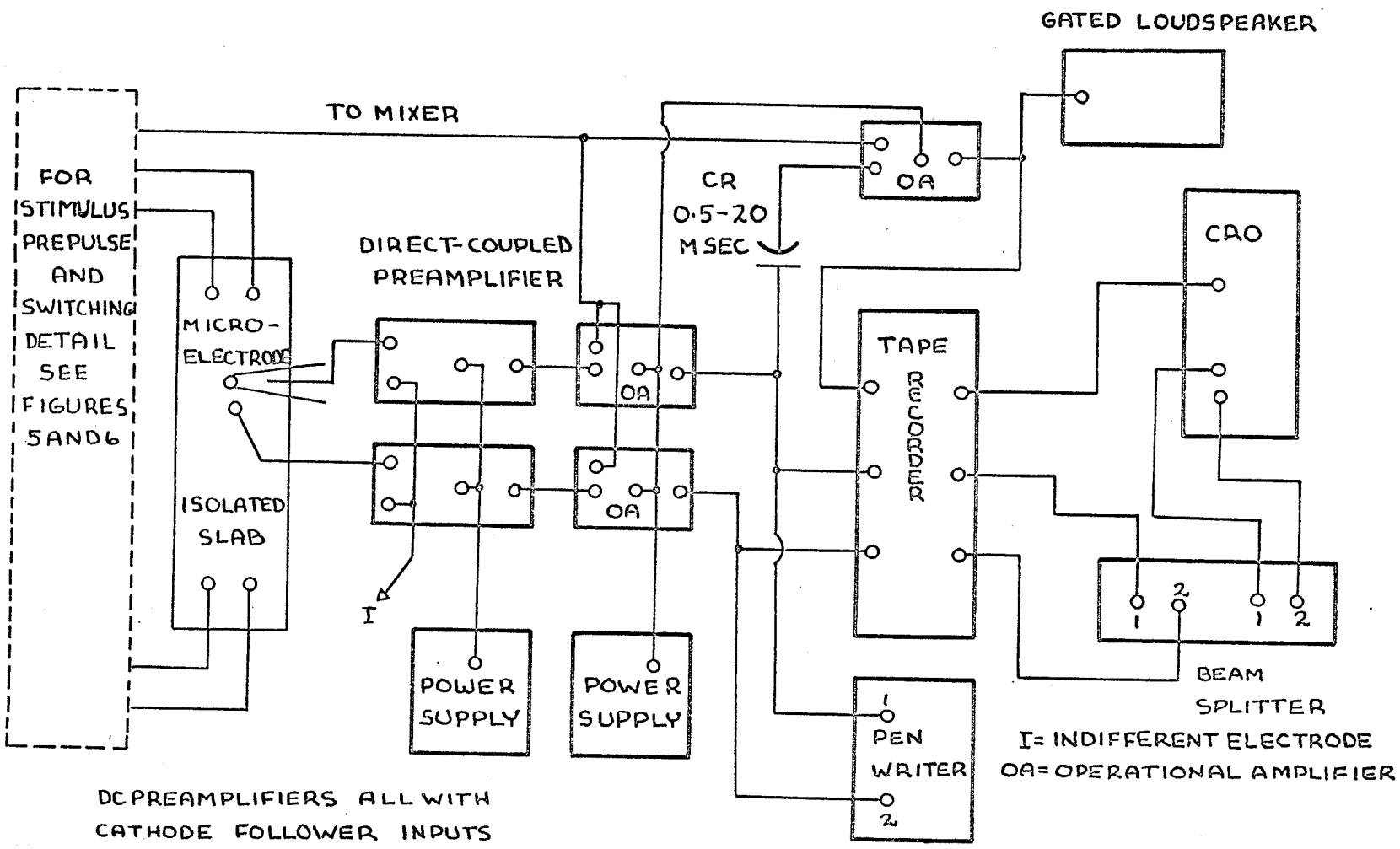



FIGURE 6

SURFACE RECORDING STIMULATING AND PREPULSE CIRCUITRY

# FIGURE 7

## UNIT ACTIVITY RECORDING CIRCUITRY



# RESULTS

# RESULTS

## (1) ANALYSIS OF STORED INFORMATION

The data that were collected and stored on magnetic tape during the experiments were analyzed in the following ways:

### a.) MEASUREMENT OF SURFACE ACTIVITY

Photographic Method:

In the earliest experiments, two recording electrodes were used. The two channels of stored information were displayed continuously on a Tektronix 502A oscilloscope whose time base was kept stationary. An oscillographic camera (Shackman, AC/25) was loaded with blue-sensitive high-contrast film (linograph Ortho, Gray base, Kodak) and placed with its shutter open in front of the oscilloscope face. The film in the camera was moved at an appropriate speed past the horizontally stationary spot.

The photographic record displayed both the stimulus artifact and the entire surface-positive burst response.

The stimulus artifact was used as the point of zero time in measuring the transmission delay of the response (Figure 8a). The onset of the surface-positive burst response was taken as the end of the transmission delay. This onset was usually abrupt enough that there was no difficulty in determining the end of the delay period. In those instances where the onset was more gradual, an objective set of criteria was established to determine an end point value. These criteria, visually represented in Figure 8b, were applied in the following way: An angle was formed by the intersection of a line projected along the horizontal axis of the trace and a line projected along the segment of

steepest slope of the earliest part of the response. This angle was bisected and the onset of the response was taken as that point where the projection of the bisector crossed the axis of the trace.

Storage Oscilloscope Method:

In later experiments, a much simpler, more convenient method of displaying the responses for analysis was employed. In these experiments, four recording electrodes were used. Responses at all four recording electrodes were displayed simultaneously on a four-trace storage oscilloscope, (Tektronix Type RM 564) and measurements of transmission delays were made directly from the face of the oscilloscope with an accuracy of $\pm$ 10 msec.

As shown in Figure 6, the waveform generator drove two pulse generators, one providing the stimulus and the second delivering a prepulse to the input of a mixer in the operational amplifier circuit. By manual triggering of the waveform generator, a sawtooth waveform of 100 msec duration was delivered to both pulse generators. The second prepulse generator gave a prepulse of 5 msec duration, 5 msec after the onset of the sawtooth waveform. The first pulse generator delivered the stimulus pulse to the experimental preparation 60 msec after the onset of the sawtooth waveform. The prepulse served to trigger the storage oscilloscope when the responses stored on the magnetic tape were later reproduced for study. The interval between the prepulse and the stimulus pulse allowed for easy and unmistakable recognition of the stimulus artifact.

This storage oscilloscope method resulted in a display of the responses similar to that achieved with the photographic method. Measurements of transmission delays were made easily from this dis-

play using the criteria described above.

The measurements of the transmission delays and the distances traversed by the responses constituted the majority of the raw data studied.

b.) MEASUREMENT OF DEEP ACTIVITY

The deep activity was recorded with a microelectrode. The signal from the microelectrode was recorded through a CR network of variable time constant to give unit spike activity and simultaneously direct-coupled to give deep mass activity. The unit spike activity was interpreted as a brief potential change representing the action potential of excitable cells. Deep mass activity was considered to be the potential changes in the field surrounding a group of excitable cells, and resulting from the sum of their activity.

The information contained on the magnetic tapes was displayed on a Tektronix 502A oscilloscope in a manner similar to that used for monitoring the tape recorder output (see METHODS and Figure 7). The rapidly rising phase of each spike was intensified on the oscilloscope screen by means of a differentiating network connected between the vertical deflection amplifier and the intensity grid of the cathode-ray tube. The prepulse triggered a single sweep of the oscilloscope whose trace was recorded on a single frame of film exposed to the oscilloscope face. Unit spike activity and deep mass activity were compared with the surface-positive burst response on the photographic records. Information of a similar nature but with much less resolution for spikes was also gained by displaying the responses on a storage oscilloscope.

## (2) STATISTICAL ANALYSIS

The slab was divided arbitrarily into three interelectrode zones by the placement of the recording electrodes (Figure 5). The transmission delays of a series of responses, after stimulation from either end of the slab, were measured and their means and standard errors calculated. As well, the transmission delay across each interelectrode zone, in either direction, was obtained by subtraction. For example, when stimulation originated at the anterior end of the slab, the value for transmission delay between electrode 2 and electrode 3 was determined by subtracting the value obtained at electrode 2 from that at electrode 3. This difference in transmission time shall be referred to as the interzonal delay. The means and standard errors of these interzonal delays were also calculated. Interelectrode distances were measured directly during the experiment with the micrometer eyepiece attachment of a Zeiss dissecting microscope. The velocity of the response across an interelectrode zone could be calculated easily from the measured values for delay and distance.

The transmission velocity of the surface-positive burst response across the whole slab was calculated using the distance from the stimulating electrode to the most distant recording electrode and the transmission delays measured at the particular recording electrode. This velocity was termed the overall velocity across the slab. A comparison was made between velocity in the anterior-to-posterior direction and the velocity in the posterior-to-anterior direction.

In most instances, all the data referred to were subjected to a factorial analysis to determine their statistical significance (Steel and Torrie, 1960).

The means and standard errors for transmission delays and inter-electrode zone velocities were calculated for experiments in which threshold parameters were determined. Similar calculations were made for the observations from chronic slabs and from slabs where transverse cuts were made or tetrodotoxin was applied.

Comparison of this material was made using the Student t test for unpaired data (Dixon and Massey, 1957).

### (3) STIMULUS THRESHOLD PARAMETERS

Variations in stimulus parameters were tested to see what effect they might have on transmission delay of the surface-positive burst response.

Stimulus parameters were tested in all experiments to determine a stimulus amplitude, duration and interstimulus interval adequate for consistent production of surface-positive burst responses. With two of the stimulus parameters held constant the third was varied and its effect determined on the transmission delay of the response. The value of the parameter to be tested was altered and measurements of transmission delay taken for comparison. As threshold was approached, not all stimuli initiated a response and finally if the value of the stimulus parameter being tested was lowered sufficiently, no responses were elicited. If the magnitude of one of the stimulus parameters was then raised, responses often returned. On occasion, when the stimulus strength parameters approached threshold, a local nonpropagated surface-positive response of short duration and low amplitude was recorded from the region lying between the poles of the stimulating electrode. No "bursting activity" (Burns, 1951) was seen with these nonpropagated responses. Responses were abolished when the stimulus amplitude was raised

to about 4 to 5X its threshold value and when the stimulus duration was raised to 10 to 15X its threshold value.

The following elaborates the results of the above procedures.

### a.) INTERSTIMULUS INTERVAL

The transmission delay was prolonged when the interstimulus interval was shortened. This is shown in Figure 9 which represents a typical experiment. At the shortest interstimulus interval tested, that is at 1 sec, only every other stimulus evoked a response. It was found that an interstimulus interval below 3 sec altered the transmission delay of the response. The measurements taken at 3 sec and 5 sec were not significantly different from each other, but were highly significantly different ($P \leq .001$) between 2 and 3 sec. Similar results were recorded from other experiments.

An afterdischarge ("afterbursts"; Burns, 1954) of surface-positive burst activity sometimes occurred when very short interstimulus intervals were used. Stimulation was stopped when the afterdischarge appeared. This activity disappeared if the slab was allowed to rest for a few minutes.

The transmission delay was prolonged or no response was seen if the stimulus followed too closely on "spontaneous" surface-positive burst activity. In most cases, the critical interval between the spontaneous response and the subsequent stimulus was 3 seconds or less.

### b.) STIMULUS DURATION

The transmission delay was prolonged when the stimulus duration was shortened. As the threshold value for stimulus duration was approached, not all stimuli initiated a response. In the example presented in Figure 10 a stimulus duration of 0.1 msec did not elicit a

response. It was found that a stimulus duration below 0.5 msec altered the transmission delay of the response. Measurements taken at 1.0 and 0.5 msec duration were not significantly different from each other, but measurements at 0.5 and 0.3 msec were highly significantly different ($P \leq .01$).

c.) STIMULUS AMPLITUDE

The transmission delay was prolonged when the stimulus amplitude as read from the pulse generator voltage control was reduced (Figure 11). As the threshold value for stimulus amplitude was approached, not all stimuli initiated a response. Responses elicited at 10 volts were inadvertently not recorded on magnetic tape in the experiment illustrated in Figure 11. However, examination of the polygraph record showed that the transmission delays at 10 volts were similar to those at 7.5 volts. If this observation is included the value of the point at which stimulus amplitude alters transmission delay of the response is between 5 and 7.5 volts. Measurements of transmission delay taken at these two values were shown to be significantly different ($P \leq .025$). At 3 volts or less, no responses were elicited.

d.) GENERAL REMARKS

The threshold value for stimulus parameters required to elicit a surface-positive burst response varied from experiment to experiment and within an experiment. The variation within an experiment occurred generally over a considerable time, that is, the effect was gradual and not abrupt, however, these variations were small and contributed little to the transmission delays seen during an experiment.

In some experiments, higher than usual stimulus strength was

required to produce a propagated response in one direction than in the other. This was not a property of all slabs tested but, when present, was directly related to the preferred direction of transmission. By employing a fixed stimulus strength 2 to 5X greater than threshold in these cases the directional preferences in transmission delays in either direction could not be attributed to the directional differences in threshold. The threshold values for stimulus in slabs of opposite hemispheres of the same cat were usually similar.

If an animal was dying or if the slab became relatively avascular, the thresholds of the stimulus parameters required to elicit even a local response were increased. The slab then became refractory to stimulation, evidences of "spreading cortical depression" (Leao, 1944; Grafstein, 1954, 1956) soon followed in most instances, and the experiment was terminated.

### e.) SUMMARY

In summary it can be said that the transmission delay of a surface-positive burst response is dependent within a critical range around threshold on the amplitude and duration of the stimulus and on the interval between stimuli. The threshold for stimulation was somewhat variable with time and direction of stimulation within any one slab. It was occasionally variable between slabs of different cats and in slabs cut in opposite hemispheres of the same cat. However, with the relatively high stimulus parameters selected in the experiments, the threshold for stimulation cannot be considered a significant factor in transmission delay.

## (4) NONUNIFORM TRANSMISSION OF THE SURFACE-POSITIVE BURST RESPONSE IN ACUTELY ISOLATED SLABS OF CEREBRAL CORTEX

Measurement of surface-positive burst response transmission delays at several points along the length of the slab was initially effected by the placement of one of the two recording electrodes progressively farther from the stimulating electrode. Measurements were made for stimuli originating at either end of the slab (Expt. 13). Two interesting properties became apparent when delays measured in opposite directions were compared. Firstly, it seemed as if the response was being transmitted over the length of the slab faster in one direction than in the other; that is, the transmission delay in the posterior-to-anterior direction was shorter than the delay in the opposite direction. Secondly, it was found that the velocity of the response across an interelectrode zone was not constant from zone to zone along the slab. These observations were immediately questioned because of the time taken between measurements at the various points, it was considered that these observations might in fact be no more than variation in the physiological state of the slab.

To rule out this possibility, recording electrodes were placed at each end of the slab and a stimulating electrode placed midway between them. With this arrangement, transmission delays of a response originating at a common point but travelling in opposite directions were compared. Again the response travelled faster in the posterior-to-anterior direction than in the anterior-to-posterior direction. The interpretation of these results were debatable because the responses were not travelling over the same portion of the slab.

In the next experiment (Expt. 14) the original recording ar-

rangement was used but the time between measurements was reduced. Again the same two properties appeared with, in this instance, directional preference for anterior-to-posterior transmission rather than for posterior-to-anterior. The nonuniform character of the response velocity was observed but did not vary from zone to zone in the same way as in the first experiment.

The problem of interpretation was finally solved by the simultaneous recording at several points along the length of the slab. In the remaining experiments four surface electrodes were used, arranged in a linear array as seen in Figure 5 and described in the METHODS.

### a.) PREFERRED DIRECTION OF TRANSMISSION OF THE SURFACE-POSITIVE BURST RESPONSE

The results of the experiments to test whether directional preference for transmission of the surface-positive burst response is an inherent property of the slab are summarized in Table 1. In Column 1 the numbers identify the experiment from which the results were obtained, the letters R and L refer to the hemisphere, right or left, in which the slabs were cut. Column 2 shows the distance in mm from the stimulating electrode to the recording electrode farthest along the slab. Columns 3 and 5 give the mean of surface-positive burst response transmission delays in msec with their standard errors across the slab in the anterior-to-posterior direction (A$\longrightarrow$P) and the posterior-to-anterior direction (P$\longrightarrow$A) respectively. Columns 4 and 6 contain the overall velocity of the response across the slab in the anterior-to-posterior direction and the posterior-to-anterior direction, respectively. Column 7 shows the preferred direction of transmission and the statistical significance of the difference in transmission delay between the two directions. It

can be seen from this table that 9 slabs showed an anterior-to-posterior directional preference while 6 slabs showed a directional preference, posterior-to-anterior. In one slab (Expt. 19) there was not a significant difference at the accepted $P \leq .01$ or $P \leq .05$ value. A Chi-square test done to determine if statistically there was any favoured preferential direction showed that there was not; that is, an infinite population of cats might be expected to yield equal numbers of slabs with anterior-to-posterior and posterior-to-anterior directional preferences.

Where slabs had been prepared in both hemispheres in a cat, the same directional preference (Expts. 18, 20, 27 and 28) was exhibited in both slabs.

Two possible objections to the above interpretation became evident during the project. Firstly, the majority of the slabs showing an anterior-to-posterior directional preference had received their first series of stimuli from the anterior end. It was suggested that these slabs might have been induced into a preferred direction of transmission by the first few stimuli. Secondly, all the slabs had been prepared through posterior sink holes. It was suggested that the trauma to the nearby cortical substance may have adversely affected the transmission characteristics of the neural networks in the posterior area and thus biased the directional preference of the slabs.

To answer the first objection stimulation was initiated in four slabs from the posterior end. The distribution of directional preference was roughly in the same proportion as when stimulation had been initiated from the anterior end. In two slabs, single stimuli were delivered alternately from one end and then the other. The transmission delays were compared for several of these alternating stimuli

and the preferred direction of transmission determined. An attempt was then made to see if the supposed preferred direction of transmission could be overcome with a large number of stimuli originating from the opposite end of the slab. Fifty stimuli were delivered in the direction opposing the preferred direction of transmission and were then followed by 12 stimuli in the preferred direction. For comparison, 5 repetitions of this basic pattern were delivered to the slab. Fifty stimuli in the preferred direction of transmission were then delivered to the slab, followed by 12 stimuli in the opposing direction for comparison. Five repetitions of this pattern were also carried out. Comparisons of the transmission delays measured showed that the preferred direction of transmission was not changed by these procedures. The two slabs had originally showed a highly significant posterior-to-anterior directional preference and it was maintained throughout the experiment.

To answer the second objection, two slabs were prepared with both anterior and posterior sink holes. Anterior-to-posterior preference was shown in the first slab in which an anterior sink hole was made (Expt. 25). It should be noted that in the one experiment where an anterior and posterior sink hole was made on one side (Expt. 27-L) and a posterior sink hole only on the other side (Expt. 27-R), both slabs showed posterior preference. These results taken with the former observation that slabs in both cerebral hemispheres have the same directional preference led to the conclusion that the presence of a sink hole at a particular end was not instrumental in establishing directional preference.

### b.) NONUNIFORM TRANSMISSION VELOCITY BETWEEN INTERELECTRODE ZONES

The use of four recording electrodes arbitrarily divided the slabs into 3 interelectrode zones, each zone having a particular number (Figure 5). The zone number remained the same regardless of the direction from which the stimulus had originated; that is, zone 1 was always the zone at the anterior end of the slab. The results of experiments in which the interelectrode zone transmission velocities were calculated are shown in Table 2. In Column 1, the number identifies the experiment from which the results were taken, the letter refers to the hemisphere in which the slab was cut. Column 2 indicates the distance in mm across zones 1, 2 and 3, respectively. Columns 3, 4 and 5 show the means of the interelectrode zone velocities for each zone in the anterior-to-posterior direction, and Columns 6, 7 and 8 show the same information in the posterior-to-anterior direction.

The results in Table 2 were studied to see if a definite distributional pattern could be established for velocity across the slab in either direction. It often appeared that the response travelled exceedingly fast across a zone in one direction as compared with the other direction; while in adjacent zones the velocities in either direction were similar. The zone with the fast transmission in one direction comprised most of the difference for the overall velocity observed when the two directions were compared. There was no definite correspondence between zones in one slab and the other of the same cat.

Statistical analysis (Student t for unpaired data) showed that in the A$\longrightarrow$P direction there was a significant difference ($P \leq .05$) between the velocities of zone 2 and 3, but no significant difference

between velocities of zones 1 and 2 or between zones 1 and 3. In the P A direction, there was a significant difference between zones 1 and 3 ($P \lesssim .05$) but no significant difference between zones 1 and 2 or between 2 and 3. These particular results indicated that zone 3 tended to have the slowest velocity. The question arose whether this slowness in velocity in zone 3 was due to its close proximity to the posterior sink hole. Several facts do not bear out this interpretation. Firstly, there is considerable trauma to surrounding cortical tissue by the movement of the blunt edge of the slab-cutting knife through the cortex. This trauma at the anterior end is at least as great as that caused at the posterior end by the sharp point of the tapered glass tube used as a suction tip in forming the sink hole. Secondly, the slabs were cut of sufficient length to ensure at least a 3 mm spacing at each end between the stimulating electrodes and the end of the slab. Histological sections of the slabs showed no remarkable changes in the cytoarchitecture beneath the anterior and posterior stimulated points. Thirdly, it can be seen from Table 2 that on two occasions the zone with the highest velocity was zone 3. Fourthly, two slabs were prepared which had both anterior and posterior sink holes. If the presence of a sink hole was instrumental in slowing the velocity of the response in an adjacent zone, it would be expected that zones 1 and 3 would both be slow while zone 2 would have the highest velocity in these cases. In one slab, zone 1 had the highest velocity ($P \lesssim .05$) while in the other slab zone 3 had the highest velocity ($P \lesssim .05$). Finally, of four slabs tested which showed posterior-to-anterior preferential transmission, on anterior stimulation zone 1 had the highest velocity in two slabs while zone 3 had the highest velocity in the other two. On stimulation from the

posterior direction zone 2 had the fastest velocity in 3 slabs while the fastest velocity occurred once in zone 3. From the data just presented, it is difficult to determine whether there is a definite pattern of zone velocity distribution. No rigid statement can be made about the influence that external factors such as sink hole placement, stimulus mode or inherent factors, such as preferred direction of transmission, would have on any such distribution. It should be noted, however, that 7 of the 8 slabs in Table 2 showed anterior-to-posterior preferential transmission, and so from the data it might be said that slabs which show an anterior-to-posterior preference for transmission, exhibit a tendency to have the zone 1 or 2 as the fastest conducting areas.

### (5) DETERMINATION OF NEURONAL PATHWAYS INVOLVED IN THE TRANSMISSION OF THE SURFACE-POSITIVE BURST RESPONSE

Any hypothesis concerning the transmission of the surface-positive burst response must take into account the organization of neuronal elements in both the grey and white matter. Interruption of the supposed pathways responsible for transmission of the response should give useful information about this organization. Three methods were employed to this end. Firstly, transverse cuts were made into the slabs both from above and below as described in the METHODS. The effect of these cuts on the transmission of the response was then studied. This approach to the problem has been used by Burns and Grafstein (1952). Secondly, tetrodotoxin, a specific inhibitor of membrane sodium conductance (Frank and Pinsky, 1966; Kao, 1966; Ochs and Clark, 1968) was applied topically to study its effects on transmission of the response. Its activity may be considered as a form of pharmacological interruption of the transmitting pathways. Thirdly, the effect of long-term neuronal

isolation upon the transmission of the response was examined in light of the concomitant changes (Weisman, Gorchynski and Pinsky, 1967).

a.) EFFECTS OF TRANSVERSE CUTS

The effects of cuts from the top or underside of the slab on the transmission of the surface-positive burst response was somewhat variable from experiment to experiment. These effects, however, followed a general pattern. The immediate effect seen after completion of the cut was a wave of spreading cortical depression which slowly traversed the slab, involving all areas. During this spreading depression phase the slab was refractory to stimulation. The duration of the spreading depression varied. This was followed by a phase of general electrical silence during which the slab was still refractory to stimulation. Recovery of electrical activity was heralded in one of two ways; either spontaneous surface-positive burst activity appeared in part or in all of the slab, or, stimulation elicited a local non-propagated response. Eventually, stimulation resulted in a response propagated at least up to the cut region but the transmission delay to that point was considerably prolonged. Finally, in most instances, transmission of the response occurred across the cut region with considerable delay. Often with time the transmission delays at all points approached the values seen prior to cutting. Recovery was usually good at points that were not too close to the cut region both proximally and distally. In other words, inter-electrode zonal velocities for zones not involved in the cut returned to their precut values.

The most severely affected area seemed to be that surrounding the cut region and it usually showed the least recovery with time.

The initial responses seen during recovery were of short duration with relatively low amplitude, and often without noticeable bursting activity. With time, all the response parameters usually returned towards precut levels, that is, the amplitude and duration increased and burst activity became evident. At times this temporal improvement in response characteristics was not seen beyond the cut region even though a response was transmitted up to that area.

Another early effect seen with cuts was that the stimulus amplitude and duration required to elicit a response was increased and the interstimulus interval prolonged. As the transmission delay approached precut levels, so did the stimulus amplitude, duration and interstimulus interval.

The recovery of transmission of the surface-positive burst response often occurred first in one direction before the other, in terms of both time and threshold. This directional preference was not always related to the directional preference seen before the slab was cut.

In most instances, histological verification of the completeness and extent of the cuts was available and will be discussed in relation to later examples.

Effects of Undercuts.

Figure 12 shows the results of one of the experiments for which there is histological verification of the cut. The cut was made approximately 14 mm away from the stimulated region. The precut transmission delays are shown in the lower part of the graph. Immediate changes with the cut have been described. Five minutes after the cut, recovery had occurred at the electrode 5 mm away from the stimulated site but with an increased transmission delay. By 12 minutes, trans-

mission occurred to an electrode 11 mm away from the site of stimulation but no responses crossed the cut region; the transmission delay at the electrode 5 mm from the stimulator was further reduced. Twenty-four minutes after the cut, responses were being transmitted across the cut region but with considerable delay. Further recovery in the form of reduced transmission delays and lowered stimulus thresholds were seen later.

Stimulation from the end nearest the cut produced only a local response initially within 10 to 12 minutes after the cut. Responses were transmitted across the cut region from this end 30 minutes after the cut was made but with considerable delay in transmission. Recovery, evidenced by reduced transmission delays and lowered stimulus threshold and interstimulus intervals necessary to elicit a response, was seen in this direction also.

Longitudinal sections of the slab examined histologically (Figure 13) show the undercut to extend through the subcortical white matter and into the cortex to within 0.44 mm of the pial surface. Several of these sections revealed the cut to be essentially complete from side to side and relatively uniform in depth.

Other experiments in which undercuts were employed showed similar results for transmission delay and recovery with time.

In some experiments, as many as three undercuts were made and responses studied. The recovery pattern in these preparations was similar to that just described. Positive histological verification was made in most instances.

Effects of Surface Cuts:

The two methods of cutting into the cortex from the surface have been described in the METHODS section. It was thought that the

approach directly through the pia might be adversely affecting the vasculature of the slab so a second method through a side approach was developed. No differences in recovery or effect on transmission of the response after cutting were seen when the results following the two methods were compared. The second method is less likely to produce uncontrollable haemorrhage and the extent of the cut could be seen more easily because blood clots did not form on the pial surface.

Figure 14 shows the effect of a surface cut made by the direct approach 15 mm from the stimulator. The precut transmission delays are shown in the lower portion of the graph. They were measured 10 minutes before the cut was made. The immediate effects following the cut have been described. Within 5 minutes after making the cut, responses were being transmitted up to the cut region (not shown) and by 10 minutes, responses were passing the cut region. The transmission most affected was that involved in the area proximal to the cut. By 30 minutes after making the cut, recovery of transmission was still progressing and by 60 minutes, transmission had returned to the former levels for delay, stimulus threshold and interstimulus interval. Stimulation in the opposite direction initially resulted in a local response only, but by 15 minutes, occasional responses with long delays were propagated across the cut region. Recovery in this direction was not as complete as in the first direction discussed.

Histological examination of the slab made in this experiment (Figure 13) showed the cut to extend almost completely through the cortical substance down to the underlying white matter. Serial sections revealed the cut to be essentially complete from side to side and relatively uniform in depth. However, at some points, particularly at the

edges of the slab some small amounts of layer 6 of the cortex remained intact. Figure 13 shows the cut extending into layer 6 of the cortex.

Other experiments in which surface cuts were made by either method showed similar results for delay of transmission and recovery with time. Histological studies of these slabs showed the cuts to be of the same depth as the one just described.

In most experiments, two additional cuts across the slab at the midpoint of the other interelectrode zones were made and their effect studied. The recovery pattern was similar to that described above. Positive histological verification was available in most instances.

In one experiment, the three surface cuts were made and time allowed for recovery; then an undercut was placed midway between two of the surface cuts. In this instance, recovery occurred across all cut regions including the undercut.

On one occasion, a cut from the surface irreversibly abolished transmission of the response across the cut region. Responses were propagated to the cut from both directions but no responses crossed the cut. Histological sections of this slab showed the cut to extend entirely through the cortex and well into the white matter below.

### b.) THE EFFECTS OF TETRODOTOXIN

The effects on the transmission of the surface-positive burst response in isolated cortical slabs after topical application of tetrodotoxin was extremely variable. There are many possible reasons for this variability but probably the two most important are a reduction in potency with storage of the stock solution and irregularities in diffusion of the drug through the pia and cortical substance.

Saline controls were done in all experiments and no changes in the transmission characteristics of the surface-positive burst response were seen during or after the application and removal of the control saline-soaked filter paper strip.

The effects on the transmission characteristics of the surface-positive burst response by the topical application of tetrodotoxin were somewhat similar to those seen after transverse cuts had been made in the slabs. In one experiment, no effect was seen even with relatively high concentrations of the drug. In this instance, failure of activity was attributed to deterioration of the drug during storage. However, no potency tests were carried out on this solution before use.

In one experiment (Figure 15), exposure of the slab to tetrodotoxin resulted in abolition of the transmission of the surface-positive burst response over a large area of the slab, but at either end, local responses could be elicited. With time, transmission occurred up to but not across the affected area. The use of higher stimulus amplitudes and durations and longer interstimulus intervals elicited transmission of responses across the affected region within several minutes of removal of the drug-soaked filter paper strip. The transmission delays of these responses were prolonged.

With time, recovery was evidenced by the reduction in the amplitude and duration of the stimulus and interstimulus interval required to elicit a transmitted response. At the same time, there was a decrease in transmission delay seen at all electrodes (Figure 15) although not necessarily to pretreatment levels. Recovery occurred earlier and to a greater degree with anterior than with posterior stim-

ulation in this experiment. This directional difference in recovery could easily have been due to irregular diffusion and removal of the drug. Responses with the saline control strip on the slab were recorded between 5 and 10 minutes before application of the drug-soaked filter paper strip. The latter was left on the slab in zone 2 for 10 to 12 minutes. The time annotations in Figure 15 are calculated from the moment the tetrodotoxin-soaked filter paper strip was applied. Attempts to record responses from the cortex were made on several occasions after application of the drug. Within 10 minutes, the slab became unresponsive except in the region of the stimulating electrodes. At 26 minutes, recovery was observed with the transmission of a response to the electrode just proximal to the drug-treated region but not across it. Further recovery was evidenced by a reduction in transmission time at 36 minutes. At this time, an occasional response crossed the treated area but with a much prolonged delay. By 92 minutes, responses were crossing the affected area but with considerable transmission delay, particularly in the immediate region of drug application. Further recovery was seen at 171 minutes and was reflected in reduced transmission delays at all points.

### c.) EFFECTS OF CHRONIC ISOLATION

The effects of chronic neuronal isolation on the transmission characteristics of the surface-positive burst response were studied in four cats. The responses to stimulation in chronically isolated slabs of cerebral cortex have been described (Grafstein and Sastry, 1957; Sharpless and Halpern, 1962; Reiffenstein, 1964; and Brock, 1967) and are accompanied by, but not necessarily correlated with, known histological changes (Reiffenstein, 1964; Weisman et al., 1967).

In the first two slabs, only limited transmission of the surface-positive burst response (up to 5 mm) was recorded with anterior stimulation. Stimulation was performed at zonal boundaries along the length of slab but only local responses were elicited and no response propagated in either direction. Delays in transmission were 165 ± 6 msec for 5.0 mm in the first slab (velocity 3.03 ± 0.27 cm/sec) and 420 ± 17 msec for 4.5 mm in the second slab (velocity 1.07 ± 0.10 cm/sec). High stimulus strength and long interstimulus intervals were required to elicit responses that were propagated for even a short distance in chronic slabs. The stimulus parameters used in chronic slabs were of the order of 2X those used routinely in acute slabs. The amplitude and duration of the responses seen in chronic slabs were considerably less than those in acutely isolated slabs. There was only occasionally the burst activity characteristic of responses seen in acute slabs.

In the other two chronic slabs, only local responses were elicited with anterior or posterior stimulation. One of these animals succumbed shortly after recording began and, therefore, it was difficult to explain its lack of responsiveness to stimulation in terms of inherent cortical properties.

### d.) RELATIONSHIP OF THE SURFACE-POSITIVE BURST RESPONSE TO DEEP MASS ACTIVITY AND UNIT SPIKE ACTIVITY

Further experimental information concerning the transmission of the surface-positive burst response was obtained by studying the action potential discharge activity of single cortical neurones (unit spike activity). To do this, the temporal relationship between the onset of the surface-positive burst response, the onset of the deep mass activity and the start of a train of spikes of unit activity was

studied. Unit activity was examined in 38 cells from 4 slabs for this work. In two slabs, spike trains were recorded both before and after transverse cuts were made in the slab and the results from one of these preparations was examined in detail. It may be stated that the results following were representative of the general relationships observed in all the experiments.

The surface and deep responses had much similarity in form but were not necessarily identical and were of opposite polarity. The onset and termination of the surface-positive burst response and deep mass activity were generally coincident (Figure 16). Rapid, low amplitude fluctuations of potential in the deep mass activity were not always reflected at the surface. In most instances, a train of spikes accompanied the deep mass activity and the surface-positive burst response. The start of this train was at or near the end of the steeply sloped initial phase of the deep mass activity response (Figure 16). The delay between the onset of deep mass activity and the start of a spike train depended, then, on the slope of that line; when the slope was gradual, the delay was longer. The train of spikes did not always last for the duration of the deep mass activity response. However, if there were large potential fluctuations in the trough of the response, they were usually accompanied at the time of negative shift by a train of spikes.

"Spontaneous" surface-positive burst activity and its concomitant deep mass activity were always accompanied by a train of spikes whose onset had the same relationship in time to the deep mass activity as did the spike trains that followed stimulation.

Spike height varied somewhat during a given response suggesting that the unit activity of several populations of cells was being

recorded simultaneously. In one experiment, groups of cells with recordable unit spike activity were found to be between 0.72 and 1.26 mm from the sub-pial surface of the cortex. In this preparation, the cortex was 1.48 mm thick at that point; histological examination showed the cell population studied to be in layers 3, 4 and 5.

In the same experiment, a transverse cut extending through all layers of the cortex was made between the recording electrodes and stimulating electrode after unit activity had been recorded in the intact slab for some 1 to 2 hours. Effects of this cut on the onset of the surface-positive burst response, deep mass activity and the start of the unit spike activity were studied beginning 10 minutes after the cut had been made. Cells at depths of 0.69 mm to 0.86 mm were studied.

The cut initially resulted in electrical silence and the absence of response to stimulation in both surface and deep recording electrodes. The first response to stimulation seen during the course of recovery was a train of spikes with widely-spaced intervals. This occurred before return of the surface-positive response or deep mass activity. Stimulation finally resulted in a surface-positive burst response being transmitted across the cut region though with considerable delay (Figure 17). The onset of the deep mass activity response occurred much earlier than the response at the surface but the slope of the early part of the deep mass activity response was more gradual than before the cut had been made. When this part of the response reached its most negative value the train of spikes occurred. As recovery proceeded, the delay in transmission of the surface-positive burst response shortened until it became similar to that of the deep mass activity (Figure 18); the slope of the initial phase of the deep mass activity became steeper at

this time. The train of unit spikes occurred at the most negative part of this initial phase.

### (6) "SPONTANEOUS" SURFACE-POSITIVE BURST ACTIVITY

Spontaneous surface-positive burst activity was seen at the beginning and often during the course of the experiments. This activity was "spontaneous" in that it was not related to any external source of stimulation. As has been noted by Burns (1950), the spontaneous activity seemed often to arise from some "irritative focus" in the slab. In the experiments described here, it was possible to locate the region from which the surface-positive burst activity originated by comparing the transmission delays at the four recording electrodes. Since the velocity of the response is nonuniform in the slab, it was impossible to localize more precisely the focus of origin. However, at times, no focus of origin for the "spontaneous" surface-positive burst activity could be found. During the course of some experiments, the apparent focus for spontaneous activity shifted from location to location.

The spontaneous activity was usually most frequent at the beginning of the experiment, often so much so that means had to be taken to reduce it before responses to direct cortical stimulation could be studied. This reduction in activity was accomplished by recutting the slab.

Recovery of excitability of the slabs to direct electrical stimulation after transverse cuts had been made or tetrodotoxin applied was often preceded by spontaneous surface-positive burst activity. This spontaneous activity was often transmitted along the whole length of the slab.

If a stimulus followed too closely after spontaneous activity, the transmission delay of the evoked response was considerably increased. The critical interval in these cases was similar to that described in an earlier part of the RESULTS for directly evoked responses.

If the stimulus frequency was kept at a constant interval rather than varied, then the spontaneous surface-positive burst activity would tend to occur just before, or at the time of stimulation. This phenomenon was most easily seen when the spontaneous activity originated at or near the end opposite to that being stimulated.

## (7) INCIDENTAL OBSERVATIONS

The following observations have been noted during the course of experimentation and analysis. They are not directly applicable to the development of the model and hypothesis. Nevertheless, it is possible that they may be of use to those working with a similar preparation. Consideration of these observations may suggest future work.

Observations on Changes in "Brain Temperature":

The animal's body temperature was maintained by the method previously described. "Brain temperature" was the term used to refer to the temperature recorded by a thermistor probe in the pool of mineral oil lying over the cortex. This pool of oil was subject to fluctuations in temperature. It is questionable whether this "brain temperature" truly reflects the overall temperature of such a vascular organ as the brain, particularly in its deeper reaches. Nevertheless, this measurement is probably closely related to the pial temperature and that of the superficial cortical layers. No appreciable change in transmission delay nor in stimulus threshold parameters for the surface-positive burst response was seen when the temperature of the oil pool varied

between 31°C and 37°C. From this, it may be concluded that none of the data was measurably affected by variations in temperature of the oil bath.

On one occasion, when the temperature of the oil pool was allowed to reach and remain at 40°C for 10 minutes, the slab showed "spreading cortical depression" and became unresponsive. The pool was rapidly cooled to 36°C but recovery did not occur even though the slab and blood vessels supplying it appeared healthy.

The Effect of Different Decerebration Techniques:

Decerebration was usually accomplished through a burr hole in the posterior fossa. This approach caused an initial profound drop in blood pressure if care was not taken to angle the decerebration knife blade well forward and keep it tightly against the bony tentorium cerebelli. Generally, there was adequate recovery of blood pressure so that the preparation procedure could continue. Decerebration anterior to the tentorium cerebelli as described by Pinsky (1961) was less likely to cause such a profound drop in blood pressure.

At times brain manipulations, such as cutting the slab or opening the dura, caused a drop in blood pressure. This drop was usually preceded or accompanied by swelling of the brain.

The Effect of Stimulating from Outside the Slab:

Electrical stimuli originating outside the slab but within the same gyrus failed to elicit any surface-positive burst responses within the isolated area. No responses occurred even with high stimulus strengths.

The Amplitude and Duration of the Surface-Positive Burst Response:

Some mention has already been made of the amplitude and duration of the surface-positive burst responses recorded during the experiments. The parameters were usually comparable to those reported by

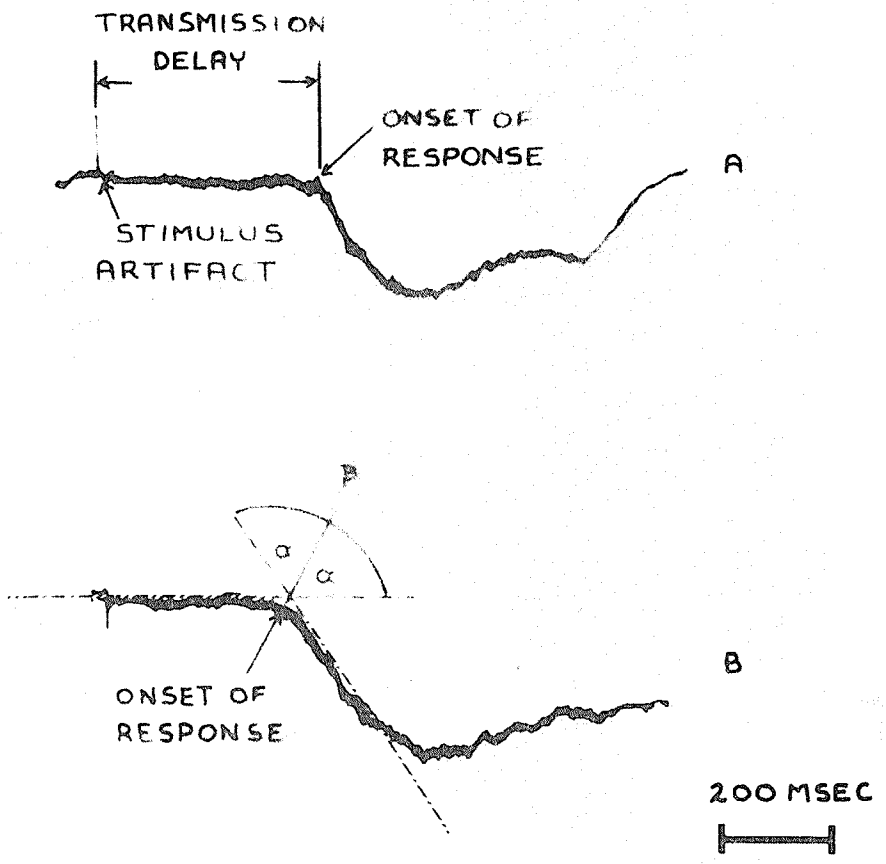
Burns (1951), the amplitude range being 0.2 to 1.5 mv and the duration range being 0.5 to 5.0 sec. At any particular recording site the pattern, amplitude, and duration of surface-positive burst responses were very similar over long periods of time. Moreover, with the stimulus delivered periodically to the same point on the surface of the cortex for periods of up to 10 minutes the responses recorded at points distant had very similar dimensions of amplitude and duration as well as obvious similarity in form. This was most clearly seen when serial responses recorded at the same point were superimposed on the screen of the storage oscilloscope. The change in amplitude and duration of the response after transverse cuts and application of tetrodotoxin have already been described.

The Effects of Gallamine on the Transmission Characteristics of the Surface-Positive Burst Response:

In the experiments described here gallamine triethiodide, 2 to 5 mg/kg, was administered routinely as a muscle relaxant. Much larger doses, 10 to 20 mg/kg were administered on several occasions to see whether there was any demonstrable effect on the transmission characteristics of the surface-positive burst response. No discernible change in transmission characteristics, form, amplitude or duration resulted from the administration of the blocking agent.

# FIGURE 8

MEASUREMENT OF THE TRANSMISSION DELAY OF THE SURFACE-POSITIVE BURST RESPONSE




THE ONSET OF THE SURFACE-POSITIVE BURST RESPONSE IS TAKEN AS THE POINT WHERE THE BISECTOR OF $\angle \beta$ CROSSES THE AXIS OF THE TRACE

FIGURE 9

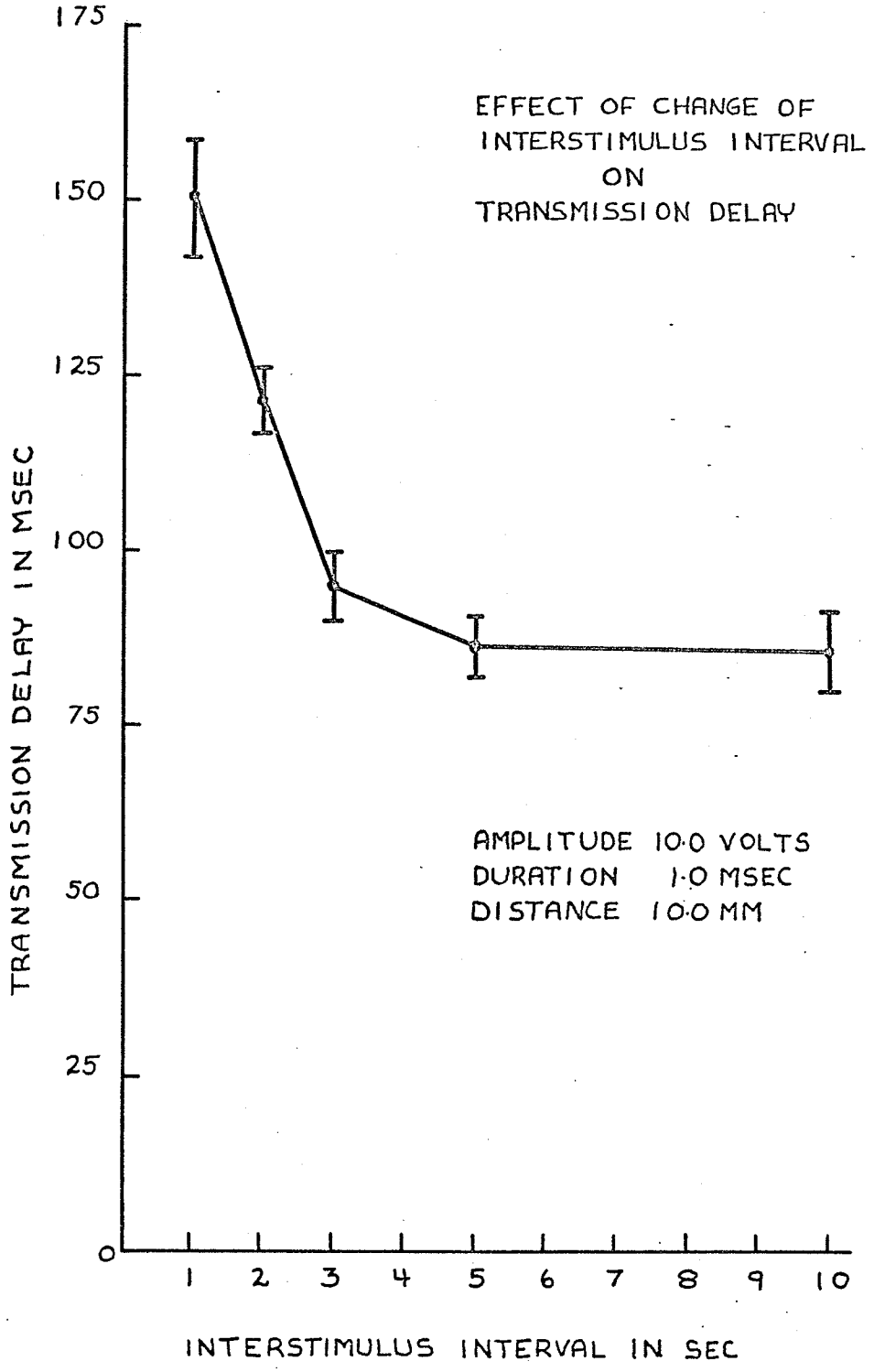

175
150
125
100
75
50
25
0
TRANSMISSION DELAY IN MSEC
EFFECT OF CHANGE OF
INTERSTIMULUS INTERVAL
ON
TRANSMISSION DELAY
AMPLITUDE 10·0 VOLTS
DURATION 1·0 MSEC
DISTANCE 10·0 MM
1
2
3
4
5
6
7
8
9
10
INTERSTIMULUS INTERVAL IN SEC

FIGURE 10

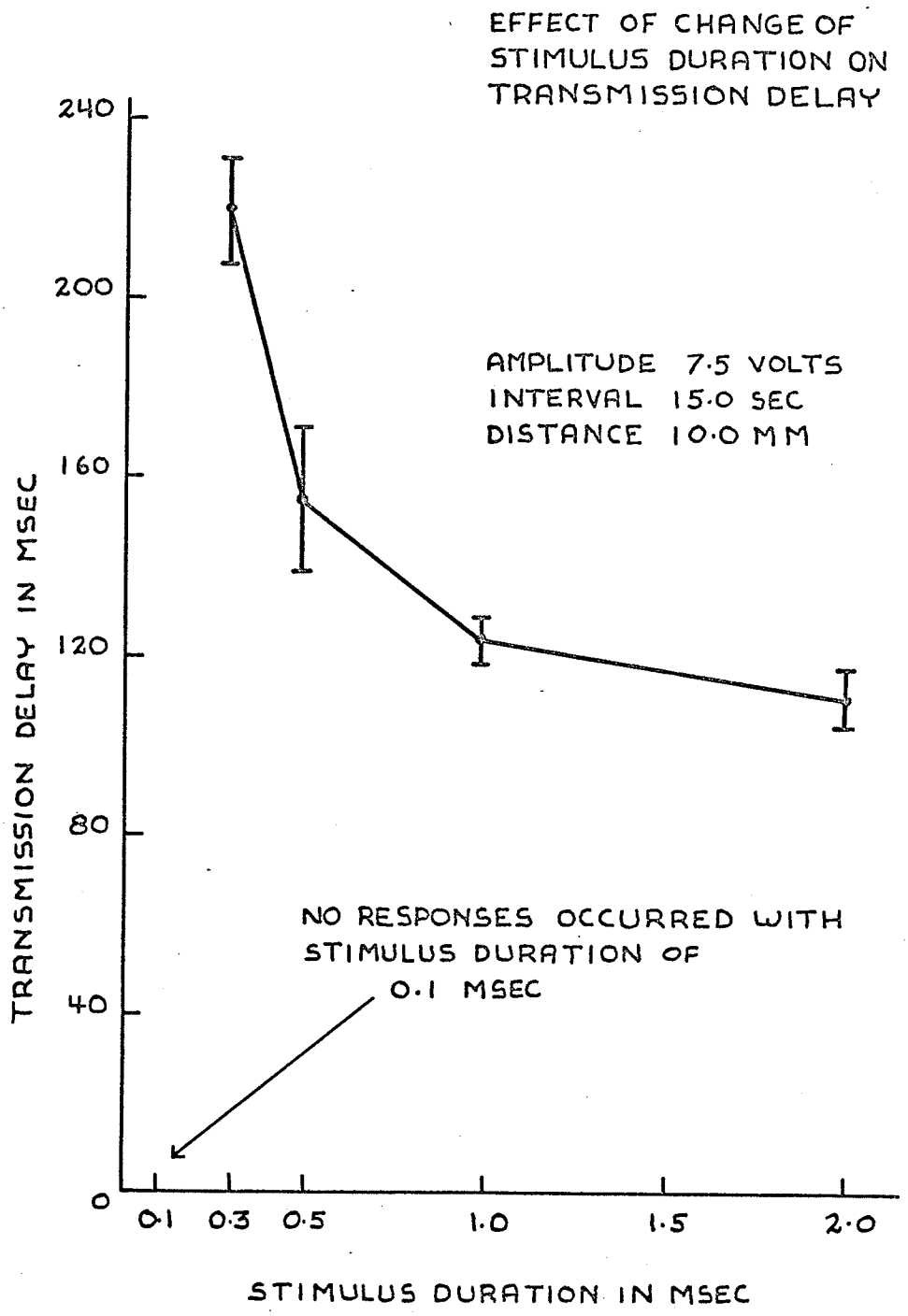

EFFECT OF CHANGE OF
STIMULUS DURATION ON
TRANSMISSION DELAY
AMPLITUDE 7·5 VOLTS
INTERVAL 15·0 SEC
DISTANCE 10·0 MM
NO RESPONSES OCCURRED WITH
STIMULUS DURATION OF
0·1 MSEC
TRANSMISSION DELAY IN MSEC
240
200
160
120
80
40
0
0·1
0·3
0·5
1·0
1·5
2·0
STIMULUS DURATION IN MSEC

FIGURE 11

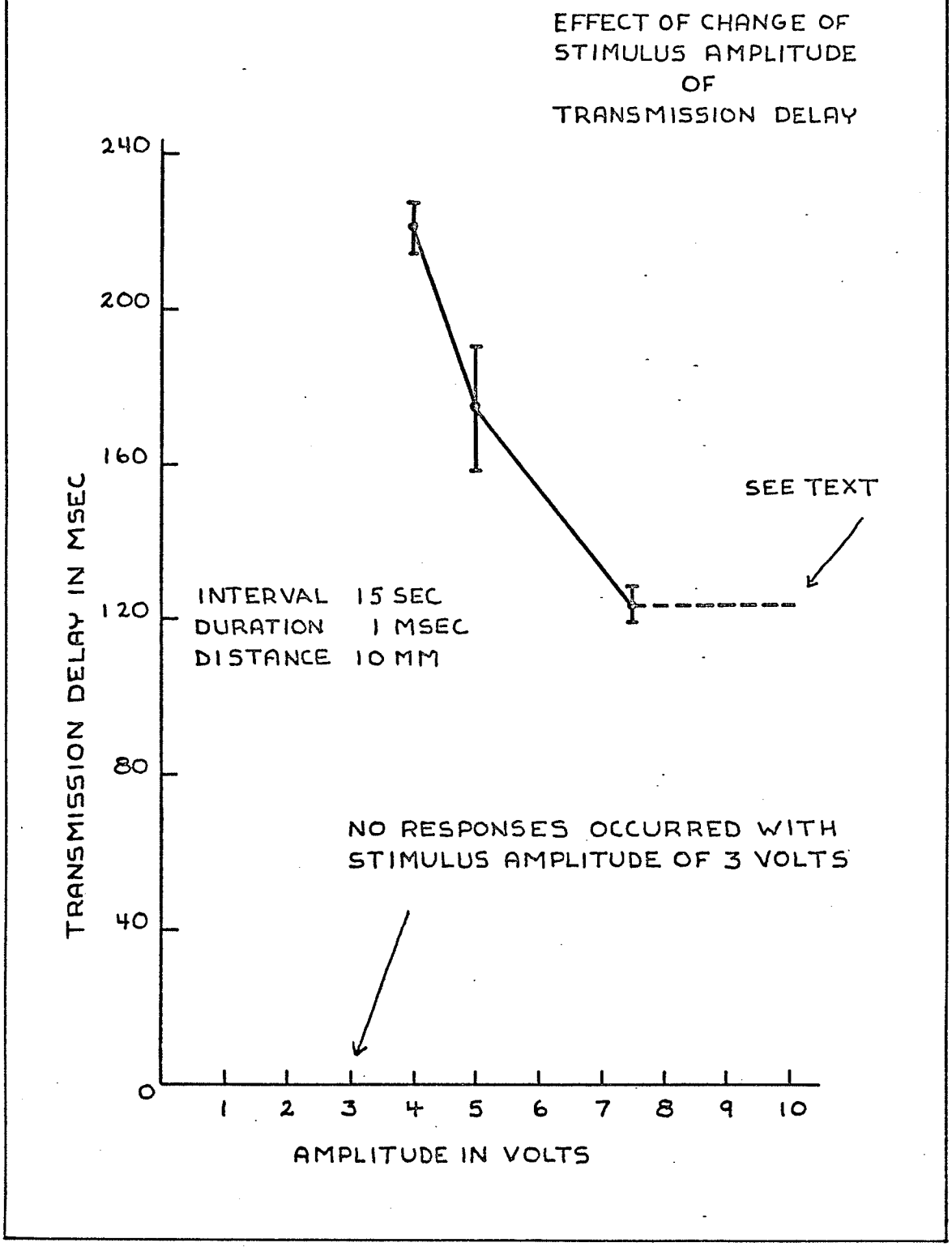
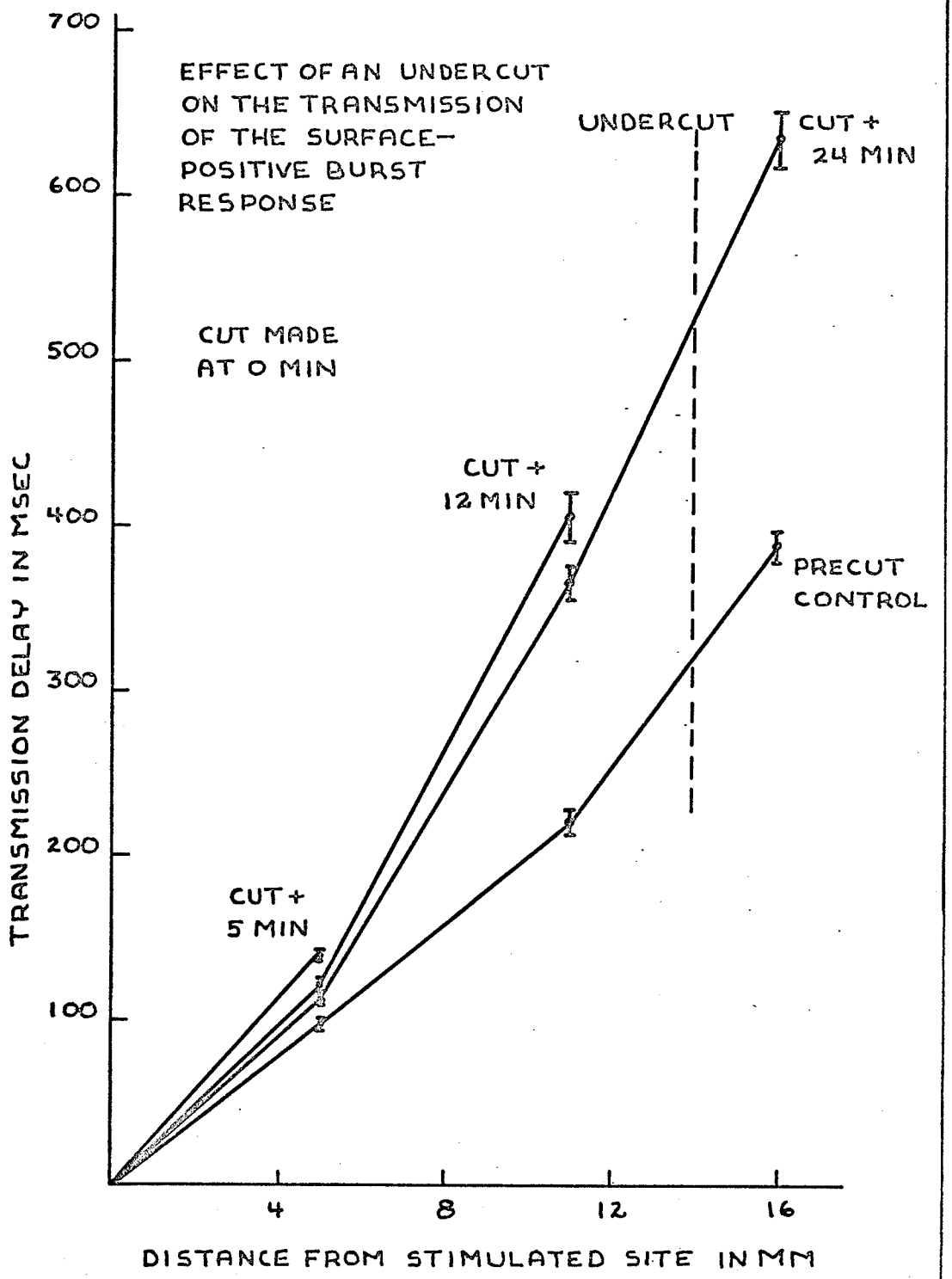
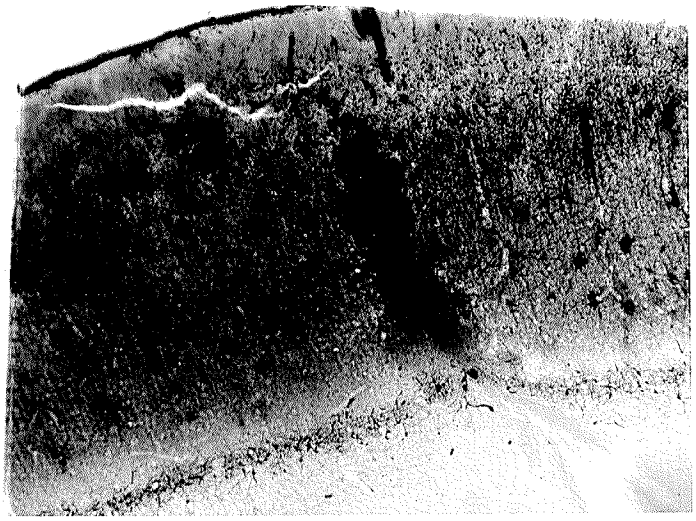

EFFECT OF CHANGE OF
STIMULUS AMPLITUDE
OF
TRANSMISSION DELAY
240
200
160
120
80
40
0
TRANSMISSION DELAY IN MSEC
SEE TEXT
INTERVAL 15 SEC
DURATION 1 MSEC
DISTANCE 10 MM
NO RESPONSES OCCURRED WITH
STIMULUS AMPLITUDE OF 3 VOLTS
1 2 3 4 5 6 7 8 9 10
AMPLITUDE IN VOLTS

FIGURE 12


EFFECT OF AN UNDERCUT ON THE TRANSMISSION OF THE SURFACE-POSITIVE BURST RESPONSE
CUT MADE AT 0 MIN
UNDERCUT
CUT + 24 MIN
CUT + 12 MIN
CUT + 5 MIN
PRECUT CONTROL
TRANSMISSION DELAY IN MSEC
700
600
500
400
300
200
100
4
8
12
16
DISTANCE FROM STIMULATED SITE IN MM

# FIGURE 13

UNDERCUT

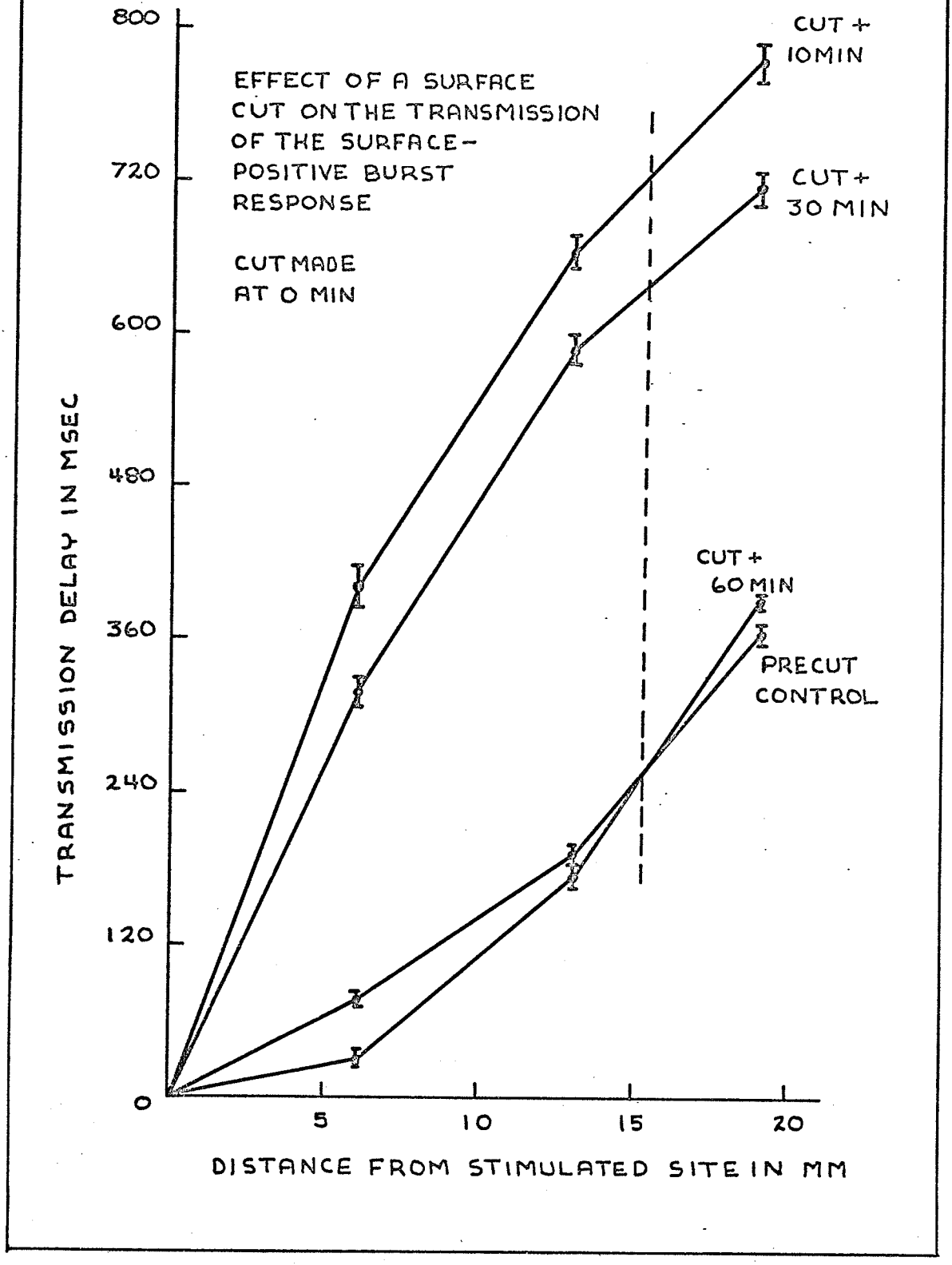


PIAL SURFACE IS AT TOP
CUT EXTENDS TO WITHIN 0·45 MM OF CORTICAL SURFACE

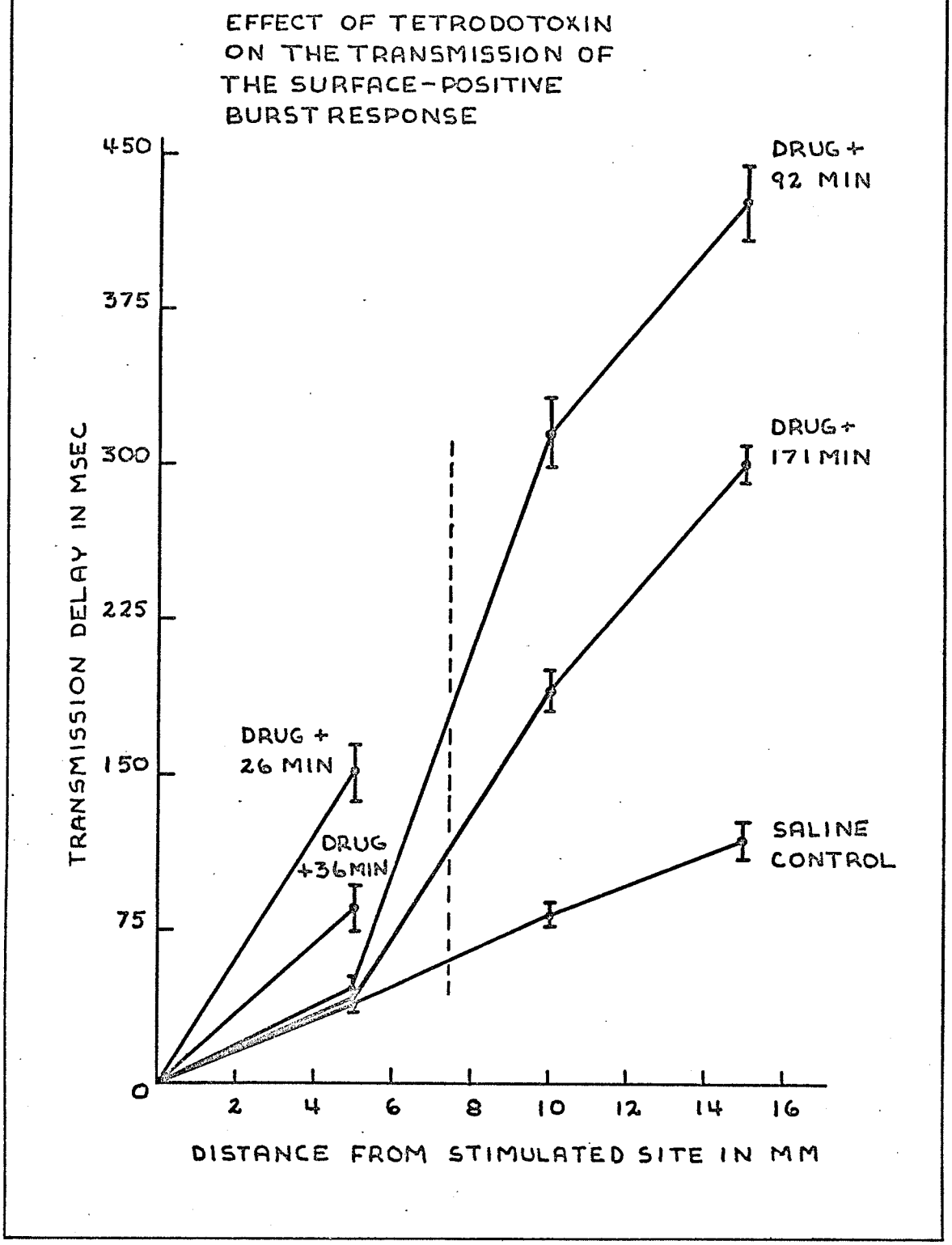
SURFACE CUT

PIAL SURFACE IS AT TOP
CUT EXTENDS THROUGH ENTIRE THICKNESS OF THE CORTEX

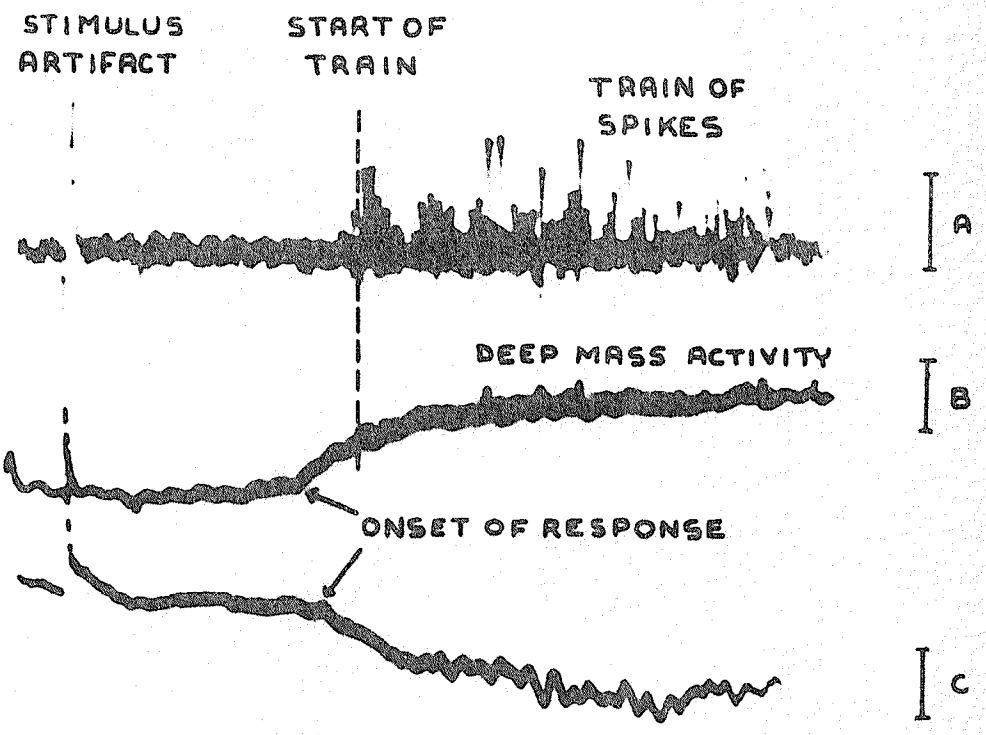
FIGURE 14


EFFECT OF A SURFACE CUT ON THE TRANSMISSION OF THE SURFACE - POSITIVE BURST RESPONSE
CUT MADE AT 0 MIN
CUT + 10 MIN
CUT + 30 MIN
CUT + 60 MIN
PRECUT CONTROL
TRANSMISSION DELAY IN MSEC
800
720
600
480
360
240
120
0
5
10
15
20
DISTANCE FROM STIMULATED SITE IN MM

# FIGURE 15


EFFECT OF TETRODOTOXIN ON THE TRANSMISSION OF THE SURFACE-POSITIVE BURST RESPONSE
450
375
300
225
150
75
0
TRANSMISSION DELAY IN MSEC
DRUG + 92 MIN
DRUG + 171 MIN
DRUG + 26 MIN
DRUG +36MIN
SALINE CONTROL
2
4
6
8
10
12
14
16
DISTANCE FROM STIMULATED SITE IN MM

# FIGURE 16




200 MSEC

AMPLITUDE CALIBRATION

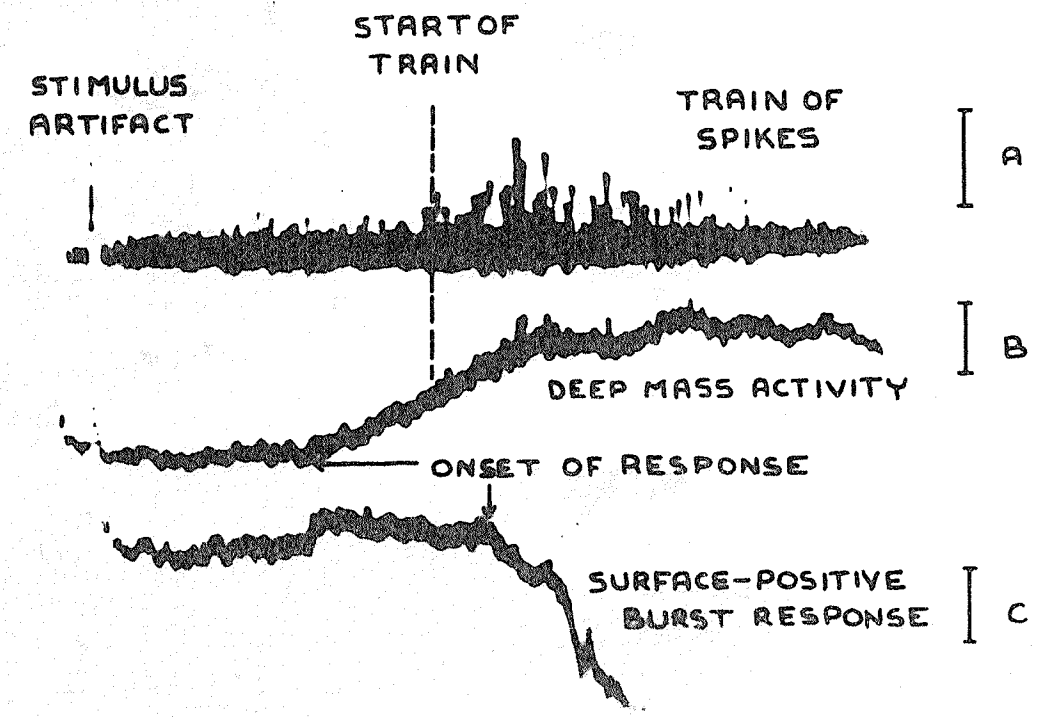
A = 50 μ VOLTS

B = 200 μ VOLTS

C = 200 μ VOLTS

RELATIONSHIP OF ONSET OF SURFACE-POSITIVE BURST RESPONSE, ONSET OF DEEP MASS ACTIVITY, AND START OF SPIKE TRAIN

# FIGURE 17




400 MSEC

AMPLITUDE CALIBRATION

A = 50 μ VOLTS

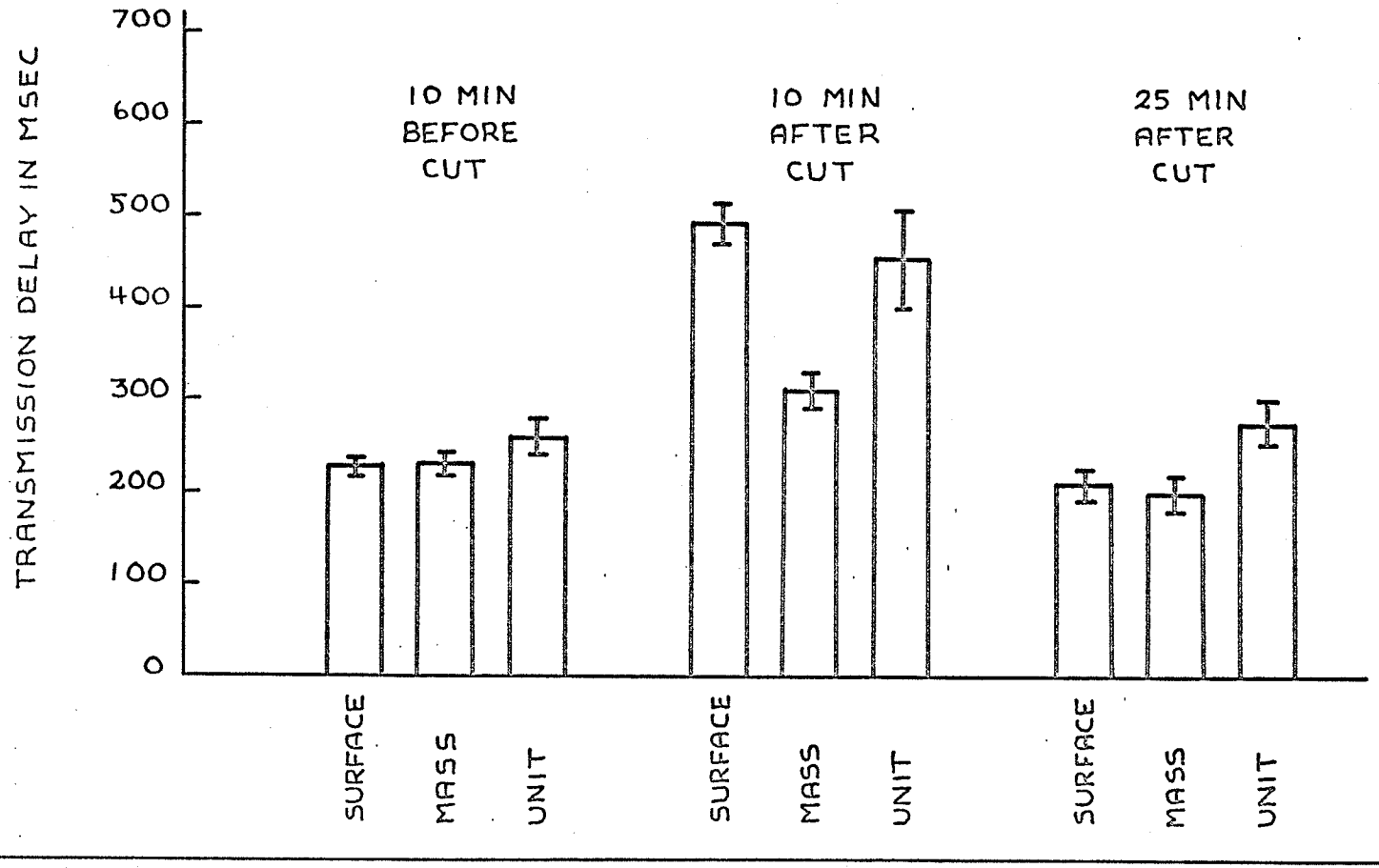
B = 100 μ VOLTS

C = 100 μ VOLTS

RELATIONSHIP OF ONSET OF SURFACE-POSITIVE BURST RESPONSE, ONSET OF DEEP MASS ACTIVITY, AND START OF SPIKE TRAIN 10 MIN AFTER A SURFACE CUT

# FIGURE 18

CHANGES IN TRANSMISSION DELAY WITH CUTS



# TABLE 1

## TRANSMISSION DELAY AND OVERALL VELOCITY OF THE SURFACE POSITIVE BURST RESPONSE

| EXPT. | DIST-ANCE | A→P TRANSMISSION DELAY IN MSEC | A→P OVERALL VELOCITY IN CM/SEC | P→A TRANSMISSION DELAY IN MSEC | P→A OVERALL VELOCITY IN CM/SEC | DIRECTIONAL PREFERENCE | SIGNIF-ICANCE |
|---|---|---|---|---|---|---|---|
| 13-L | 10.0MM | 124.0 ± 6.0 | 8.07 ± 0.59 | 78.0 ± 4.0 | 12.82 ± 0.99 | P→A > A→P | P< .01 |
| 14-R | 13.0MM | 155.0 ± 8.0 | 8.39 ± 0.60 | 282.0 ± 20.0 | 4.70 ± 0.36 | A→P > P→A | P< .01 |
| 16-R | 16.5MM | 322.0 ± 11.0 | 5.12 ± 0.26 | 409.0 ± 14.0 | 4.03 ± 0.19 | A→P > P→A | P< .01 |
| 18-L | 16.0MM | 271.0 ± 4.0 | 5.90 ± 0 19 | 427.0 ± 3.0 | 3.75 ± 0.03 | A→P > P→A | P< .01 |
| 18-R | 19.0MM | 365.0 ± 3.0 | 5.21 ± 0.11 | 573.0 ± 4.0 | 3.32 ± 0.06 | A→P > P→A | P< .01 |
| 19-R | 20.5MM | 331.0 ± 8.0 | 6.19 ± 0.23 | 347.0 ± 9.0 | 5.91 ± 0.23 | | NSD |
| 20-R | 21.0MM | 369.0 ± 17.0 | 5.69 ± 0.33 | 712.0 ± 17.0 | 2.95 ± 0.11 | A→P > P→A | P< .01 |
| 20-L | 18.0MM | 739.0 ± 18.0 | 2.44 ± 0.09 | 1079.0 ± 26.0 | 1.67 ± 0.06 | A→P > P→A | P< .01 |
| 21-R | 18.0MM | 428.0 ± 5.0 | 4.21 ± 0.10 | 542.0 ± 8.0 | 3.32 ± 0.08 | A→P > P→A | P< .01 |
| 22-R | 17.0MM | 356.0 ± 8.0 | 4.78 ± 0.18 | 552.0 ± 12.0 | 3.08 ± 0.11 | A→P > P→A | P< .01 |
| 24-R | 15.0MM | 300.0 ± 7.0 | 5.00 ± 0.20 | 142.0 ± 5.0 | 10.56 ± 0.55 | P→A > A→P | P< .01 |
| 25-R | 17.5 MM | 181.0 ± 5.0 | 9.67 ± 0.42 | 268.0 ± 10.0 | 6.53 ± 0.35 | A→P > P→A | P< .01 |
| 27-L | 16.5MM | 232.0 ± 7.0 | 7.11 ± 0.33 | 158.0 ± 9.0 | 10.44 ± 0.41 | P→A > A→P | P< .01 |
| 27-R | 16.5MM | 190.0 ± 5.0 | 8.68 ± 0.36 | 131.0 ± 4.0 | 12.60 ± 0.57 | P→A > A→P | P< .01 |
| 28-L | 17.5MM | 165.0 ± 10.0 | 10.61 ± 0.80 | 96.0 ± 8.0 | 18.23 ± 1.77 | P→A > A→P | P< .01 |
| 28-R | 18.0MM | 185.0 ± 15.0 | 9.73 ± 0.92 | 116.0 ± 7.0 | 15.52 ± 1.16 | P→A > A→P | P< .01 |

# TABLE 2

TRANSMISSION VELOCITY OF THE SURFACE-POSITIVE BURST RESPONSE ACROSS INTERELECTRODE ZONES

| EXPT. | INTER-ELECTRODE DISTANCE IN MM | A→P ZONAL VELOCITIES IN CM/SEC | | | P→A ZONAL VELOCITIES IN CM/SEC | | |
|---|---|---|---|---|---|---|---|
| | | ZONE 1 | ZONE 2 | ZONE 3 | ZONE 1 | ZONE 2 | ZONE 3 |
| 16-R | 6.0,5.5,5.0 | 9.95 ± 1.04 | 4.48 ± 0.21 | 3.54 ± 0.13 | 9.84 ± 1.03 | 4.66 ± 0.23 | 2.17 ± 0.05 |
| 18-L | 5.0,6.0,5.0 | 6.84 ± 0.21 | 9.32 ± 0.40 | 3.74 ± 0.06 | 4.34 ± 0.08 | 2.59 ± 0.03 | 6.27 ± 0.18 |
| 18-R | 6.0,7.0,6.0 | 7.36 ± 0.23 | 5.90 ± 0.15 | 3.65 ± 0.06 | 6.13 ± 0.16 | 2.45 ± 0.03 | 3.16 ± 0.05 |
| 19-R | 7.5,7.0,6.0 | 4.13 ± 0.13 | 9.83 ± 0.73 | 7.64 ± 0.44 | 9.54 ± 0.69 | 5.35 ± 0.22 | 4.38 ± 0.14 |
| 20-R | 7.0,7.0,7.0 | 4.27 ± 0.24 | 10.40 ± 1.37 | 4.09 ± 0.22 | 2.99 ± 0.12 | 3.80 ± 0.19 | 2.35 ± 0.07 |
| 20-L | 6.0,6.0,6.0 | 5.52 ± 0.72 | 2.42 ± 0.13 | 1.69 ± 0.07 | 2.66 ± 0.16 | 1.24 ± 0.03 | 1.61 ± 0.06 |
| 21-R | 6.0,6.0,6.0 | 4.04 ± 0.13 | 4.19 ± 0.15 | 4.41 ± 0.16 | 3.39 ± 0.14 | 3.82 ± 0.12 | 2.86 ± 0.07 |
| 22-R | 6.0,6.0,5.0 | 4.78 ± 0.24 | 7.01 ± 0.51 | 3.48 ± 0.13 | 5.54 ± 0.32 | 3.22 ± 0.11 | 1.94 ± 0.04 |

# DISCUSSION

## DISCUSSION

Many authors have made the comment that electrical stimulation of the cerebral cortex must unquestionably excite the simultaneous firing of many neuronal elements which otherwise would never fire together (Adrian, 1936; Sholl, 1956; Burns, 1958). This unphysiological excitation and its following response are difficult to relate functionally to the seemingly irregular melange of heterogeneous neurones and their processes seen on histological examination of the cerebral cortex. There is a multitude of hypotheses concerning various aspects of the role and significance of the many artificially produced responses that have been recorded from the more accessible regions of the central nervous system. Their very number brings to mind the adage that the number of treatments in vogue for a disease is inversely proportional to the knowledge which man has of the disease. The neurophysiologist must, nevertheless, continue to probe the mysteries of the brain and speculate on the functional significance of his findings. These hypotheses and speculations are important at least in that they stimulate others to appraise them critically. Hopefully from their criticism new concepts will be engendered to help form a sounder basis to the knowledge of this most complex of all systems.

The observations presented in the RESULTS section of this thesis will now be discussed in the light of previous findings and for their own merit.

### 1) DISCUSSION OF PREVIOUS WORK

Further consideration of the hypotheses and observations of Burns and his associates would be appropriate and useful. An outline

of the basic experimental data which led to the characterization of the surface-positive burst response has already been presented in the HISTORICAL REVIEW. A summary of currently accepted interpretations of this work will serve at this point to introduce the discussion of the experimental results reported in this thesis. The basic interpretations as suggested by Burns, follow.

Burns regarded the surface-positive burst response as a component of normal physiological activity, and the neurones and their interconnecting processes mediating the response as a functional unit (Burns, 1951, 1954, 1955, 1958; Burns and Grafstein, 1952). The characteristics of the response depend less on the nature of the exciting stimulus than on local cortical conditions; that is to say, once excitation occurs and has passed from cell to cell, the characteristics of the response as recorded at any particular moment are determined only by the cells beneath the recording point.

Synapses are undoubtedly involved in transmission of the response; this followed from its all-or-none character, its spread without attentuation and its sensitivity to general anaesthetics.

It has been suggested that there are two types of neurones mediating the surface-positive burst response; these were named primary and secondary type-B neurones by Burns, Grafstein and Olszewski (1957). The primary type-B neurones are presumed to be concerned with the tangential propagation of the response while the secondary type-B neurones are responsible for the oscillatory phenomenon superimposed on the mean positivity of the response. This oscillatory phenomenon is believed to be maintained by a process of self-reexcitation through synaptic interconnections of the second group of neurones concerned.

The excitation of this second group is brought about through connections with the group of primary type-B neurones. Neither of these cell groups have been identified other than by their functional properties in isolated slabs of cerebral cortex. They have not been identified at all, in intact cortex, nor histologically in isolated cortex.

It was assumed that cells which lay deep in the cortex and gave unit potentials that could be recorded only during the passage of a surface-positive burst response were part of the type-B population. Such cells were found generally between 0.2 and 1.3 mm deep in the cortex but especially between 0.9 and 1.2 mm; that is to say, at the same depth as that found for the deep, negative potential peak of the "burst response" (Burns and Grafstein, 1952).

It was also found that undercuts made to within 0.6 mm of the cortical surface abolished transmission of the response, as did a cut from the surface 1.25 mm deep. From this and other data it was concluded that the tangential spread of the response depended on the integrity of a network of neurones laminally organized and contained within layers 3, 4 and 5 of the cerebral cortex. Burns suggested that the excitatory pathway within a network must be randomly connected since responses initiated by the same stimulus at the same point never had the same pattern of potential change twice recorded at a distant point.

There are points both of agreement and disagreement between the results of Burns and the results presented in this thesis.

## 2) DISCUSSION OF PRESENT RESULTS

### a.) GENERAL STATEMENT

The results of the work reported in this thesis suggest that there are neurones, interconnected in functional groups which mediate

the transmission and determine the characteristics of the surface-positive burst response. These groups seemed to lie mainly in layers 3, 4 and 5 of the cerebral cortex. Whether or not there are two populations of neurones, one responsible for propagation of the response and one for oscillations superimposed on the mean positivity is difficult to say from the results obtained. It would appear that the propagation of the response may normally be served by a layered organization of these groups of neurones, but that alternate pathways for transmission, held in functional abeyance by the normal organization of the system, may become available to carry the response whenever the usual network of neurones is interrupted in any way. The data from the experiments on velocity of transmission all strongly suggest that the organization of the neurones is such that the response may be transmitted preferentially in one direction through any group. From this it may be argued that the preferred direction of transmission changes from group to group so that the velocity of the response changes from point to point.

This property of preferential transmission, and the observation in this work that serial responses recorded at a point distant from the stimulated point are similar in shape, duration and amplitude, suggests that the network of neurones responsible for these characteristics is highly organized as a functional unit; that is, the response is not transmitted by randomly interacting neurones within the cortex.

Certain evidence suggests that a minimum number of neurones; that is, a "critical mass" of neurones, must be excited before the response can be propagated. A precise determination of this minimum number would be difficult. However, the evidence from the experiments using cuts and tetrodotoxin suggest that this is so. After a cut had

been made or the drug applied there was a period of time before transmission would occur. Presumably during this time there were not sufficient excitable neurones within the stimulated area to propagate the response, the number excitable being less than the critical number.

Some evidence supports the postulate that transmission of the surface-positive burst response is synaptically mediated. This evidence includes the fact that the response travels very much more slowly than would be expected for the transmission of action potentials in unmyelinated fibres of the size predominantly observed in the cortex; that the response spreads from a single stimulated point without attenuation to all points on the slab; and that it is sensitive to general anaesthetics but relatively insensitive to small decreases in temperature.

It would be useful at this point to consider, if only qualitatively, some possible characteristics of the neuronal networks which seem to transmit the surface-positive burst response. The exciting stimulus may cause excitation to spread synaptically through a limited portion of the network, without bringing into repetitive activity groups of neurones through which it passes, and without propagation of the response. However, if a certain number of neurones are excited more or less simultaneously, and then, through a process of self-reexcitation, continue to bombard through their synaptic connections the receptive poles of neurones at the edge of the field of excitation, then the response is propagated. Depending on the neuronal elements of the unexcited cells with which synapse occurs, several more or less simultaneous trans-synaptic events would have to sum to excite those peripheral cells and thus include them in the excited mass. Transmission of the

wave of excitation could then occur by the continuing inclusion of more and more cells into the excited state. The rate at which the wave of excitation spreads would, therefore, be dependent upon the ease with which neurones on the periphery of the excited group are brought into the excited state. If these peripheral neurones are included only slowly then transmission of the response will be slow. However, if there is a high degree of synaptic interconnections between cells in the "critical mass" and cells at the periphery of this mass then there will be a greater likelihood of peripheral cells being included in this excited field of neurones much faster than when orientation and interconnection of the neurones are not as favourable.

The electrical activity in a self-reexciting chain of neurones could serve two purposes. A negative potential field, albeit small, surrounds this repetitively firing mass, and this could serve to make cells at the periphery of the field, but not included in the active mass, more excitable by partially depolarizing them. Secondly, with several cells in an area firing more or less repetitively, the chances of several impulses crossing the synaptic connections more or less simultaneously with many other cells, and including them within the excitable mass, are higher than if these cells fire once and are subsequently silent. Furthermore, if insufficient numbers of cells are excited initially, the process of self-reexcitation in an interconnected mass of neurones is less likely to occur and excitation is less likely to be transmitted. There could be, then, a situation in which enough neurones are excited to produce a brief period of self-reexcitation, but where there are not enough synaptic events occurring within a critical period of time to include sufficient peripheral neurones in

the excited sphere to propagate the response. In this case a local non-propagated response occurs in the stimulated region. As was presented in the RESULTS section such local responses were indeed often recorded. Examination of Figures 10 and 11 show that there was a critical point for both stimulus duration and amplitude below which no responses whatsoever occurred. Also, as threshold values were approached not all stimuli initiated a response, or on occasion a local non-propagated surface-positive burst response was seen. Moreover, such restricted responses were most characteristic of the chronically isolated preparation. These observations maybe explained by the supposition that the excitation of a critical number of neurones is required before a response can be initiated and propagated.

Within limits, if a greater number of neurones is initially excited it is likely that they in turn will excite a greater number of neurones at their periphery and there will be a shorter time elapsed for these peripheral neurones to be included in the excited mass. Thus if a suprathreshold stimulus current is gradually decreased it would be expected that the transmission delay would increase as less neurones are being initially excited. If less neurones are sending excitatory impulses to the peripheral neurones there is less chance of the peripheral neurones being excited. These predictions are well supported by the data illustrated in Figures 10 and 11.

As the interstimulus interval was shortened, the transmission delay became prolonged and eventually at a relatively high stimulus frequency (1/sec) only every second stimulus elicited a response. If the time required for complete recovery of the network of cells firing were longer than the interstimulus interval, then not all cells that

are normally included in a response would be excited with each stimulus and the transmission delay would be prolonged. At a certain stimulus interval a sufficient number of neurones in the network would be refractory to stimulation so that the next stimulus would not excite the critical mass and no response would occur. This is illustrated in Figure 9. Also, increase in delay and missed responses were almost invariably seen when a stimulus followed too closely after a spontaneous surface-positive burst.

At the other end of the scale if too large a stimulus strength were used then the area would be swamped with stimulus current and many of the cells which normally are excited serially would be simultaneously depolarized and no self-reexcitation would occur. In this instance, no response would be propagated. This phenomenon was seen when the values for stimulus amplitude and duration were raised to high levels.

The studies done here on transmission velocity have led to the postulate that the directional preference of transmission is explicable in terms of the organization of the neurones transmitting the response. The bulk of evidence supporting this has been summarized in the data presented in Table 1. The direction showing the faster transmission has the shorter delays and thus the faster overall velocity.

One possible explanation of these data is that the difference in rate of spread related to direction may be due to a differential richness of synaptic connections from cell to cell in a particular direction. This would permit a faster rate of excitation in the preferred direction at any given time; that is, there may be a greater number of synapses through which excitation can be passed in one direction over the other. Similarly, the more repetitively active is a group of cells the greater

will be the likelihood that transmission will occur to surrounding neurones since more excitatory impulses per unit time will be passing to the synaptic connections with other cells.

Table 2 shows clearly that the velocity of the response across the slab may change from point to point. From these data it may be inferred that there are functional groups of cells in the cortex, that behave as individual units. Different groups might pass excitation along the slab at different rates. The overall delay is the sum of the individual delays across the interelectrode zones. It follows that the interelectrode delays represent the sum of the individual group delays within that zone.

It was observed in this study that when differences in threshold to stimulation between the anterior and posterior ends of the slab occurred, they were well correlated with the preferred direction of transmission. The lower threshold was always at the end from which the response spread fastest. This further supports the mechanism just proposed to explain the preferential direction of transmission. The region where synaptic connections are richest might reasonably be expected to coincide with that region where the smallest stimulus current density will excite the critical mass of neurones.

From the data presented it appears that the consistent preferential direction of transmission in any slab is an inherent property of that slab; this preference has been shown not to be related to the experimental conditions. Furthermore, this property entails a high degree of organization within the network transmitting the response and argues against the concept that the response is transmitted by the random interaction of neurones. The fact that the velocity changes

from point to point along the length of the slab but remains relatively constant with time even over many hours, at any one point, argues again in favour of a high degree of organization of the transmitting network. The data also suggests that within the network there is a further degree of directional organization within groups of cells. The observation that serial responses initiated by equal stimuli at the same point and recorded at a point distant from the origin of the stimulus are similar in shape, duration and amplitude provides further support for the concept of a highly-organized neuronal network in the cortex. In the light of all this evidence it is highly unlikely that transmission of the response is due to the random interaction of the neurones. The progression of the wave of excitation must depend, then, on the organization in the group of neurones through which it passes.

Burns, Grafstein and Olszewski (1957) have postulated that there may be two types of neurones subserving generation and transmission of the response, however, this postulate is not necessary to explain the results presented here.

The next question to consider is to what degree does the spread of the response depend upon the layered organization of the neurones.

## b.) DISCUSSION OF THE INVESTIGATIONS RELATED TO NEURONAL ORGANIZATION

Burns' results on cuts made into the cortex from above and below suggest that the response is carried by a group of cells located in layers 3, 4 and 5 of the cortex. When he cut through these layers, the transmission of the response was abolished permanently. In experiments done here, transmission of the response was abolished permanently past a cut region only when the cut passed through the full thickness of the cortex

and well into the subcortical white matter. The responses were abolished only temporarily after undercuts to within 0.45 mm of the surface of the cortex (Figure 13a and Figure 12) and when cuts were made through the entire thickness of the cortex from above (Figure 13b and Figure 14), but not into the white matter below. After a recovery period, responses could be propagated past the cut region.

Alternate pathways for transmission, held in functional abeyance by the normal organization of the system, may become available to carry the response past the cut region whenever the usual network of neurones is interrupted. There is sufficient anatomical evidence to suppose that alternate connections could be made through the neuronal interrelations seen in the superficial layers of the cortex (for example, dendritic arborizations), and made through the connections of neurones in different parts of the cortex via the remaining subcortical fibres (Sholl, 1956; Collonier, 1967).

It is likely that the act of cutting the slab has an initial adverse effect on neurones well beyond the immediate area of the cut. This is evidenced by the sequence of spreading cortical depression and the relatively electrically silent period with refractoriness to stimulation which follows the completion of a transverse cut. This is reminiscent of the well-known phenomenon of spinal shock and may very well be a cortical analog of that condition. Apparently, cortical neurones recover from this condition within 10-30 min and can then be excited through the alternate cortical connections. How long it takes for this recovery to occur, and how plentiful are the alternate connections are factors which seem to determine the changes in transmission delay near and across the cut region. It is reasonable to expect that if the cells

recover well and if there is a large number of alternate interconnections, then the transmission delay should recover to, or nearly to, its former value. However, if there continues to be some of the formerly excitable cells that do not recover and remain refractory to stimulation, or if the alternate pathways are not plentiful enough, then the time for recovery will be prolonged. Increase in stimulus threshold during this recovery period could be due to fewer neurones being available for excitation in a given area. As recovery occurs, more neurones become available and the threshold decreases. Usually the best recovery was seen at points furthest away from the cut region. This progression in recovery can well be seen in Figure 12. Recovery was in evidence first as a local non-propagated response. As illustrated in Figure 12 further recovery was accompanied by propagation of the response first to one electrode and then to the electrode just proximal to the cut. Finally, transmission occurred across the cut region. As more and more cells recover and become available for excitation the transmission delay should decrease. This is illustrated in Figure 14 where the response recovers to its precut delay values after 60 minutes. Also, the results of the application of tetrodotoxin (Figure 15) support the view that the number of functioning cells is important for the propagation of the response and is a determinant of transmission delay. It is postulated that, as the tetrodotoxin is removed from the area, more and more cells become capable of being excited and of transmitting excitation. In the drug experiments, local non-propagated responses at the stimulating electrode were observed even at the peak of drug action suggesting that effect of the drug did not reach the cells in that region. As the drug effect wore off, transmission recovered up to

the region where the drug had been applied and finally crossed it. Further recovery was evidenced by a decrease in transmission delay. It can be seen from Figure 15 that the most severely affected area, where the drug was in the highest concentration, showed the longest-lasting prolongation of delay. Recovery was also evidenced by a reduced stimulus threshold.

Histological studies of chronically isolated slabs show an irregular degeneration of neurones throughout the slab (Weisman et al, 1967). The observations in chronic slabs from the present experiments suggest a disruption of the pathways involved in the generation and propagation of the response. Transmission is limited (5 mm) and thresholds are raised. The responses are of low amplitude, short duration, all suggesting a decrease in the number of potentially active neurones responding to stimulation.

The passage of a surface-positive burst response was accompanied by trains of spikes from cells in the cortical layers which have been suggested as those most likely to contain the neurones carrying the response. Studies of deep mass activity show that the onset of the surface-positive burst response and of the deep mass activity response were coincident or nearly so. The spike activity almost invariably began after the onset of the deep mass activity, as described in the RESULTS section of this thesis. It may be that the shape of the initial rising phase of the deep mass activity is a reflection of the field surrounding the advancing group of excited neurones. Thus this field would be invading the region in the vicinity of the microelectrode tip before the advancing front of firing neurones. The advancing field of negativity, through partial depolarization of cells not yet included in

the response, might be a critical precondition to firing in cells participating in the surface-positive response, a point already considered.

Trains of spikes are seen when actual invasion of a given cortical region by excitatory impulses occurs. Shifts toward negativity greater than the plateau of the deep mass response are usually accompanied by further trains of spikes, suggesting that further excitation is invading the region or that the self-reexcitation cycle has suddenly involved a large number of neurones in the area more or less simultaneously.

After a transverse cut was made, the slope of the initial rising phase of the deep mass activity became quite gradual as compared with that of the pre-cut response (see Figures 16 and 17). As represented in Figure 19, and suggested previously there is likely a zone of cells adjacent to the cut that remains refractory to stimulation for a period of time, longer than those cells more distant from the cut. The surface and deep electrodes due to their different location will be receptive to different cortical areas. The surface electrode is most receptive to changes in potential directly beneath it whereas the deep electrode is receptive to changes in potential surrounding it. These areas are qualitatively delineated in Figure 19.

It has been postulated that for transmission to occur past such a cut as that represented in Figure 19, excitation must be transmitted through the deepest layer of the cortex or the subcortical white matter.

As the wave front of the negative potential field surrounding the excited mass of cells advances it will be sensed first by the deep electrode. As the wave of excitation passes the cut region, it will

initially excite relatively few cells on the opposite side of the cut region. This is due to the post-cut refractory state of many of the cells in this region. Because relatively few cells are excited the negative potential field will be small and the wave of excitation will be travelling slowly. As the response advances into a region of more excitable cells, more and more cells are included in the response. The potential field will become larger and the wave of excitation will travel faster. This transition from a small, slow moving potential field to a large, fast moving field in one response is seen by the deep electrode but for the most part is still too far away to be registered by the surface recording electrode. This transition from a small, slowly moving field to a larger, faster moving field is seen as a gradual potential change at the deep electrode (Figure 17). As the potential change seen at the deep electrode approaches its maximum, trains of spikes are seen and suggest that the wave of excitation has invaded the region around the deep microelectrode tip. As well, once the wave of excitation has invaded the receptive field of the surface electrode it will be in a region of healthy neurones and will exhibit the characteristics of a normal surface response.

With further recovery of the transmitting pathway and of the neurones surrounding the cut region, more and more cells will be included at a faster rate into the response and the slope of the initial phase of the deep mass activity will become steeper and resemble the precut responses in Figure 16. Also, the delay in transmission will be reduced.

Figure 18 shows the relationship of the onset of the deep mass activity response, the surface-positive burst response and the

first train of spikes before, and at two intervals after the transverse cut was made from the surface of the cortex. From this figure it can be seen that the precut values for onset of the surface response and deep response are almost coincident while the first train of spikes lags somewhat but is not very different. In the representation of delays 10 minutes after the cut, it can be seen that the onset of the surface response and start of the first train of spikes are still very similar while the onset of the deep mass activity is significantly earlier ($P \leq .05$). The later onset of the surface response and train of spikes reflects the slowness with which the advancing wave front of excitation approaches the area beneath the surface recording electrode. At 25 minutes after the cut it can be seen that all values have recovered to the precut levels.

In almost all single unit experiments, the onset of both the surface-positive burst response and of the deep mass activity occurred before the onset of the train of spikes.

All the above observations support the suggestion that an electrical field spreads into the receptive regions of the recording electrodes before the repetitive firing of neurones in that region begins.

# FUNCTIONAL SIGNIFICANCE OF THE OBSERVATIONS PRESENTED

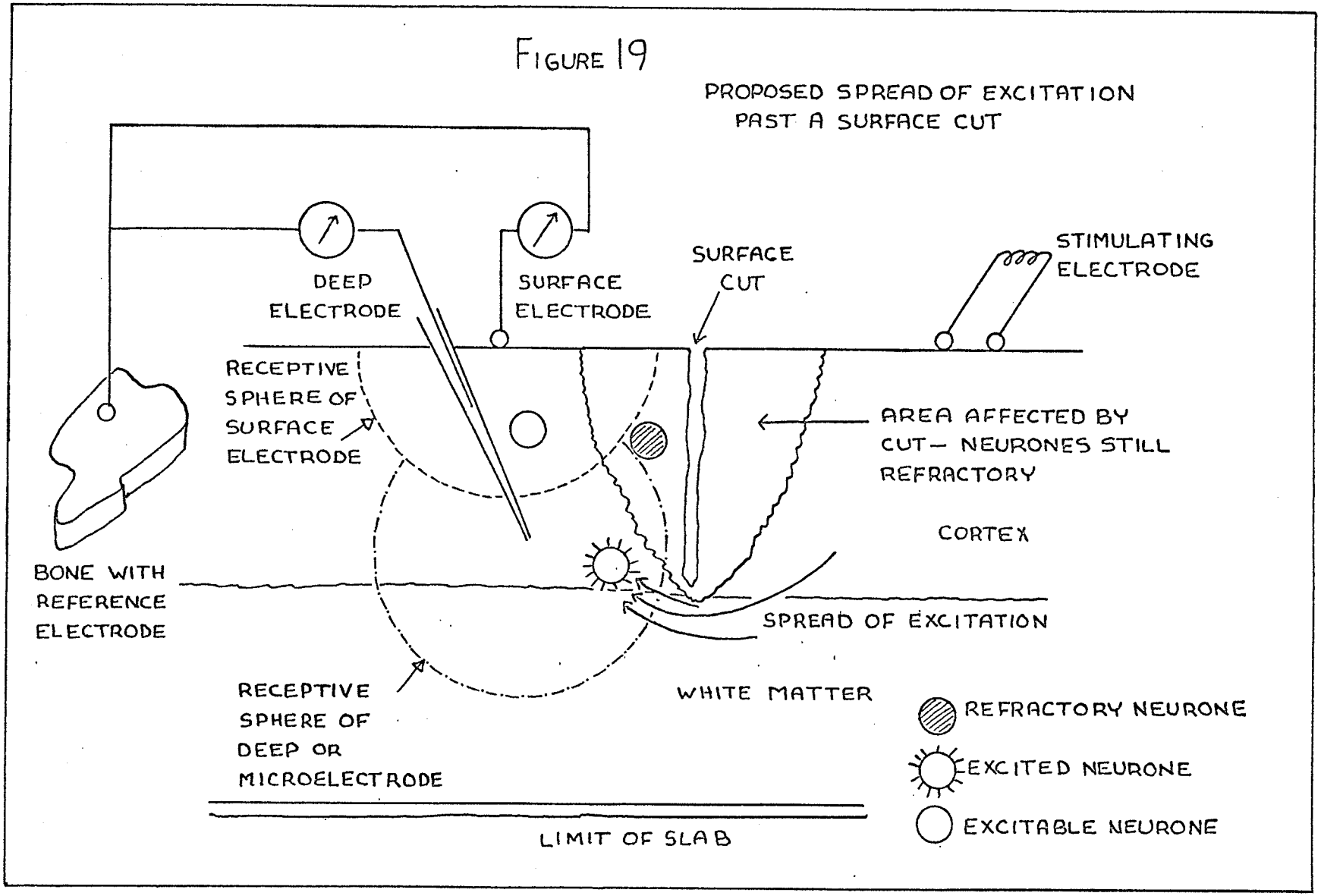


FIGURE 19
PROPOSED SPREAD OF EXCITATION
PAST A SURFACE CUT
DEEP ELECTRODE
SURFACE ELECTRODE
SURFACE CUT
STIMULATING ELECTRODE
RECEPTIVE SPHERE OF SURFACE ELECTRODE
AREA AFFECTED BY CUT— NEURONES STILL REFRACTORY
CORTEX
BONE WITH REFERENCE ELECTRODE
SPREAD OF EXCITATION
WHITE MATTER
RECEPTIVE SPHERE OF DEEP OR MICROELECTRODE
REFRACTORY NEURONE
EXCITED NEURONE
EXCITABLE NEURONE
LIMIT OF SLAB

## FUNCTIONAL SIGNIFICANCE OF THE OBSERVATIONS PRESENTED

Although nothing directly resembling the surface-positive burst response of isolated cortex is seen in intact cortex, Burns (1951, 1958) has presented evidence that the response, or at least the network carrying the response is probably a component of normal physiological activity. Burns feels that the surface-positive burst response is important because it is a mechanism by which excitation could be spread from cortical region to cortical region. Moreover, it has been shown that ongoing electrical activity of the intact cortex may be correlated with the firing of single cortical neurones in temporal epochs (Burns and Smith, 1962; Smith and Smith, 1964; Smith, 1965; Robertson, 1965). This phenomenon has also been shown to exist with the surface-positive burst responses of neuronally isolated cortex.

Brazier (1964) has categorized the features of a "stimulus" which could be recognized by the nervous system. Each of the parameters she listed may function as a trigger for signal recognition. These are intensity, duration, frequency, locus and form of the "stimulus". The author of the present thesis would like to suggest that the rate and facility with which a wave of excitation is transmitted to various regions of the nervous system should be regarded as another parameter by which information could be recognized by the brain. The data presented showing the preferred direction of transmission and the nonuniform velocity of transmission of the surface-positive burst response supports this view.

Because of the anatomic complexities of the central nervous system, particularly the myriad of interconnections between neurones in

the cerebral cortex, it has been suggested (Sholl, 1956; Burns, 1958) that the surface-positive burst response is transmitted by a random interaction of neurones. However, the results of this thesis showing the preferred direction of transmission, the nonuniform velocity of transmission of the surface-positive burst response, the consistent similarity in serial responses recorded at the same point, and the results seen in chronic experiments do not support this view. Rather this evidence suggests that neurones in the cerebral cortex are highly organized into functional groups, and the interaction of these groups determines the mode of excitation as it spreads in any direction. This functional organization may depend on the density of packing of the neurones in a particular area, or the arrangement of their axonal and dendritic processes. From this it would appear that a detailed study of the histology of the cortex coupled with a knowledge of the electrical function in the same region should reveal a clear correlation between structure and functions in the brain. Moreover, such important cerebral functions as memory, learning, and pattern recognition might all be determined by a structured organization of neuronal networks.

Burns (1952, 1957, 1958) has suggested that the transmission of the surface-positive burst response depends on a layered organization of neurones and bases this suggestion partly on the observations that in his experiments, cuts to a certain depth of the cortex abolished transmission of the response permanently. The results of the experiments presented in this thesis suggest rather, that alternate pathways of transmission are available if there is local disruption of the normal network. These findings and this interpretation suggest that the cerebral cortex has a wide margin of safety built into its transmission systems.

# CONCLUSIONS

## CONCLUSIONS

1. Isolated slabs of cat cerebral cortex exhibit a directional preference in the transmission of the surface-positive burst response.

2. The velocity of transmission of the surface-positive burst response is not uniform from point to point in any direction along the length of the slab.

3. Serial responses recorded at a point distant from the stimulated site show similarity in form, duration and amplitude.

4. Transmission of the surface-positive burst response is possible past cuts made into the cortex provided that an alternate pathway is available; that is, cuts may be made through the full thickness of the cortex and transmission will occur provided the subcortical white matter remains essentially intact, or cuts may be made through the subcortical white matter and into the cortex and transmission will occur provided there are some superficial cortical connections remaining. Thus, there are alternate pathways through which excitation may spread if the local pathways are interrupted.

5. Transmission of the surface-positive burst response in chronically isolated slabs of cerebral cortex is of limited extent probably due to disruption of regular pathways.

It is inferred from all these results that there is a high degree of organization of neurones into functional groups in the normal cerebral cortex and that these groups subserve the transmission of the surface-positive burst response. This organization appears to be an inherent property of the cortex. It is possible that it serves to identify signals arriving from several different directions in

the brain. Alternatively, it may function to direct the flow of several different signals to appropriate target regions in the brain, by selective alterations in their velocity.

# RECOMMENDATIONS

# FOR

# FUTURE WORK

## RECOMMENDATIONS FOR FUTURE WORK

Several interesting possibilities for future work arose during the course of the experiments. Their study would have constituted a separate project in themselves and they have not been further pursued. It is necessary that they be recorded, however, if they are not to become lost in the inevitable shuffle that occurs after completion of a major project. These possibilities include the following:

(1) Much useful information about the interaction of neurones subserving the transmission of the surface-positive burst response and, as a logical extension, the functional organization of the cerebral cortex could be gained by careful study of other parameters of this response, such as amplitude and duration.

(2) Because it is highly probable that the surface-positive burst response has some physiological function, it would serve as an excellent tool with which to study the effects of drugs on the nervous system.

(3) Several observations on the so-called "spontaneous" surface-positive burst activity were presented in this thesis and it is suggested that the results of a more careful study of this phenomenon in the brain may be usefully compared with other spontaneously active tissue such as the heart and gut.

(4) A careful histological study, correlating a more detailed study on the velocity of transmission of the response from point to point with the underlying cytoarchitecture, would aid in relating neurone structure to its function.

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