SPATIAL ORGANIZATION IN THE CEREBRAL CORTEX: EVIDENCE FROM STUDIES ON THE N-WAVE DCR

A Thesis

Presented to the

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

by

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October, 1971

ABSTRACT

The manner in which the negative-wave direct cortical response (nDCR) spreads tangentially from a point of stimulation was determined for several different locations of the stimulating electrodes along the length of the cat suprasylvian gyrus. All experiments (N = 14) were done on freshly-prepared islands of cerebral cortex in cats whose forebrain had been acutely transected. Responses were averaged and measured with the aid of an on-line digital computer.

The logarithmic rate of nDCR decline with distance of the nDCR peak was found to be non-uniform over the length of nDCR tangential spread. There occurred regions of slow and regions of fast rates of nDCR decline. Regions of slow rate of nDCR decline ("reinforcement" regions) tend to occur at the same location on the gyrus independent of the position of the stimulating electrodes. Corollary to this, regions of fast rate of nDCR decline which immediately precede or follow a reinforcement region also tend to be constant in their gyral locations, independent of the position of the stimulating electrodes. This suggests the existence of a functionally important spatial organization of the cerebral cortex extending beyond the limits of the vertical columns described by Mountcastle (1957) and Hubel and Weisel (1962).

The tangential spread of the nDCR was found to be asymmetrical about the point of stimulation; i.e., responses have a greater amplitude and a shorter peak latency at electrodes located the same distance away but in the opposite direction. In the suprasylvian gyrus, the directionality of asymmetry is mainly posterior (87 out of 110 trials). Almost all of the asymmetry is produced within 3 mm of the stimulated site, with the greatest amount being produced at about

1 mm from the stimulated site. The element responsible for asymmetry is postulated to be a cortical afferent which branches into two unequal portions within the molecular layer. The portion lying in the direction of greater nDCR amplitude branches extensively while the other portion is branch-poor. Directionality of nDCR asymmetry is related to stimulus location on the suprasylvian gyrus. In the middle regions of the gyrus (corresponding to the somatic association area) the asymmetry is always posteriorly oriented. Regions nearer to either end of the gyrus (corresponding to visual association areas) show an admixture of anterior directionality.

No difference was found in nDCR transmission across a cut 0.5 mm deep as compared with that across a cut which severed the island into two portions ("complete cut"). The transmission of the nDCR across these cuts is postulated to be due mainly to volume conduction. The mean logarithmic rate of decline of the "volume conducted" response was found to be -0.83 log mV/mm. This is about twice the average rate of decline for the physiologically conducted response. In some trials, the nDCR peak latency increased with distance across either type of cut. This transmission was postulated to be due not only to volume conduction but also, in part, to very superficial axonal fibres which were missed in the cutting procedure.

The relationship between response size and stimulus strength was tested for various distances from the site of stimulation. It was found to be the same for all distances up to 4.8 mm from the stimulated point.

Paired-pulse experiments were done to test nDCR excitability from 0.1 to 10 seconds after a control response. Two components of test pulse depression were found: the first component has a time

constant of 100-200 msec and the second component has a time constant of about 4.5 seconds. The latter component was postulated to underlie a form of short-term memory. Repetitive stimulation (10 per second for 10 seconds) caused an increase in both time constants and therefor was believed to alter the processes underlying each of the two components of test pulse depression.

ACKNOWLEDGMENTS

I am indebted to the following people for:

The opportunity to study in this department	Dr. I.R. Innes
Overall supervision, inspiration and encouragement	Dr. Carl Pinsky
Consultation and guidance	Dr. A.J. Vazquez Dr. J.W. Phillis Dr. H. Weisman
Help in animal surgery and technical assistance	Mr. David Brown
Technical consultation	Prof. E.W. Mazerall
Animal care	Mr. John Kiekush and staff
Valuable discussions and assistance	Dr. R.C.A. Frederickson Dr. G. Krip Mr. D. Kahanovitch
Preparing the figures and helping to edit the text	Miss Gayle A. Peterson
Equipment repair and maintenance	Mr. John Delaive
Photographic work	Mr. Roy Simpson
Typing the manuscript	Mrs. Louise Robin
The warmth of friendship	Fellow Graduate Students Teaching and Support Staff of this department

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A. INTRODUCTION

I. History

a) Adrian

The history of the negative-wave direct cortical response (nDCR) started in 1936 with Adrian's classical paper on the spread of activity in the cerebral cortex. In this paper (Adrian, 1936), he described a monophasic potential, negative in polarity, which lasted about 10 msec and was approximately 100 μV in amplitude. It occurred in response to a brief electrical stimulus applied directly to the surface of the cerebral cortex. Adrian considered the response to be an activation of elements in only the most superficial layers of the cortex, hence his term "superficial response".

Because the time course of the superficial response was much slower than that of a nerve action potential, Adrian thought it unlikely that the response was due mainly to nerve fibres. He believed it to be caused either by nerve cells with laterally spreading dendrites, such as the horizontal cells of Cajal, or else the upper branches of the apical dendrites from pyramidal cells.

b) The early 1950's

Except for a brief report by Rosenblueth and Cannon (1942), there was no systematic study on the nDCR for about a decade and a half after Adrian's 1936 paper. Interest quickly picked up in the early 1950's starting with Burns' work on isolated cortex (Burns, 1950; Burns, 1951; Burns and Graftein, 1952). Almost simultaneously, Chang (1951) published his views on the nDCR, heavily emphasizing the importance of the apical dendrites in this response. A theoretical

paper by Eccles (1951) appeared about this time, applying information obtained from spinal cord research to the interpretation of various responses obtained in the cerebral cortex. These workers, together with Adrian, established the three major theories which have postulated specific anatomic sites for the electrogenesis of the recorded nDCR. In brief, these are: (1) directly excited axons (Burns and Grafstein); (2) directly excited dendrites (Chang, Adrian), and (3) synaptically excited dendrites (Eccles).

1) Burns

Burns' work is unique in that his observations were the first to be made on neuronally isolated slabs of cerebral cortex (see Burns, 1950). Burns was also the first to investigate the nDCR in unanaesthetized cats (Burns, 1951); he found that it was no different from the response in chloralose-anesthetized animals.

Burns found that the response attenuated rapidly with distance and could be recorded no farther than 10 mm from the stimulated site. He concluded that the outward spread of the response could not be accounted for by activity passing from one element to another across synapses since he believed that synaptic transmission would maintain the response to a far greater degree. He postulated instead the existence of a random arrangement of surface conductors which spread outward, parallel to the pial surface, to a distance not exceeding 10 mm. Only those elements whose end happened to be directly under the stimulating electrodes could conduct the response to a maximum of 10 mm; those elements which were stimulated somewhere in their middle region would conduct the response in 2 directions but for a shorter distance.

This theory was more fully developed by Burns and Grafstein (1952) on the basis of their results with laminar recording. They found that the nDCR was recorded as a positive-going wave from about 0.6 to 3.0 mm beneath the pial surface. This distribution of potential suggested to them that the surface elements responsible for the nDCR have "limbs" which run downward through the grey matter and even below it. These limbs would then act as sources to the sinks constituted by the depolarized portions in the superficial layers. Burns and Grafstein postulated the limbs to be axons which originate from the lower layers of the cortex and divide in the upper layers to give rise to long branching filaments responsible for the tangential spread of the nDCR.

Laminar stimulation indicated two peaks of excitability for producing the nDCR. They occurred at 0.4 mm and at 2.0 mm below the pial surface. To account for this, Burns and Grafstein postulated the existence of two families of branches on the axon limbs at the depths where the excitability peaks occurred. They thought that branches would make the stimulating current more effective since their presence would increase the number of neurons which could be affected by the stimulating current. The upper branches would be consistent with the surface branching already referred to. The lower peak of excitability could be accounted for either by a second family of branches or alternatively, by the stimulation of the cell bodies giving rise to the axon limbs.

The axon theory of Burns and Grafstein has found little support among subsequent investigators. It is especially difficult to reconcile it with the work of Ochs and Suzuki (1965). These workers found that γ-amino butyric acid (GABA) is ineffective in

blocking the nDCR when placed on either the stimulated site or on the cortex between stimulating and responding sites. However, when placed on the responding site, GABA quickly abolished the nDCR. They interpreted these results as indicating that the primary responding elements are non-axonal. They argued that the transmission of the response must depend on axons in the molecular layer since GABA is relatively inert on axons (Curtis, 1961). The blockade by GABA of the responding site was thought to be due to a post-synaptic depressive effect of this agent on the apical dendrites (Ochs, 1965).

2) Chang

On the basis of the histological information of his day, Chang (1951) noted that of all the elements present in the molecular layer of the cerebral cortex, the apical dendrites of pyramidal cells were the most abundant. Also, since the molecular layer is the most superficial layer of the cerebral cortex, its elements should be the first to be activated by surface stimulation. Chang therefore proposed that the negative potential wave caused by a weak surface shock was due primarily to the direct activation of apical dendrites in the molecular layer. He considered any contribution by other elements (ascending axons of Martinotti cells, recurrent collaterals of pyramidal cells, Cajal cells) to be insignificant since he believed that they were small in number compared to the apical dendrites. In addition, he found that a cut 1.0 mm deep from the pial surface did not completely block the tangential transmission of the response.

On slightly stronger stimulation, Chang noticed a small positive wave preceding the negative wave. He interpreted this as indicating activity starting from the lower layers of the cortex

(initial positive wave) and propagating upwards into the apical dendritic bushes to add to the negativity already achieved by direct stimulation of these dendrites. The lower activated sites were postulated to be the bifurcating points of apical dendrites and the cell bodies of the pyramids. The latter were assumed to be activated indirectly by small cells in the upper layers of the cortex which were directly excited by the stimulus.

3) Eccles

Early proponents (Adrian and Chang) of apical dendrites as being responsible for the nDCR postulated these elements to be directly excited by the stimulus current. A departure from this view was proposed by Eccles (1951) on the basis of advances which had been made in studies on spinal motoneurons. Microelectrode recordings of the excitatory postsynaptic potential (EPSP) in motoneurons (Brock, Coombs and Eccles, 1951) were found to have a time course which Eccles noticed was similar to that of the nDCR. He therefore suggested that the superficial response be regarded not as a direct activation of the apical dendrites, but as a postsynaptic potential generated in the apical dendrites by impulses in the molecular layer axons. By this theory, only the axons are directly stimulated. They in turn activate the apical dendrites across synapses to produce the recorded negative wave.

c) Delineating the nature of the dendritic response

Except for Burns (Burns and Grafstein, 1952; Burns, 1958), the theory that the recorded nDCR was produced by the apical dendrites of pyramidal cells had widespread acceptance by the mid 1950's. Research interest now swung towards delineating the nature of the

dendritic response.

Clare and Bishop (1955) believed that surface stimulation directly activated the apical dendrites (producing the nDCR) and that strong stimulation to the optic radiation indirectly activated the apical dendrites by way of cell body and basal dendritic excitation. Stimulation and recording at different depths (Bishop and Clare, 1953) indicated to them that the apical dendrites conduct actively upward (antidromically) in the cortex but not downward toward the cell body. They therefore postulated that conduction down the apical tree towards the soma of the activated upper branches was electrotonic in nature while antidromic conduction propagated actively but decrementally. The two types of graded response then known were the local response and the synaptic response. The latter seemed to them to be more closely related to the dendritic response since local responses have the same time dimensions as an all-or-none response (Hodgkins, 1938). This would be too short to account for a response such as the nDCR. It must be noted that Clare and Bishop believed dendritic responses to resemble synaptic responses but they did not believe that the dendrites are synaptically activated in response to surface stimulation. On the contrary, they considered that the apical dendrites were electrically excitable and hence would be directly activated by surface stimulation.

Purpura and Grundfest (1956) rejected the notion of direct dendritic excitability and returned to Eccles' theory of EPSP's. They did so on the basis of their findings with d-tubocurarine (d-TC), which they found to inhibit the nDCR and various other cortical dendritic responses. Purpura and Grundfest interpreted this result

as being due to a synaptic blocking effect of d-TC. They also found that d-TC blocked the negative but not the positive wave of the positive-negative sequence which is recorded at the cortical surface in response to pyramidal-tract stimulation. From this, they concluded that the negative wave was caused not by antidromic activation of the apical dendritic tree but by recurrent collaterals and/or interneurons synaptically impinging on the apical dendrites. This led them to propose that the apical dendrites are composed of a synaptically excitable membrane which is not capable of responding to an electrical stimulus. Hence, the nDCR was in their opinion a synaptically excited response in apical dendrites, much the same as Eccles had proposed in 1951.

This work was later called into question by Ochs (1959) who showed that low blood pressure produced effects on the nDCR similar to those observed with d-TC. This drug has well-known hypotensive effects due to its histamine-releasing properties (Goodman and Gilman, 1965). Also, systemically injected d-TC is unlikely to penetrate ths blood-brain barrier (Curtis, 1961).

Ochs criticized the postsynaptic potential theory of Eccles on the basis of nDCR refractoriness which he found with spatial and temporal interaction studies (Ochs and Booker, 1961). Postsynaptic potentials are expected to summate (Curtis and Eccles, 1960). The refractoriness (lasting 20 to 40 msec) of the nDCR suggested to Ochs that the response involved a regenerative process such as occurs in an action potential but differed from the all-or-none action potential by being graded in nature and also by propagating decrementally down the apical tree. It would fail at the soma and

would thus be unable to fire the axon unless other axosomatic activity were occurring simultaneously (Ochs, 1966, 1962: Ochs and Booker, 1961). The refractoriness found with spatio-temporal interaction could then be considered to be due to occlusion of a common pool of apical dendrites; i.e., once activated, a dendrite would respond less effectively for a period of about 30 msec, to a second stimulus applied either at the same site or at a different site close by. An alternative to the hypothesis of decremental propagation in apical dendrites also was suggested by Ochs (1965), based on experiments with frog sartorius fibres. It was found that the electrotonic spread from a region was much greater with a maintained depolarization than with brief pulses of current. Hence, Ochs suggested that a spread into the initial segment of pyramidal cells may occur as a result of sustained depolarization caused by repeated synaptic activation (which would lead to refractoriness) of the apical dendrites.

Ochs, however, still maintained his views on the postsynaptic character of the nDCR in that he believed the response was activated in dendrites across synapses by molecular layer axons. The difference between his and Eccles' theory was that Ochs did not consider the nDCR to behave like a motoneuron EPSP, but rather to involve an active process which exhibited refractory properties.

d) Microelectrode studies on the nDCR

Microelectrode studies on the nDCR fall into two categories: field potential studies and single-unit recordings. The former is accomplished by using low-resistance microelectrodes (1 to 2 megohms, cf. Ochs and Clark, 1968a) whose tips are too large to pick up unit responses. They record the field potentials generated by all the

active elements in the volume conductor medium of the cerebrum. Single-unit firings can be recorded either extracellularly or intracellularly with ultramicroelectrodes whose tip resistance is of the order of 20 to 40 megohms (Phillis and Ochs, 1971).

1) Field potential studies

Field potential studies of the nDCR were originally carried out by Burns and Grafstein (1952). They found that the response was recorded as a negative wave with respect to an indifferent electrode placed on inactive tissue from the pial surface to a depth of about 0.6 mm. Below this depth, the response was recorded as a positive-going wave. Similar laminar recordings were made by Ochs and his co-workers (Suzuki and Ochs, 1964; Ochs and Clark, 1968a and b) who found that the response reversed from a negative-going wave to a positive-going wave most often at depths of about 200 - 300 μ.

The distribution of nDCR potential with depth as reported by these investigators resembles very closely that described by Nicholson and Llinás (1971) for the Purkinje cell response to parallel fibre activation in alligator cerebellum under conditions of reduced blood flow. They term this type of potential distribution with depth as a "reversing negative-positive field potential" and have found that it corresponds very closely to the field-potential distribution predicted by their mathematical model of cerebellar cortex for the case of passive field potentials. In this model, it is assumed that the parallel fibres cause synaptic potentials in the dendrites of the superficial Purkinje cells. These potentials are further assumed to be conducted down the dendritic tree by passive dendritic processes only; i.e., as a function of the passive electrical properties of the

Purkinje cell dendrites. If, on the other hand, the dendrites were assumed to be able to conduct active responses down the Purkinje dendritic tree, the model would predict a field potential distribution (for a response generated by the parallel fibre system) which remains negative in polarity at all depths of the cerebellar cortex down to the level of the Purkinje cell body. This latter type of field potential occurs under conditions of normal blood flow in the alligator cerebellum. Under these non-ischemic conditions, the Purkinje cell dendrites are known to conduct spikes (Nicholson and Llinás, 1971; Llinás and Nicholson, 1971). This "nonreversing negative field potential" does not at all resemble that of the nDCR as seen by the laminar recording studies in the cerebral cortex.

2) Unit firing

Li and Chou (1962) recorded intracellular unit discharges in response to surface stimulation which was at the threshold for the nDCR. They found that 95% of the cells which produced a spike discharge responded within an interval of 22 msec after stimulation. This interval approximately corresponded to the time course of the nDCR. The spike discharges which occurred more than 1 msec after the stimulus were preceded by a depolarizing potential which they considered to be equivalent to an EPSP. Li and Chou concluded that the nDCR recorded at the surface arose as a result of the summation of these depolarization potentials from all the units activated by the stimulus. Sugaya et al. (1964) concur with Li and Chou on the postulate that the nDCR arises out of a summation of EPSPs. However, they seldom recorded intracellular spikes during the time course of the nDCR. These occurred most often when the

stimulus was strong enough to cause one or more positive deflections preceding the nDCR.

Phillis and Ochs (1971) found unit spikes and intracellular depolarization potentials in response to surface stimulation during the time course of the nDCR. However, many of the cells required a low level of L-glutamate (5-10 nA) released iontophoretically in their near vicinity to show spike discharges. They considered the intracellular depolarization potentials to arise from dendritic depolarizations which fail to propagate fully into the soma. These depolarizations, unlike EPSPs, were postulated to be active in nature and to propagate decrementally down the dendritic tree such that an additional depolarization (e.g., that produced by L-glutamate), is required for some cells to fire. The depolarizing potentials exhibited both temporal and spatial refractoriness similar to that described for the nDCR (Ochs and Booker, 1961). Spatial occlusion was considered to be a result of a graded spread of activity from excited synaptic endings into adjacent dendritic areas occupied by the endings of other axonal inputs, rendering these regions refractory. To explain the refractoriness of the dendrites, Phillis and Ochs postulated that the synaptic transmitter carries the membrane potential toward an equilibrium potential and that further transmitter release onto the dendritic membrane is unable to produce further depolarization even though it may be increasing membrane conductance.

These workers theorized that the nDCR is the surface representation of the intracellularly-recorded depolarizations, even though the latter have a duration of 100 msec and more. They point out that the surface-negativity of the nDCR is curtailed by the occurrence

of the late positive wave, restricting the nDCR to a time course of 10 to 20 msec. Stohr et al. (1963) consider the late positive wave to be a manifestation of an inhibitory synaptic action. The laminar studies of Ochs and his co-workers indicated to them that the late positive wave is the response of a deeper population of neurons with a resulting surface positivity on a volume conductor basis (Ochs and Clark, 1968b; Ochs and Suzuki, 1965; Suzuki and Ochs, 1964).

e) Tangential spread of the nDCR

There is little agreement in the literature on the extent of tangential spread of the nDCR. Adrian (1936) reported 4 mm, Chang (1951) 5 mm, and Burns (1950) 10 mm. The graphs of Brooks and Enger (1959) indicate that they recorded the response as far as 14 mm from the stimulating electrodes. More recently, Suzuki and Ochs (1964) reported a tangential spread of about 4-5 mm. Grafstein and Sastry (1957) did not find any significant difference between the tangential spread (about 7 to 10 mm) of the nDCR in acute and in chronic cortical slabs.

Radial cortical cuts have been used by several workers in an attempt to find the layer or layers responsible for tangential nDCR transmission. Widely varying results have been reported: Chang (1951) was unable to abolish transmission by a 1 mm incision; a 3 mm cut was required to do so. Burns and Grafstein (1952) found that a cut only 0.13 mm deep was enough to abolish the response. In a study on rabbits, Ochs (1956) reported that little change in nDCR transmission occurred even when all the cortical layers had been severed. Transmission was blocked only after the cut severed both cortex and the underlying white matter. Similar results were layer reported in the cat (Ochs and Booker, 1961). Ochs later reversed his position (Ochs

and Clark, 1968a) when he observed that, in preparations where the only intact layer was the molecular layer (i.e., all other cortical connections and some underlying white matter had been severed), the nDCR was transmitted as far and with the same latency as in the intact cortex (Ochs and Suzuki, 1965). He concluded that in his previous experiments a few superficial fibres in the molecular layer had escaped the cutting procedure and were responsible for transmitting the response beyond the cuts.

Brooks and Enger (1959) found in cats that with weak stimulation the nDCR amplitude decayed in height linearly with an increase in stimulus-recording distance, and travelled up to 3 to 6 mm from the stimulating electrodes. With stronger stimulation, the response again declined in height up to about 5 or 6 mm away from the stimulating electrodes but then, from 6 to 10 mm, underwent an increase in height and then entered a new region of decline. Brooks and Enger called this phenomenon "reinitiation" if the response had declined to zero before reaching the area of renewed strength. If it had not declined completely to zero the phenomenon was termed "reinforcement". Supra-maximal stimulation produced reinforcement at both 5 mm and at 10 mm. The reinforced or reinitiated responses were reported to occur 1 to 3 msec earlier than what could be expected from a simple conduction away from the stimulus site.

On the basis of these reinforced responses, Brooks and Enger distinguished between slowly and rapidly conducted nDCRs. They suggested that the slowly conducted nDCRs are due either to direct or to synaptic excitation of pyramidal cells. Those with a faster conduction velocity were proposed to be synaptically initiated on

other pyramidal cells after fast conduction of about 10 M/sec in tangential fibres such as the corticocortical axons, recurrent collaterals of pyramidal cells, axons of stellate cells, or a mixture of these.

II. Anatomical considerations

It has been argued that the recorded nDCR is probably due mainly to activity of elements in the molecular layer of the cerebral cortex (Adrian, 1936; Burns, 1951; Ochs and Clark, 1968a). Nevertheless, it is well to note that the elements presumably activated during the response (axons and dendrites) are derived mostly from neurons in the other cortical layers, and also from certain cortical afferents. An understanding of the anatomy of the entire cerebral cortex is therefore necessary in order to try to arrive at a fuller understanding of what is happening in cortical elements during the nDCR. With this in mind, a brief summary of some of the known morphology of the cerebral cortex is presented in this section.

a) General anatomy of the cerebral cortex

On the basis of cell body distribution shown by Nissl staining, the cerebral cortex is usually described as a six-layered sheet of grey matter. Dendritic and axonal stains, however, show that these cell processes do not restrict themselves to the cell body layers but freely transgress the laminal boundaries. This is in contrast to the cerebellar cortex where dendritic and axonal ramifications spread within laminar confines.

The neurons of the cerebral cortex can be classified into two main categories: pyramidal and stellate. On the basis of structural

resemblance, other neuron cell-types can be considered to belong to either of these two categories. For instance, the star-pyramids of the second layer may be considered to be essentially a pyramidal type. The fusiform cells of the fifth and sixth layers may be considered either as inverted pyramidals (Martinotti cells) or else as a type of stellate cell (Szentágothai, 1969).

The pyramidal cell has a conical perikaryon whose summit gives rise to a large apical dendrite which ascends to the first layer by dichotomous branching. Basilar dendrites arise from the base of the cell body. They are thick but short, and branch into numerous segments. Colonnier (1964) has shown in the visual cortex of the cat that the basilar dendrites may spread as a circle, as a cross or as an elongated figure in the tangential plane. Both apical and basilar dendrites possess a large number of dendritic spines (Colonnier, 1966). The size of the pyramidal cell tends to be greater the deeper it is located in the cortex. Small pyramids are located in layer II, medium and large pyramids in layer III, and giant pyramids in layer V. An exception to this tendency are the small pyramidal cells with arciform axons which are located in the upper part of layer IV.

Scholl (1956) subdivided pyramidal cells into four classes on the basis of their axonal arborization:

(1) those with unbranched axon to the white matter

(2) those with branched axon to the white matter

(3) those with branched axon to the white matter and recurrent collaterals

(4) those whose axon has recurrent and horizontal collaterals

but does not go to the white matter.

Scholl found that the pyramidal cells with recurrent collaterals occur mainly in layers V and VI whereas those without recurrent collaterals occur in layers II, III, V, and VI.

The distribution of synapses on the pyramidal cell have been worked out by Colonnier and Rossignol (1969). They found only synapses of the symmetric type on the cell body, the spine-free portion of the apical dendrite, and on the parent stem of the basilar dendrites. These synapses have "flattened" vesicles and have been assumed to be inhibitory in nature. They occupy only 10 percent of the cell body surface and 5 percent of the initial portions of the dendrites. On the other hand, 92 percent of the synapses on dendritic spines were of the asymmetric type. These synapses have spheroidal vesicles and are considered to be excitatory in nature. Only about 3 percent of spine contacts were of the symmetric type and the remaining 5 percent could not be easily classified. Finally, the dendritic trunks of the spine-bearing dendrites have only 2 percent of their surface covered with synapses. Of these, the ratio of symmetric to asymmetric synapses is 4:1.

If we assume that synapses with flattened vesicles are inhibitory and those with spheroidal vesicles are excitatory, then even though the latter outnumber the former by a factor of about 10:1 (Colonnier and Rossignol, 1969), the inhibitory synapses are far more strategically located for control of cell firing than are the excitatory synapses.

The stellate cell has an oval or circular perikaryon from which dendrites arise abruptly. Their dendrites are thinner than those

of pyramidal cells and are symmetrically arranged around the cell body. Most stellate cell dendritic fields have the form of disks or cylinders elongated in the tangential plane (Colonnier, 1964).

Scholl (1956) subdivided stellate cells into 3 classes on the basis of where the axon travelled:

(1) those whose axon distributed itself within the dendritic field of the cell

(2) those whose axon went to the white matter

(3) those whose axon went to the molecular layer.

The first two types were found to occur mainly in layer IV. The third type was small in number and occurred mainly in layers II and III.

Scholl's classification of stellate cells may be considered a gross over-simplification since it leaves out a number of stellate cell types whose description might give a clearer picture of how the cerebral cortex is organized. One of these is a type of stellate cell similar to the basket cells of the cerebellum (Ramón y Cajal, 1911). In layer II they have a tangential axonal spread of only a few cell diameters whereas in the third and fourth layers this spread extends up to a distance of 500 μ and perhaps even further in deeper layers (Szentágothai, 1969). In analogy to the basket cells of the cerebellar cortex these cells are postulated to be inhibitory in nature. Their axon terminal arborizations embrace closely the bodies and the adjacent large dendritic segments of pyramidal cells. Another type of stellate cell mentioned by Ramón y Cajal is the "cellule à double bouquet dendritique". Colonnier (1966) describes it as having two bouquets of beaded dendrites sprouting from the upper and lower poles

of the cell body. It possesses an extremely ramified, vertically oriented axonal arborization which extends both above and below the level of the cell body and may traverse the whole thickness of the cortex including layer I. They are found in layers II, III, and IV and are extremely numerous, especially in human cerebral cortex. Since vertical spaces, about the size of apical dendrites of large and medium-sized pyramidal cells, exist within the axonal fascicles formed from the branches of the "cellule à double bouquet", Cajal has postulated that this type of cell makes multiple synaptic contacts with the apical stalks of pyramidal cells, similar to the repeated contacts which the climbing fibres make with the Purkinje cells of the cerebellum (cf. Colonnier, 1966).

Colonnier (1966) suggests that the "cellule à double bouquet" together with the basket-type stellate cells provide anatomical correlates to the physiological columns described by Hubel and Wiesel (1962) for the visual cortex. These columns extend radially throughout the cortex and respond to a specific orientation of a visual stimulus. The "cellule à double bouquet" is postulated by Colonnier to be excitatory in nature, it would therefore provide an excellent means of transmitting activity from the specific afferents to all layers of the cortex. Simultaneously, the basket-like stellate cells, presumed to be inhibitory in nature, would inhibit cells on all sides of the active focus and thus would provide an inactive periphery.

Szentágothai's observation of the synaptic "cartridge" around the apical shafts of pyramidal cells (Szentágothai, 1969) is consonant with Cajal's postulation of multiple synapses between the vertical

branches of the "cellules à double bouquet" and the apical dendrites of pyramidal cells. The synaptic cartridge is created by thin terminal axons forming a vertical "horsetail" which envelopes an apical stalk and establishes repeated synaptic contacts with the dendritic stalk by means of the dendritic spines. Szentágothai estimates that the number of contacts between presynaptic and postsynaptic neuron established through a synaptic cartridge to be of the order of 10^2 to 10^3. He has found the axonal contribution to synaptic cartridges to arise from stellate cells in the fourth layer. Szentágothai (1969) considers the cartridge axons to bring only a few specific cells into action. This contrasts with Colonnier's interpretation of vertically oriented axons leading to excitation of many cells in a columnar focus.

Stellate cells receive both asymmetrical ("excitatory"?) and symmetrical ("inhibitory"?) synapses on both cell body and dendritic trunk. Their dendritic trees possess virtually no spines (Colonnier, 1966).

b) Input to the cerebral cortex

Szentágothai (1969) has divided the afferent fibres of the cerebral cortex into 5 categories: (1) specific afferents (well-defined only in primary sensory regions) arising from the specific subcortical source of that region; (2) nonspecific afferents arising from non-specific subcortical source; (3) callosal afferents; (4) association afferents from cortical source; (5) association afferents from sub-cortical source.

The specific afferents terminate mainly in layer IV. Since the descending or ascending horsetail-shaped terminal arborizations of these afferents are obliquely oriented, it is concluded that they

do not contribute to the synaptic cartridges around the apical stalk of pyramidal cells. Instead it is inferred that they contribute to the synaptic terminals surrounding the basilar dendrites of pyramidal cells (Szentágothai, 1969). The main receivers of specific afferents, however, are most likely the stellate cells of the fourth layer. These cells are embedded in numerous degeneration fragments after transection of the sensory radiation (Szentágothai, 1969). Globus and Scheibel (1967) have shown that in the rabbit a loss of spines on dendritic shafts of pyramidal cells occurs after lesions of the lateral geniculate body, and similar results have been obtained by Valverde (1968), after enucleation in the mouse. Szentágothai (1969), however, considers this to be a transneuronal effect.

Callosal fibres have been shown by Heimer et al. (1967) to have their terminals in all the cortical layers. Most often, however, they have their greatest concentration in layers I and II. A gradient of terminals was also found for cortical ssociation fibres by Szentágothai (1969). He reported that the greatest concentration of these terminals occurs in layer I.

Very little is known about the termination of nonspecific afferents and association afferents coming from a subcortical source.

c) Efferent fibres of the cerebral cortex

Efferent fibres of the cortex originate from all levels of the cortex with the exception of layer I (Scholl, 1956). Krieg (1965) classifies cortical efferent fibres into 4 categories: (1) projectional, (2) callosal, (3) thalamic, and (4) associational. Except for a few instances (e.g., the Betz cells of area 4 give rise to fibres in the pyramidal tracts), little is known of the cells of origin of cortical

outflow for the above categories. There is some rather sparse histological evidence that the cells of origin for callosal fibres reside in layers III to VI (Jacobson, 1965; Pines et al., 1939).

d) Heterogeneity of the cerebral cortex

It has become increasingly evident that the cerebral cortex cannot be considered to have a homogeneous structure in all regions of the neocortex (Colonnier and Rossignol, 1969). Pyramidal and stellate type cells vary considerably from area to area both as to their size and their relative number in the different layers (e.g., the visual cortex has giant stellate cells in the fourth layer). Afferent and efferent fibres of the cerebral cortex also contribute extensively to the heterogeneity of the cerebral cortex. For instance, area 17 of the monkey has no callosal fibres to join the primary visual regions of the two hemipheres (Krieg, 1965). In some cortical fields, such as the motor cortex, a powerful efferent tract arises from layer V pyramidal cells; in other fields such as the occipital fields, the main efferent tracts arise from the upper layers (Lorente de Nó, 1949). Area 46 is an example of where the efferent outflow is relatively meager and is almost exclusively limited to associational connections with areas 8 and 9 (Krieg, 1965).

e) Component-elements of the molecular layer

Layer I is a dense neuropil consisting mainly of dendrites and axons. These elements generally run parallel to the pial surface, giving the molecular layer neuropil a tangential orientation as a whole. The only neurons whose cell bodies are present in layer I are the horizontal cells of Cajal, but these are very small in number (Szentágothai, 1965a).

One of the major components of the molecular layer in the cerebral cortex are the terminal branches of the apical dendritic tree of pyramidal cells (Chang, 1951). The extent of tangential spread of these branches from the apical stalk is not precisely known but it is most likely in the order of 0.2 mm or less (Scholl, 1956).

Axons also form a major component in layer I. The origin of a large number of these axons has been partially worked out in the last decade. Szentágothai (1965a and b) used the method of "persisting elements" to determine if any of the axonal elements in layer I were of intracortical origin. This method, in conjunction with the Bielchowsky staining technique, showed that undercutting just below layers I and II leads to an almost complete loss of axons in the molecular layer. If the cut is made below layers III and IV a few more axons survive but it is not until the undercut is made below layers V and VI that a large portion of the axon population remains intact. Szentágothai interprets this as meaning that very few of the axons in layer I come from layers II to IV but that a large number are derived from layers V and VI. He suggests that the latter come from the fusiform cells of the sixth layer (Szentágothai, 1965a and b). Colonnier (1966) suggests that these axons may be arising from the recurrent collaterals of pyramidal cells since Scholl (1956) reported that the greatest concentration of pyramidal cells with recurrent axon collaterals occurs in layers V and VI. However, the initial axon collaterals of pyramidal cells are found to establish synapses with the basal dendrites and the apical arches of surrounding pyramids, and do not seem to contribute significantly to the molecular layer

(Szentágothai, 1965b; Scheibel and Scheibel, 1970).

Other axonal elements which have been shown to contribute a significant part of the first layer's fibres and terminals are the callosal and association afferents of the cortex. These have been discussed in the foregoing. Although the numbers of these fibres in layer I are considerable, most of the axons in the molecular layer are still thought to be derived from an intracortical source (Colonnier and Rossignol, 1969).

The horizontal cells of Cajal are the only type of neuron whose cell body lies within the molecular layer. Their axons run for "long distances" in the tangential direction (Lorente de Nó, 1949). However, as mentioned above, these cells are very few in number (Szentágothai, 1965a).

f) Tangential spread of axons in the molecular layer

Szentágothai (1965a and b) investigated the extent of tangential spread of axons in the molecular layer of the cat cerebral cortex. With the aid of the Nauta-Gygax impregnation procedure, he found that axon degeneration, after a superficial cut into the first layer, never extends any further than 4 mm tangentially from the cut. The greatest majority of degenerated fibres disappear within the first two and a half millimeters from the cut. Since most of the axons in layer I appear to be derived from layers V and VI, Szentágothai (1965a) concluded that the longer running axons (3 to 4 mm) are largely from the axons of local Martinotti-type cells located mainly in layer VI.

Jones and Powell (1968) found that after making lesions in the somatic sensory area of cats that there were fine degenerating fibres within the molecular layer of cortical areas immediately

surrounding the lesions. These fibres travelled in the most superficial part of the molecular layer and were found consistently to radiate out to a distance of 5-7 mm from the edges of the lesion. Jones and Powell mentioned also unpublished observations which showed these long axons to be present in the molecular layer "in all parts" of the neocortex.

III. Aims of the thesis

a) To look for large units of organization in the cat's cerebral cortex.

The basic unit of functional organization of the cerebral cortex appears to be a vertical column of cells extending throughout much of the depth of the cortex (Mountcastle, 1957; Hubel and Wiesel, 1962). Histological elements which might explain the existence of these physiological columns are the basket-like stellate cells (Colonnier, 1966; Szentágothai, 1969) and perhaps also the "cellule à double bouquet dendritique" (Cajal, 1911) which has recently been described by Colonnier (1966). Analagous to this arrangement is the probable functional organization of the cerebellar cortex. Here the unit of functional organization appears to be a narrow line of Purkinje cells activated by a beam of parallel fibres. This line of activated cells is enclosed on either side by Purkinje cells which are inhibited by stellate cells in the molecular layer (Eccles et al., 1966a and b; Hillman, 1969; Llinás and Hillman, 1969). The axons of the stellate cells are oriented lateral (i.e., perpendicular) to the parallel fibre system.

The physiological columns of the cerebral cortex are very

small in diameter (about 0.5 mm; cf. Hubel and Wiesel, 1962; Mountcastle, 1957) and have been determined for primary and secondary sensory areas such as visual and somatic cortex. The question might be raised whether or not the size of the columns is adequate to account for the association of information which goes on in a large scale in the cerebral cortex. Is there a larger unit of cortical organization which accomplishes this?

One point of inquiry, then, which this thesis has attempted to solve is whether or not there is a unit of functional organization in the cerebral cortex which exceeds the boundaries of the columns of primary and secondary sensory regions, and has escaped the eye of the anatomist because of the largeness of field required to apprehend it. The involvement of very small diameter fibres (e.g., axons in the molecular layer) together with several cortical interneuronal connections could easily overtax existing histological techniques and equipment.

Axons are known to travel within the molecular layer for distances of at least 7 mm (Jones and Powell, 1968) - far exceeding the boundaries of the physiological columns of Hubel and Wiesel. The possibility exists that some of these tangentially running axons (perhaps the axons from the fusiform cells of layers V and VI; see Szentágothai, 1969, 1965a and b) might be analagous to the parallel fibre system in the cerebellar cortex. Unlike the parallel fibre system, however, these axons are probably oriented in all directions in the horizontal plane to give rise to a circle rather than a line of activated cells. Analysis of any given radius, then, should lead to an understanding of the circle as a whole. Surface stimulation was therefore used, in the present study, to activate the molecular layer

of the cerebral cortex at a fairly discrete point. The ensuing response (nDCR) was recorded at several points along a straight line extending from the site of stimulation. In this way, the manner in which the nDCR varied with distance could be determined and analysed for information about the systems transmitting it or affecting its transmission along the molecular layer.

The nDCR was used since it is a response which is widely accepted as being associated with the molecular layer of the cerebral cortex (Adrian, 1936; Burns, 1951; Chang, 1951; Ochs and Clark, 1968a). It is monophasic in nature and hence lends itself very readily to analysis in terms of peak amplitude and peak latency.

The suprasylvian gyrus of the cat was chosen for this study both because of its largeness of size and accessibility, and for the fact that it is believed to be associative in nature and therefore should be more likely than other regions to have a unit of functional organization large enough to permit a high degree of information mixing for associative purposes.

b) To see if there are regional differences in the suprasylvian gyrus which could be detected by use of the nDCR

As just discussed, it is becoming increasingly obvious that the neocortex is not homogeneous throughout. Each area of cortex has a unique function to carry out and hence, its morphology and function has evolved in a unique manner in order to meet the demands of that function. Histology reveals differences between areas (e.g., in the number of layers, in the number and distribution of cell types, in the extent of spread of axonal and dendritic processes, and in the number and origin of afferent fibres). Externally, however, there is

little evidence of differences between one area of cortex and the next. Viewed from the pial surface, visual cortex does not appear different from motor or association cortex. Hence, when neuroscientists study different regions of the cortex, they must rely on topographical features as landmarks in order to locate the cortical region of interest. They rely first of all on the relative position of the cortical region (e.g., visual cortex is located caudally, the somatosensory frontally, and the auditory at the lateral aspect of the cerebral hemispheres in mammalian species). Next, they rely on the convolutions of the cerebral cortex, and in general are able to match certain gyri and sulci of a given species with a given functional and morphological cortical area. This, of course, is not a perfect correlation since the location of gyri and sulci are not entirely constant from one animal to the next. Also, the resolution of this technique is poor, e.g., one could not locate the boundaries between areas 17 and 18 of the cat with an accuracy any greater than to within several millimeters.

A technique which could locate cortical areas from the pial surface with greater precision than just discussed would be a useful contribution to the neurobiologist in that it would enable much greater control over his experimentation on cerebral cortex. At present, much of the variability which the physiologist encounters between experiments involving neocortex can undoubtedly often be accounted for by differences in the location of his stimulating and/or recording electrodes. The greater the experimenter's ability to locate his apparatus precisely in or on the cortex from experiment to experiment, the more likely is he to obtain a more constant result and the more likely will he be

able to detect differences in the properties of different regions of the cortex.

The manner in which the nDCR spreads along the length of the suprasylvian gyrus was examined with the foregoing considerations in mind. It was hoped that differences would be observed in the pattern of spread with slightly different locations (as close as 0.8 mm apart) of the site of stimulation. If such differences were observed then resolution of the technique being in the sub-millimeter region would make it a valuable asset in future research. The next step, in future studies, would be to try and correlate these differences with a close examination of the morphology of the suprasylvian gyrus so that the observed pattern of nDCR spread could be used as a tool in telling the neurobiologist the exact location of various subregions within this gyrus.

c) To check for long-term excitability effects of a conditioning stimulus

Experiments involving paired-stimuli were conducted to determine what interval of time was required before the nDCR excitability returned to its pre-stimulus level. A period of test-response depression lasting 20-40 msec had already been well established by Ochs (Ochs and Booker, 1961; Ochs and Clark, 1968b). Other workers (Berry and Hance, 1965; Merlis, 1965) had reported a test-response depression lasting up to 800 msec. Since certain forms of "very short-term" memory may last in the order of seconds (Kincaid and Wickens, 1970; Posner and Keele, 1967; Turvey et al., 1970), it was hoped that some form of change of excitability (either a depression or enhancement) might be detected (using the technique of response-

averaging) within intervals up to 10 seconds.

The effect of a tetany of stimuli on nDCR excitability was also tested. It was conjectured that some facilitation of the nDCR might occur after the tetany, somewhat analagous to the post-tetanic potention which occurs in neuromuscular preparations. If such a phenomenon were to occur, it would form a good substrate for a hypothesis correlating it with a form of cortical memory.

B. METHODS

I. Biological preparation

a) Initial anesthesia

Adult cats of either sex, weighing between 2 and 5 kg, were anesthetized with diethyl ether (Squibb) for the surgery which took place before the brainstem was transected. Ether induction was achieved by placing the animal in a closed wooden box slightly larger than 1 cubic foot in volume, and introducing an ether-soaked cotton pad into an upper chamber of the box. The ether fumes travelled from the upper chamber into the animal's chamber through a wire-gauze partition. An observation window permitted a close check on the animal's condition. When it was judged that surgical anesthesia had been attained, the animal was quickly removed from the box and transferred to the operation table. A wire-gauze cone containing an ether-soaked cotton pad was placed over the animal's nose to maintain the anesthesia while the trachea was being cannulated. The trachea was then connected by a short rubber tube to a variable-bypass ether bottle and the cone removed from the animal's nose. The right femoral artery and vein were cannulated to permit subsequent blood pressure recordings and fluid replacement.

b) Craniectomy

The animal's head was placed in a Czermak holder (Palmer) to position and steady it for surgery. The scalp was shaved and a midline incision made. The scalp was then separated with a blunt instrument from the underlying muscle. Both temporal muscles were

scraped back from the skull and their bases tied tightly with a loop of strong thread. This cut off the blood supply to these muscles and permitted the portion of muscle above the loop to be removed with a minimum of blood loss. The skull over the left hemisphere was removed by circumscribing a large area with a high-speed dental drill (30,000 r.p.m.). Bone rongeurs were then used to extend this hole posteriorly to the tentorium cerebelli and laterally to include most of the temporal bone. Anteriorly the hole extended to the nasal sinuses and medially to within 2-3 mm of the mid-line. Bone wax (beeswax with 1% phenol) was used to seal the cut edges of the bone and thus prevent blood loss during and after the craniectomy.

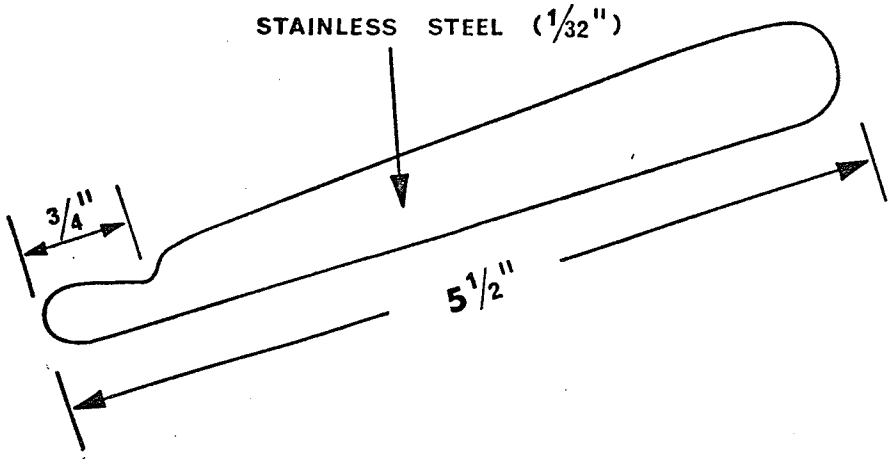
c) Forebrain isolation

The dura mater was cut away and the exposed brain kept moist with warm (37°) saline. The forebrain was isolated by passing a flat, blunt-edged spatula (Fig. B-1A) through the brainstem, using the anterior surface of the tentorium cerebelli as a guide for the flat edge of the spatula. The anesthetic was removed as soon as the forebrain isolation had been completed.

d) Isolated slabs

The on-going electrical activity in the cerebral cortex of the unanesthetized cat is considerable and often leads to amplifier overloading at gains suitable for recording the nDCR. This problem is all the more acute when one is trying to record from 8 channels simultaneously. Hence, it was considered advantageous to use isolated slabs of cerebral cortex for this work because they are known to be

A. SPATULA FOR FOREBRAIN ISOLATION



B. CORTICAL SLAB ISOLATION KNIVES

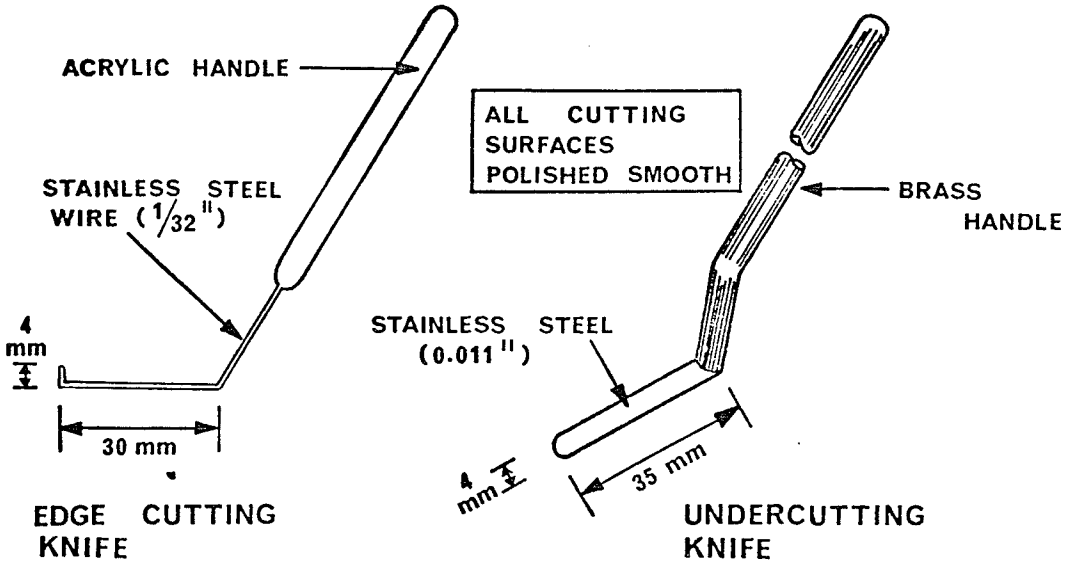


Fig. B-1. Instruments for midbrain transection (A) and isolation of cortical slab (B).

far more electrically silent than intact cortex (Burns, 1958, 1951).

A small area of cortex was electrocauterized at the posterior end of the suprasylvian gyrus where the gyrus turns laterally. A drainage hole to the lateral ventricle was made in this area by aspirating the brain tissue away with a tapered glass tube connected to a water-tap aspirator. A slab was then cut anterior to the sink hole in the manner described by Burns (1950). Fig. B-1B shows the undercutting and edge cutting knives used for this purpose. Slabs were generally about 4 mm deep, 20-25 mm long and 4-5 mm wide. Fig. B-2 is a diagram of a sagital section at the middle of the suprasylvian gyrus. It shows the position of the brainstem cut, the drainage hole and the isolated slab.

Once the slab had been cut in the left suprasylvian gyrus, craniectomy and slab-cutting procedures were duplicated on the right hemisphere to produce another slab in the right suprasylvian gyrus. This was done to provide an alternative preparation should the left slab be damaged during the experiment. The slab which appeared to be in the best condition was chosen for the experiment.

e) Preparation of the animal for recording

With the operation completed, the animal was moved to the recording table and its head mounted in a stereotaxic instrument (David Kopf #1504). The edges of the scalp were stitched around a metal ring so that a reservoir was formed into which paraffin oil at 37°C could be poured to cover the exposed brain to a depth of a few millimeters. Body temperature was maintained at 37°C by a heating pad connected to a temperature control unit (Yellow Springs Instrument

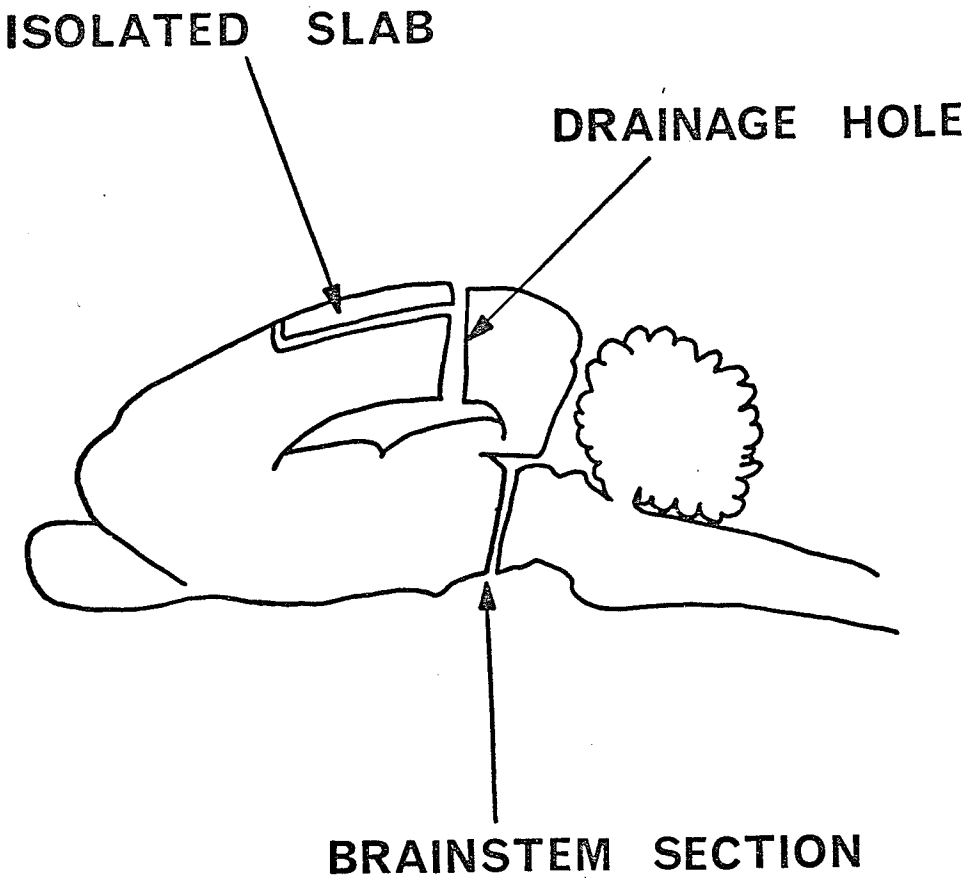


Fig. B-2. Saggital section of cat brain preparation through middle of suprasylvian gyrus.

Co., model #71) which regulated the temperature by means of a rectal thermistor probe. Brain temperature was kept between 34°C and 37°C by placing a heat lamp at an appropriate distance from the cat's head.

Femoral arterial pressure was measured continuously by means of a Statham pressure transducer coupled to a 2-channel Offner pen recorder. Mean blood pressure was normally in the range of 80 to 100 mm Hg. An intravenous drip of 5% dextrose in normal saline was fed through the femoral vein cannula. The flow rate was adjusted so as to deliver about 5 ml of fluid per hour to the animal. Approximately 10-20 ml of this solution was initially injected into the femoral vein to help compensate for blood lost in the operation.

II. Stimulation and recording

a) Stimulation

Bipolar surface stimulation was used in all the experiments. Two beaded Ag/AgCl electrodes were placed about 1.5-2.0 mm apart and positioned so that they straddled one of the recording electrodes (see Fig. B-3). In most of the experiments, especially those requiring stimulus scans (see below), several stimulating sites were used for the same position of the recording assembly (described below).

To obtain an accurate measurement of the nDCR peak amplitude, it was essential that electrode polarization be minimized. This was achieved by:

(1) The use of non-polarizable electrodes (Ag/AgCl).

(2) The use of a constant-current stimulator (Fig. B-4). Any polarization potential would be maximally dropped across the high

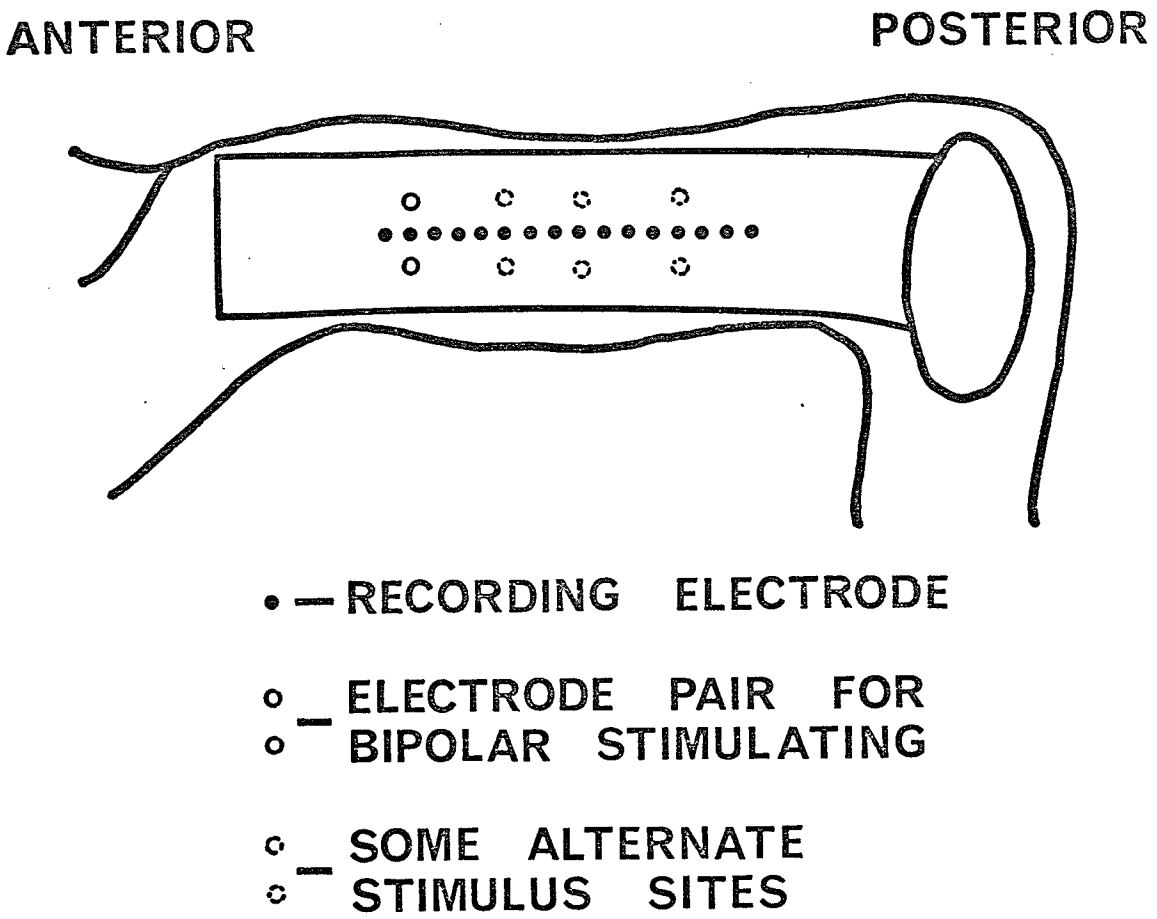


Fig. B-3. Approximate position of stimulating electrodes and recording electrode assembly on cortical slab. Several stimulus sites other than those depicted were also used (see text on stimulus scans).

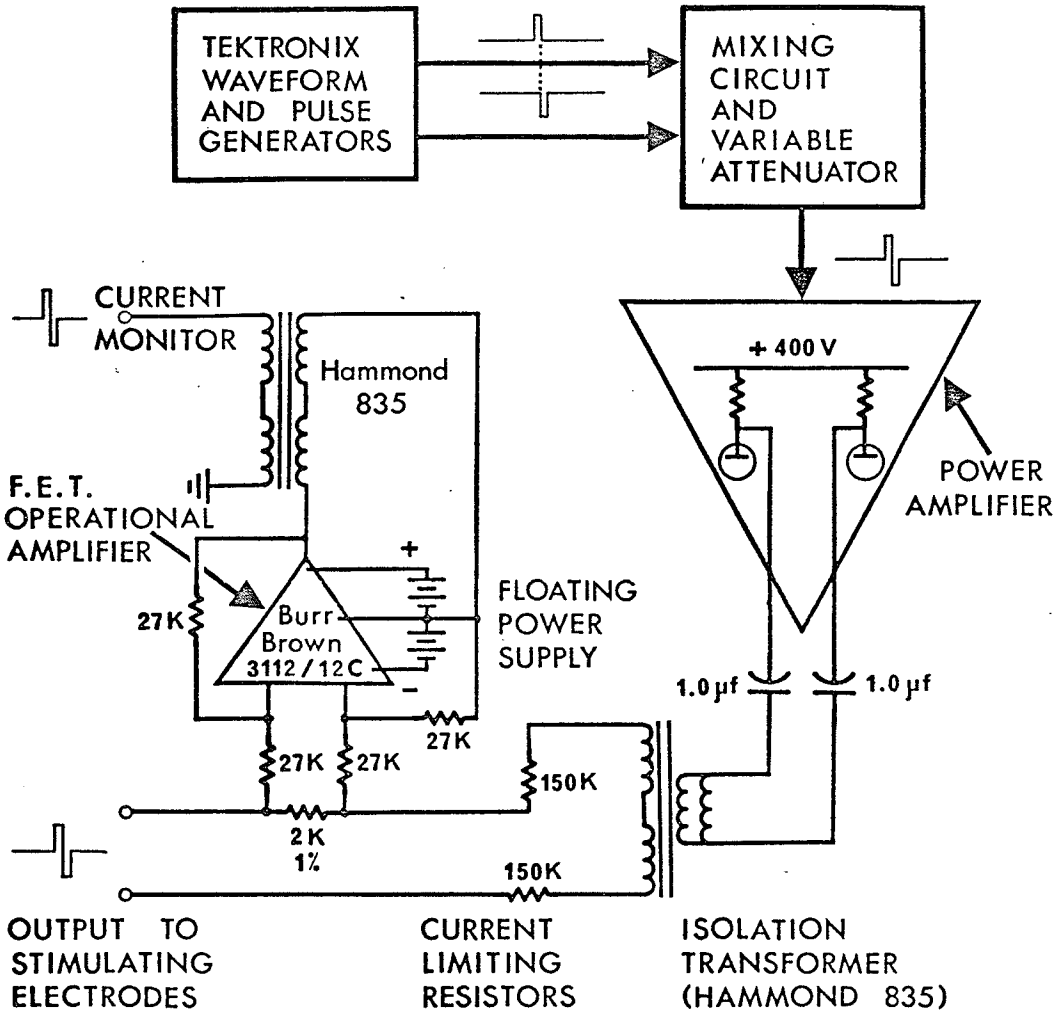


Fig. B-4. Constant-current stimulator used to minimize polarization artifacts (see text). It was constructed out of materials already available in the laboratory (e.g., Tektronix generators, power amplifier). Stimulus-current was monitored by measuring the voltage drop across a 2K precision resistor in the output circuit.

internal resistance (300 K) of such a stimulator and only minimally across the preparation (< 5 K).

(3) The use of bipolar pulses for stimulation. The second phase of the pulse helped to cancel any polarization caused by the first phase.

Cancellation was ascertained by examining the baseline of the monitored stimulus pulse (see Fig. B-4). The amount of deviation from zero of the baseline following the second phase was tuned to a minimum by adjusting the size of the second phase.

An effort was made to deliver stimuli of comparable strength to each preparation and to the different stimulus sites within a given preparation. This was attempted by adjusting the stimulus strength so as to be a little below (usually from 10 to 20%) that of the threshold of the late positive wave (Iwase et al., 1961). Most often, this gave an nDCR of about 1 mV in peak height at the site of stimulation (Iwase et al., 1961; cf. their Fig. 2). Occasionally, (especially after a long session during which the preparation seemed to fatigue) a response of only 0.5 mV could be obtained without eliciting the late positive wave. Current strength required for this size of response was quite variable, ranging from 0.1 to 1.0 mA with a pulse of about 0.07 msec in duration for each phase. The reason for this variability is almost certainly due to the variable amount of fluid which tended to accumulate between the stimulating electrode poles. This fluid would shunt much of the stimulus current. When the fluid was removed by soaking it up with an absorbent cotton ball, the stimulus current required for the response was greatly reduced.

Stimulation rate was never greater than 1 pulse per 3 seconds.

b) Recording

An assembly of electrodes was constructed specifically for the recording of the longitudinal spread of the nDCRs. Construction details of this assembly are shown in Fig. B-5. It contained 16 electrodes placed 0.8 mm apart from one another in a straight line. A silver wire coated with AgCl at its tip was used for the portion of each electrode which touched the brain surface. Since silver dissolves in mercury, platinum wire had to be used for the portion of each electrode which made contact with the mercury pool. Each electrode could be individually adjusted in height, and this permitted the electrode assembly to fit the contours of the suprasylvian gyrus by lowering each electrode in turn until they all rested gently on the pial surface. A gauge made out of heavy paper was used as a final guide to positioning the electrodes 0.8 mm apart. The leads from the sixteen electrodes were soldered on to consecutive contacts of a circuit board. The first eight contacts of the receptacle for the circuit board were each connected to one of the eight recording preamplifiers. With this arrangement, any 8 consecutive electrodes could be connected to the preamplifiers simply by plugging the appropriate contacts of the circuit board into the first eight contacts of the receptacle. The procedure most often used for each stimulated point was to record first from eight electrodes at one end of the assembly such as electrodes 1-8. Having measured the response at these points, recordings were then taken from electrodes at the other end of the assembly. One electrode, however, was

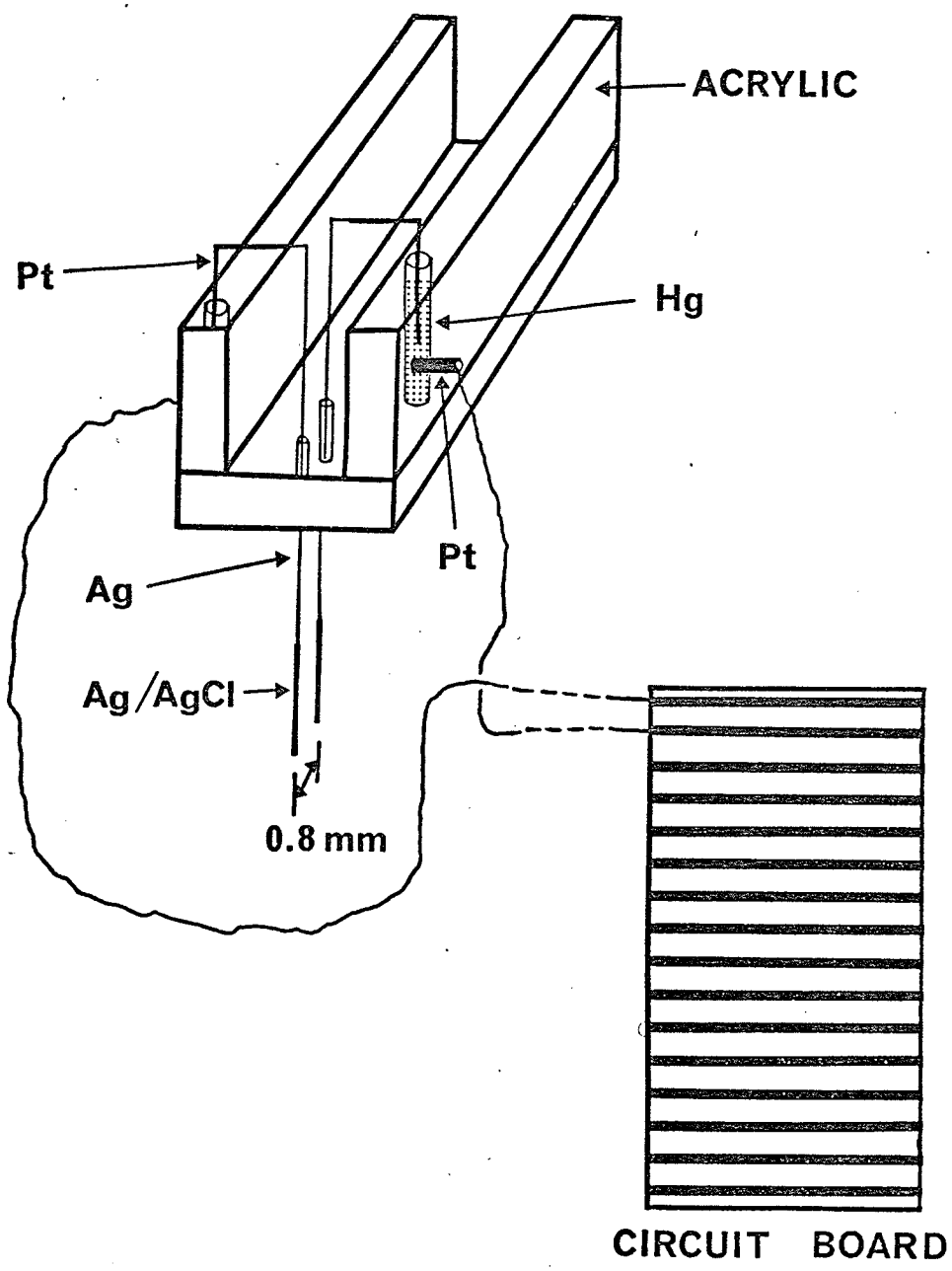


Fig. B-5. Recording electrode assembly. Only the first 2 electrodes are shown.

recorded from in both groups to provide continuity. In the example used, electrodes 8-15 would constitute the second group with electrode #8 as the control.

Four of the amplifiers used were Grass P6's and a fifth was a Grass P5. These were followed by an operational amplifier which served two purposes: (a) to give an extra stage of gain for the Grass P6's and (b) to provide a DC level shift capacity to enable adjustment of the output bias to a level suitable for the analogue-to-digital converter. The other three amplifiers were designed and constructed in the laboratory where this research was done. They consisted of two operational amplifier gain stages coupled in series to a cathode-follower for high input impedance. All 8 amplifiers were set with a high-frequency cut-off (half-down point) of 10 KC or greater. A low-pass filter was inserted either before or immediately after the cathode-follower stage of each amplifier to give a time constant of 1 second or greater.

c) Stimulus scans

It was found that the manner in which the nDCR spread tangentially from the site of stimulation was different for different locations of the stimulating electrodes (see Results). In order to determine whether or not there was a pattern of cortical organization which gave rise to these differences, a series of ten experiments was conducted in which the cerebral cortex of the suprasylvian gyrus was stimulated at several points along its length. This procedure may be defined as a "stimulus scan". The stimulus sites were located along the recording assembly. Distances between the stimulus sites were 0.8 or 1.6 mm (i.e., the distance between one, or two, consecutive

recording electrodes). The spread of the nDCR was measured at each of these stimulus sites.

III. Data acquisition and analysis

A Lab-8 digital computer (Digital Equipment Corporation) was used for response averaging and analysis. This instrument is equipped with an 8-channel multiplexor and an analogue-to-digital converter (ADC). The multiplexor permitted simultaneous digitilization of data on all 8 channels. The timing and control of the ADC (see Fig. B-6) was partly determined by external pulse logic (generated by Tektronix waveform and pulse generators) and partly by software routines. The interval between ADC samples was about 26 μSec. Since this was multiplexed among 8 channels, it meant that the ADC took a sample of each channel about every 208 μSec (i.e., 8 x 26 μSec).

Two methods were used for averaging: one was designed chiefly for accuracy, and the other for speed. Each of these will be discussed separately.

a) Method #1 (Accurate method)

Method #1 consisted simply of adding 100 consecutive responses to one another and storing the result in digitalized form on magnetic tape along with voltage and time calibrations. The peak latency and peak amplitude of these responses were then measured manually at a subsequent date with the aid of computer-generated cursors on an oscilloscope screen (see Fig. B-7). Each cursor could be positioned anywhere on the screen and the computer would print out on demand the voltage and time co-ordinates of the desired

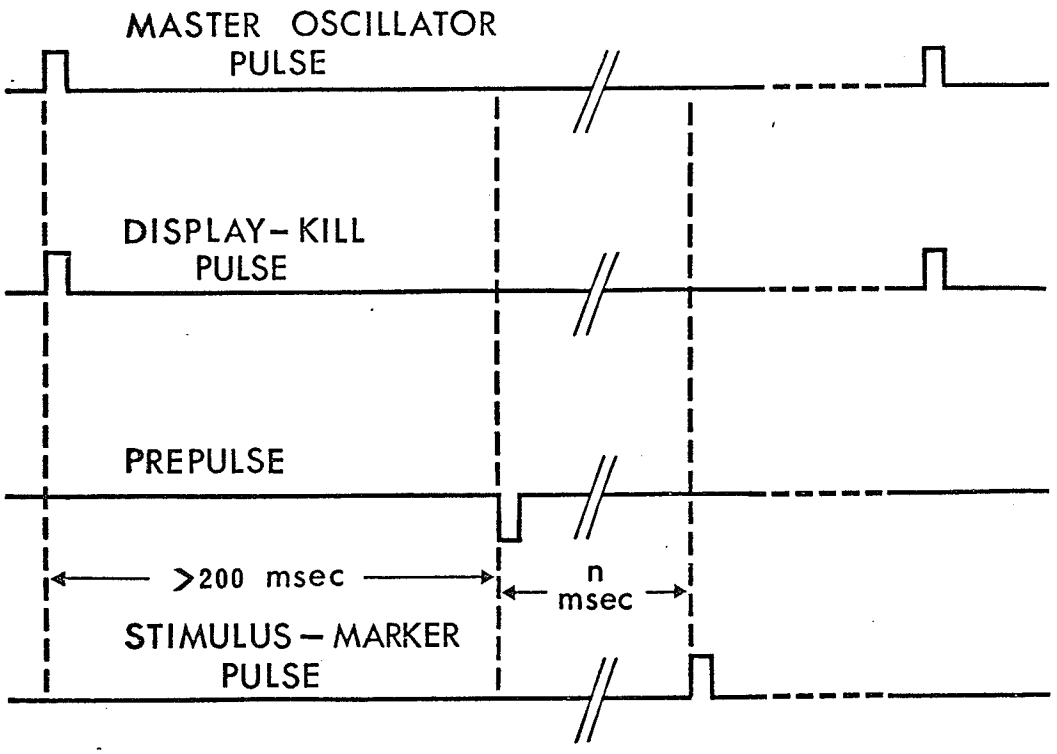


Fig. B-6. Pulse logic for external timing of computer.

The master oscillator controlled the rate at which stimuli occurred. It's pulse initiated 3 pulses in sequence which were fed into the computer: The display-kill pulse shuts off the average response display routine and prepares the computer for the analogue-to-digital conversion (ADC) of an nDCR. The prepulse starts the ADC. It occurs at a fixed interval (usually about 6 msec) before the stimulus pulse to provide a pre-stimulus baseline for the nDCR. The stimulus-marker pulse is sent to the computer to inform it when the stimulus occurs in relation to the prepulse.

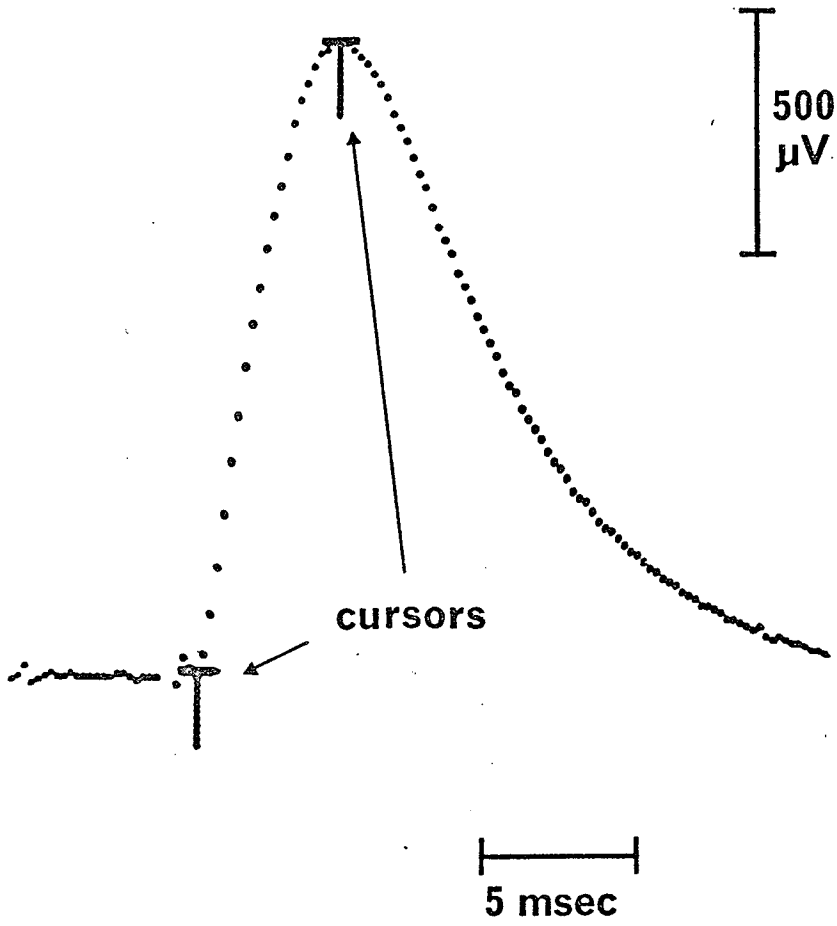


Fig. B-7. Cursor technique to measure averaged responses for Method #1 (accurate method).

cursor. The error due to slight baseline shifts caused by polarization of the stimulating electrodes was minimized by measuring the amplitude peak in reference to the part of the trace immediately following (within 0.5 msec) the stimulus artifact. This method was highly accurate especially with regard to peak amplitude measurements where precision was approximately $\pm$ 1.0 μV between channels. It suffered, however, from being slow and tedious. Therefore it was used mainly to demonstrate, with a high degree of reproducibility, the occurrence of regions of reinforcement (see Results). Programs to handle the data acquisition, processing and analysis were written in FOCAL language (a high-level language) using special commands which could call up routines written in machine language.

b) Method #2 (Fast method)

Method #2 is depicted in Fig. B-8. From 1 to n (where $n \leq 10$) consecutive responses were added to one another. The computer then searched automatically for the peak of the response and printed out its latency and amplitude (for all eight channels). This process was repeated N times (where N usually was 10-15) and the mean and standard error were then calculated for these individual measurements. The size of n was chosen on the basis of the signal-to-noise ratio. The whole purpose of adding responses was to increase the signal-to-noise ratio to a point where the computer could detect the response peak as contrasted with peaks of random noise.

The advantages of this method lie in its speed since the measurements were performed automatically by the computer. This enabled response curves to be obtained from several points of stimulation on the cortex without making the data analysis an impossible

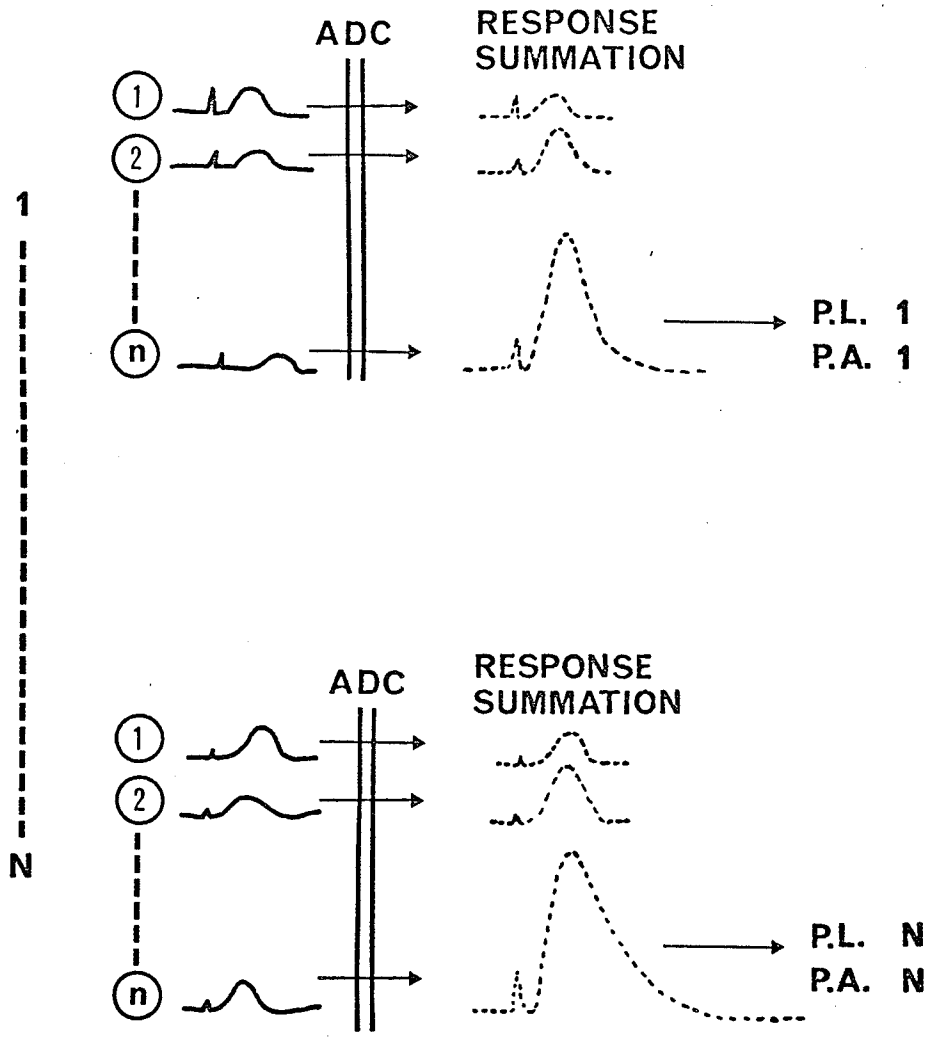


Fig. B-8. Method #2 (fast method) for measuring nDCRs. The computer measured the peak latency (P.L.) and peak amplitude (P.A.) of the summed response after the n'th response had been added onto it. This process was repeated "N" times after which the means and standard errors were calculated for the P.L.'s and the P.A.'s (see text).

task. The chief disadvantage in the method was its susceptibility to a baseline-shift artifact (due to stimulating electrode polarization) since all peak amplitudes were in reference to the pre-stimulus baseline. It was because of this that extensive measures were taken to ensure that shifts in baseline were minimal (see above).

Method #2 was used chiefly to determine in what manner the form of the nDCR longitudinal spread depended on the position of the stimulated point. It was also used to see whether the nDCR could be transmitted across a cut from various stimulating distances from the cut.

IV. Cuts

Transverse cuts were used to study the pathway requirements for longitudinal transmission of the nDCR. A flattened bead, 0.5 mm in diameter, on the end of a 0.01-in platinum wire was used as the cutting tool for the 0.5 mm cut. The bead was slipped under the pial membrane and its upper edge pressed up against the underside of the pia. The bead was then drawn back and forth across the width of the slab to cut the uppermost layers of the cortex (see Fig. B-9); this whole procedure was carried out with the aid of a micromanipulator.

The method for making the complete cut was the same as that used to cut the front edge of the slab. The edge cutting knife (see Fig. B-1B) was simply introduced through the posterior drainage hole and the cut made at the appropriate location. All layers, including the underlying white matter, were severed, leaving only the pial membrane connecting the two slab portions produced.

Before making a cut, careful note was made of the position of the electrode assembly with respect to blood vessel configurations.

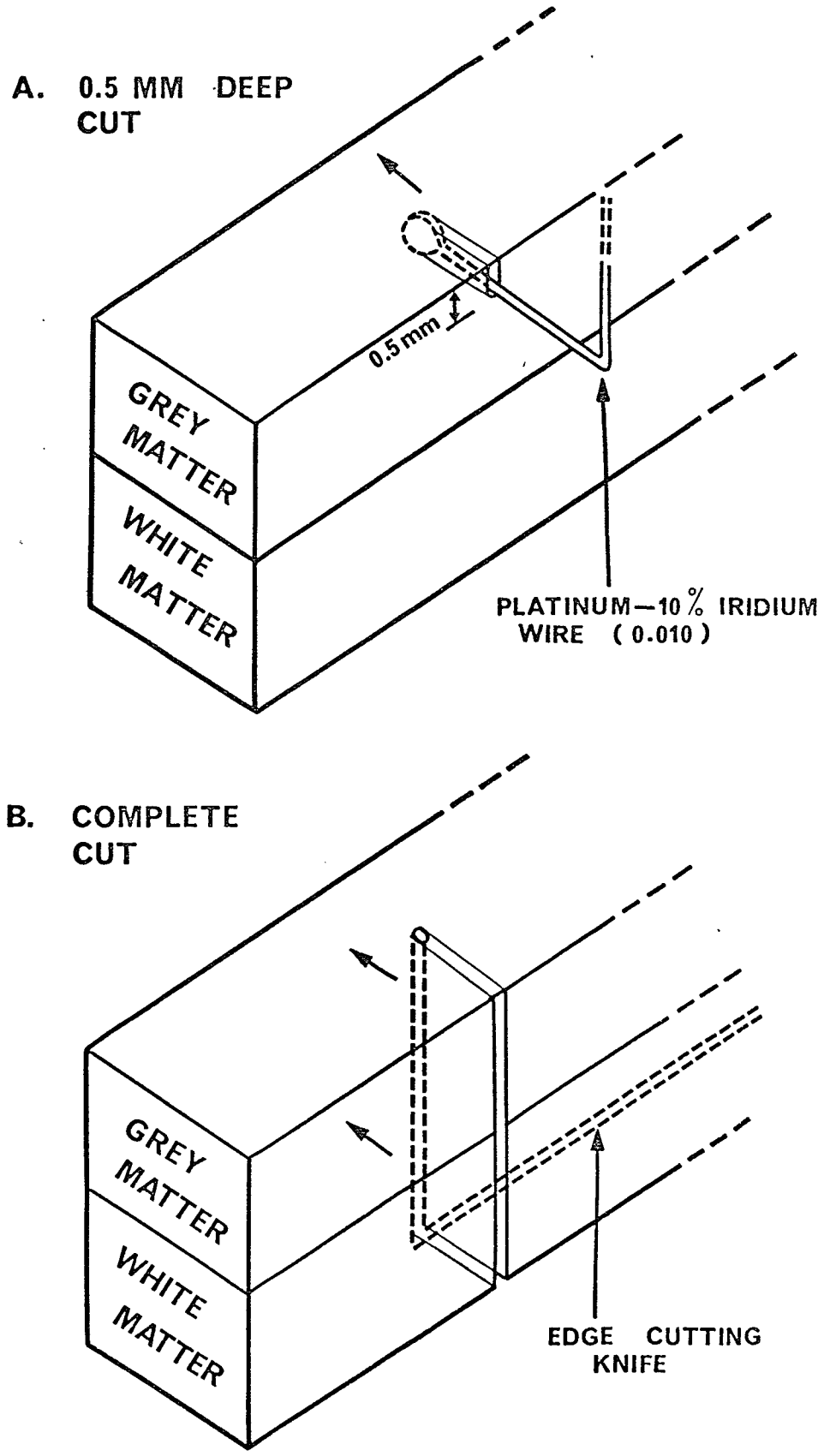


Fig. B-9. Procedure for making 0.5 mm deep cuts (A) and complete cuts (B).

The assembly was then removed to permit the cut to be made and replaced as close as possible to its original position when cutting was completed.

V. Paired-pulse and experiments with repetitive stimulation

Experiments were carried out with paired pulses to determine the length of time required for nDCR excitability to recover to control levels after a response had been evoked. Trials were conducted also to measure the amount of time required for the nDCR to recover after a period in which the nDCR was evoked at the rate of 10 per second for 10 seconds. The latter form of stimulation shall be referred to as "tetany".

Unfortunately, the computer programs did not allow the concomitant averaging of control and test response amplitudes. Separate trials were therefore necessary in order to measure the average amplitude of either the control or the test response. Hence, it was efficient to use the same control for both the paired-pulse trials and the tetany trials.

Referring to Figs. B-10A and B-10B, the following sequence of trials was used to obtain an average measure of control and test nDCR amplitudes for a specified test pulse interval:

(1) a trial to obtain an average measurement of the amplitude of the control response (paired-pulse trial)

(2) a trial to obtain an average measurement of the amplitude of the test response (paired-pulse trial)

(3) a trial to obtain an average measurement of the amplitude of the test pulse following a tetany (tetany trial)

(4) a trial identical to (1).

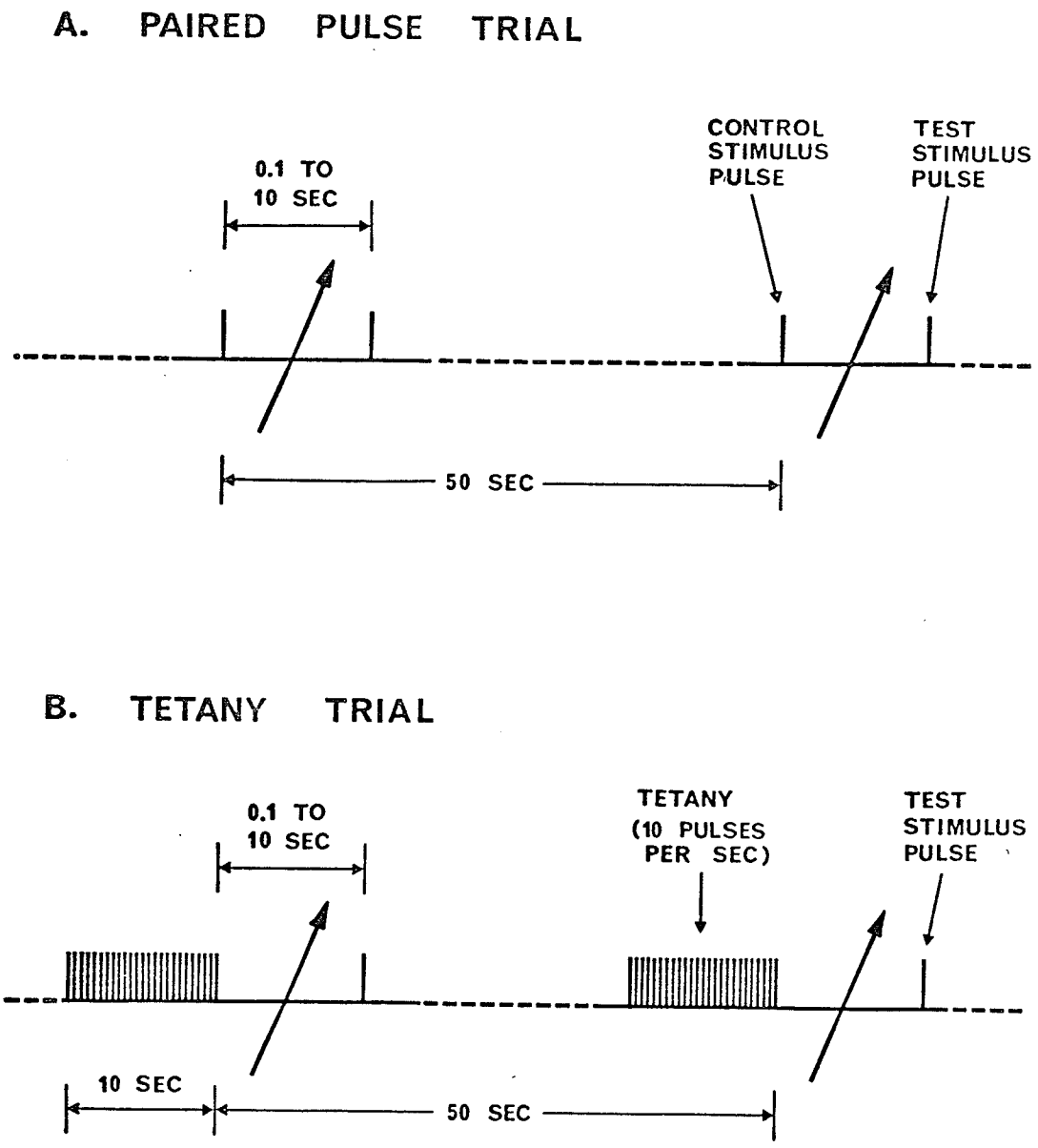


Fig. B-10. Timing of stimulus for paired pulse (A) and tetany (B) experiments. Each stimulus sequence was presented approximately every 50 seconds.

The test pulse interval was the same for both the paired-pulse and tetany trials except when the interval was 0.1 sec for the paired-pulse trial. In this instance, 0.16 sec was used for the test pulse interval in the tetany trial. The longest test pulse interval used was 10 seconds. The interval between control pulses or tetany bursts within each trial was about 50 seconds.

To help control for the non-specific changes which might occur between trials (e.g. a change in the amount of fluid shunting the stimulus current), the mean amplitude of the two control trials (i.e., numbers (1) and (4) in the above sequence) was used as the control nDCR amplitude. All eight recording channels were used to measure the response not only at the stimulus site but also at sites anterior and posterior to this. The recording electrode assembly was used for this purpose; responses were recorded from 3 electrode sites immediately anterior, from 4 electrode sites immediately posterior to the stimulus site and, as well, from the stimulus site itself. The amplitude measurements of the nDCR recorded at all eight sites were summed and this sum was used as the measure of response size. This procedure helped eliminate any nonspecific variability occurring on an individual channel.

Method #2 (fast method; see foregoing) was used to measure the amplitude of the response at the different channels. Since the response was measured at sites close to the stimulated point, its peak was large enough on all eight channels to be discerned easily from peaks generated by noise or by cortical activity which was random with respect to the occurrence of the stimulus pulse. Hence, "n" was made equal to 1 and "N" was set between 10 and 15.

C. RESULTS

I. Pattern of nDCR spread

a) Extent of spread

The technique of response averaging confirms the presence of the nDCR at distances up to at least 10 mm from the stimulus site (see Fig. C-1). This extent of spread is a fairly consistent finding although the response was found occasionally to be transmitted no further than 7 or 8 mm (see Fig. C-2). Whether or not the response recorded at the farther distances is generated by the same elements which produce the response closer to the stimulus will be considered in the DISCUSSION section.

b) Occurrence of regions of reinforcement

(Brooks and Enger (1959) observed that in some trials the nDCR disappeared completely at an intermediate distance from the site of stimulation and reappeared at distances further away. They called this phenomenon "re-initiation" of the response. In other trials the response did not disappear completely at intermediate distances but declined to a minimum and then became larger at a region more distant from the stimulus site. This phenomenon they called "reinforcement".)

None of the trials in this present series of experiments showed the phenomenon of re-initiation but a type of "reinforcement" region was observed in almost all experiments where the nDCR was measured at distances beyond 6 or 7 mm from the stimulus site. The reinforcement region observed here differed from that of Brooks and Enger in that the response most often was no larger in

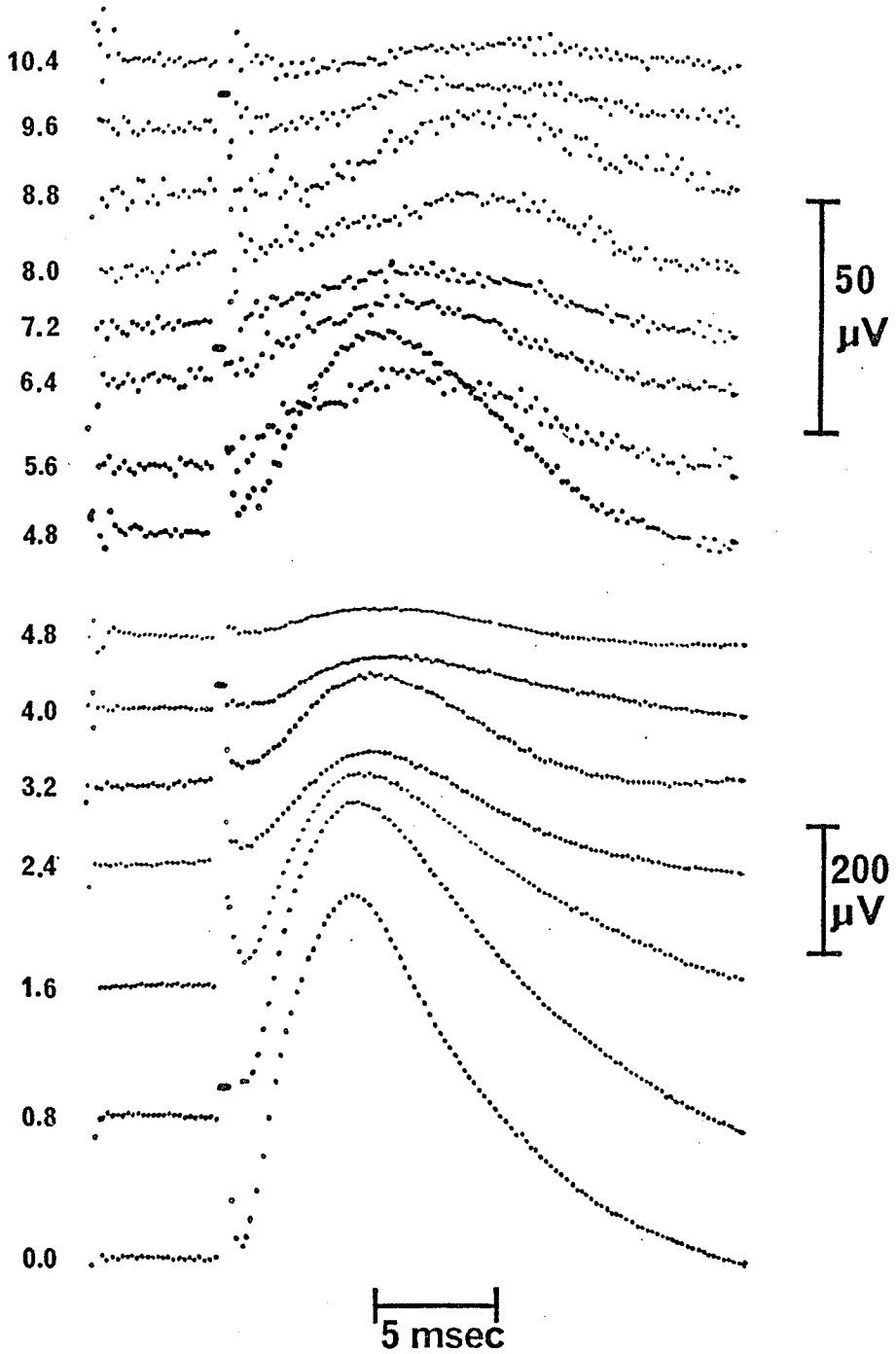


Fig. C-1. Trial showing spread of nDCR up to 10.4 mm from site of stimulation. Numbers to the left of responses indicate the distance (in mm) at which they were recorded. See Fig. C-3 for a graph of their amplitudes against distance from stimulus.

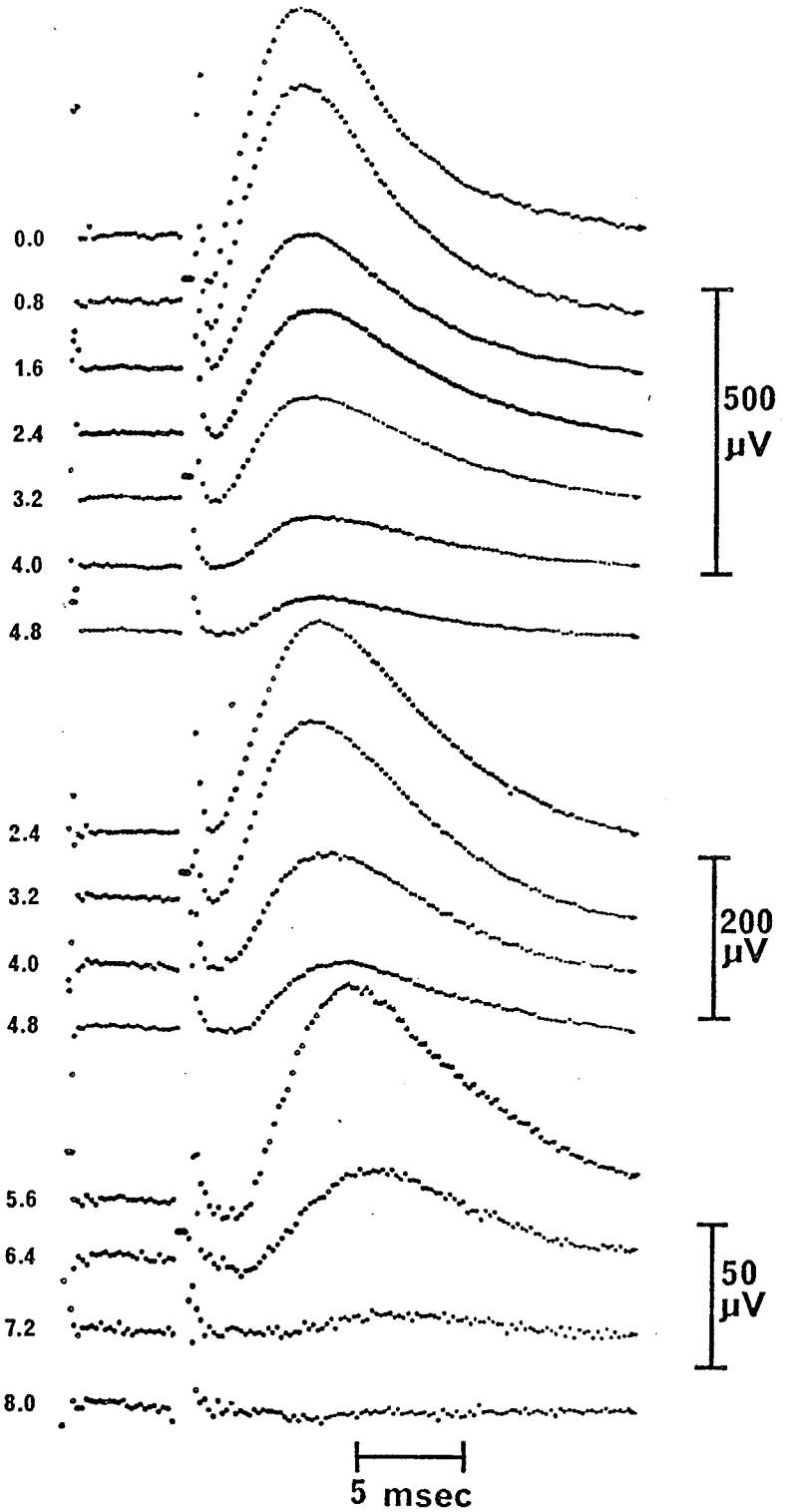


Fig. C-2. Trial showing instance in which spread of nDCR extends less than 8 mm. Numbers to the left of responses indicate distance (in mm) from site of stimulation. See Fig. C-5 for a graph of their amplitudes against distance.

this region than in the more intermediate regions. Rather, the region of reinforcement manifested itself as a marked decrease in the rate of nDCR decline with increase of distance from the stimulus site. An example of the type of reinforcement similar to that observed by Brooks and Enger is shown in Fig. C-3. The region of reinforcement is where the response has increased in size beyond 7.2 mm distance from the site of stimulation. With this kind of reinforcement region, the size of reinforcement was always much less than that shown in Brooks and Enger's diagrams (e.g., their Fig. 5, Brooks and Enger, 1959). An example of the most often encountered type of reinforcement is shown in Fig. C-4 where the slope of the curve plotting peak amplitude against distance changes from a steep to a much more gentle incline at approximately 4.0 mm distance from the stimulus site. The region of slow decline (reinforcement region) extends from this point to 7.2 mm where the slope of the curve again becomes steep.

c) Variability in the pattern of nDCR spread

The pattern of longitudinal spread of the nDCR (as characterized by peak amplitude and peak latency measurements) was quite variable from trial to trial and from experiment to experiment. In some trials, the rate of decline of the nDCR amplitude with distance was slow for the first 4-5 mm of spread before entering into a region of rapid decline (see Fig. C-5). In other trials, a region of rapid decline began at distances closer to the site of stimulation, sometimes as close as 0.8 mm from the stimulus site (see Fig. C-6).

d) Possible sources of variability

There are a number of possible reasons for this variability

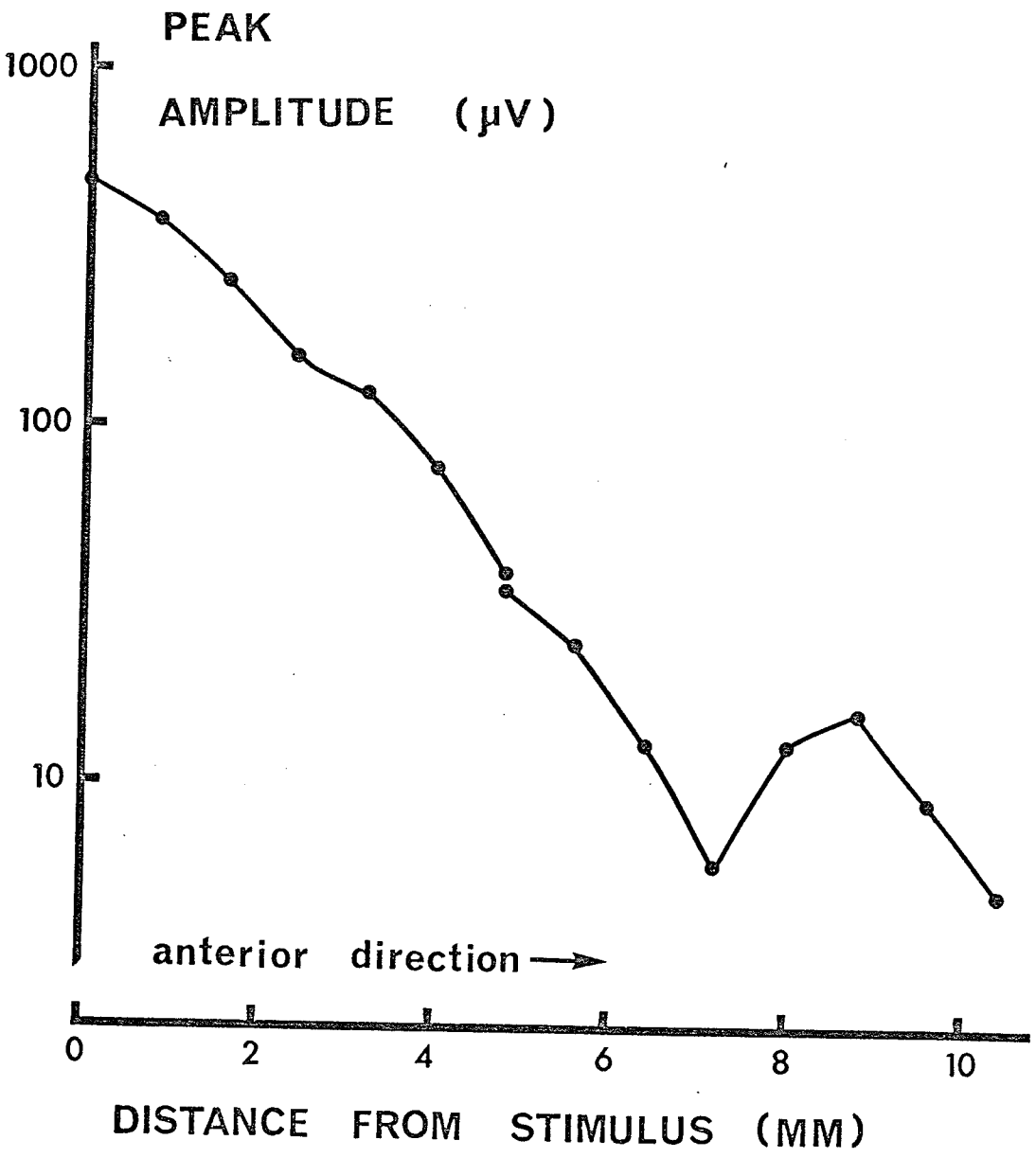


Fig. C-3. Trial showing reinforcement region of type observed by Brooks and Enger (see text). Responses for this graph (measured by Method #1) are shown in Fig. C-1. Peak amplitude in this and all subsequent graphs is plotted on a logarithmic scale.

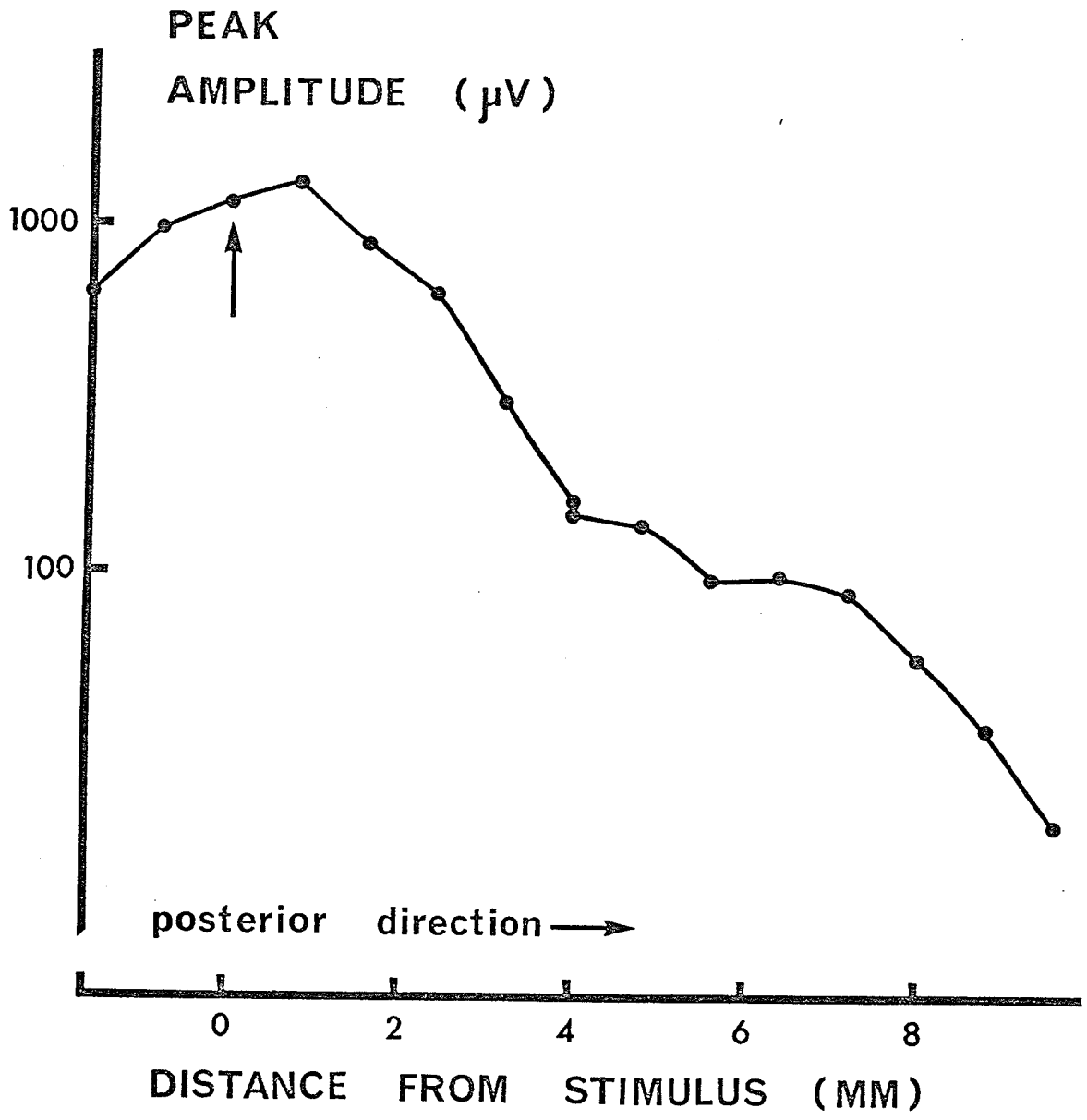


Fig. C-4. Trial showing usual type of reinforcement region (see text). Reinforcement region occurs between 4.0 and 7.2 mm. (Responses measured by Method #1).

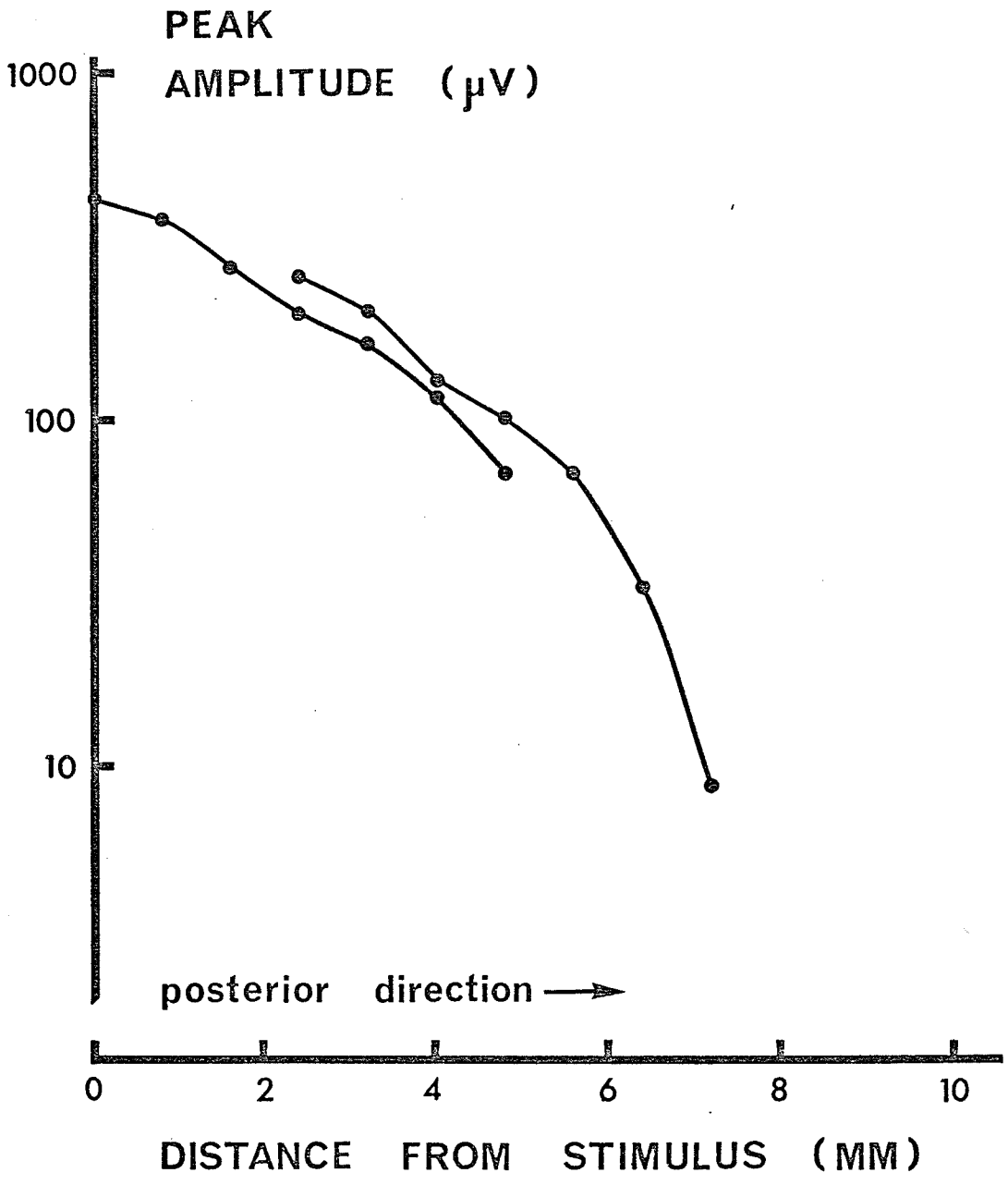


Fig. C-5. Trial showing long region of slow rate of nDCR decline extending from site of stimulation (see text). Responses for this graph (measured by Method #1) are shown in Fig. C-2. The nDCR spread is less than 8 mm from the site of stimulation in this trial.

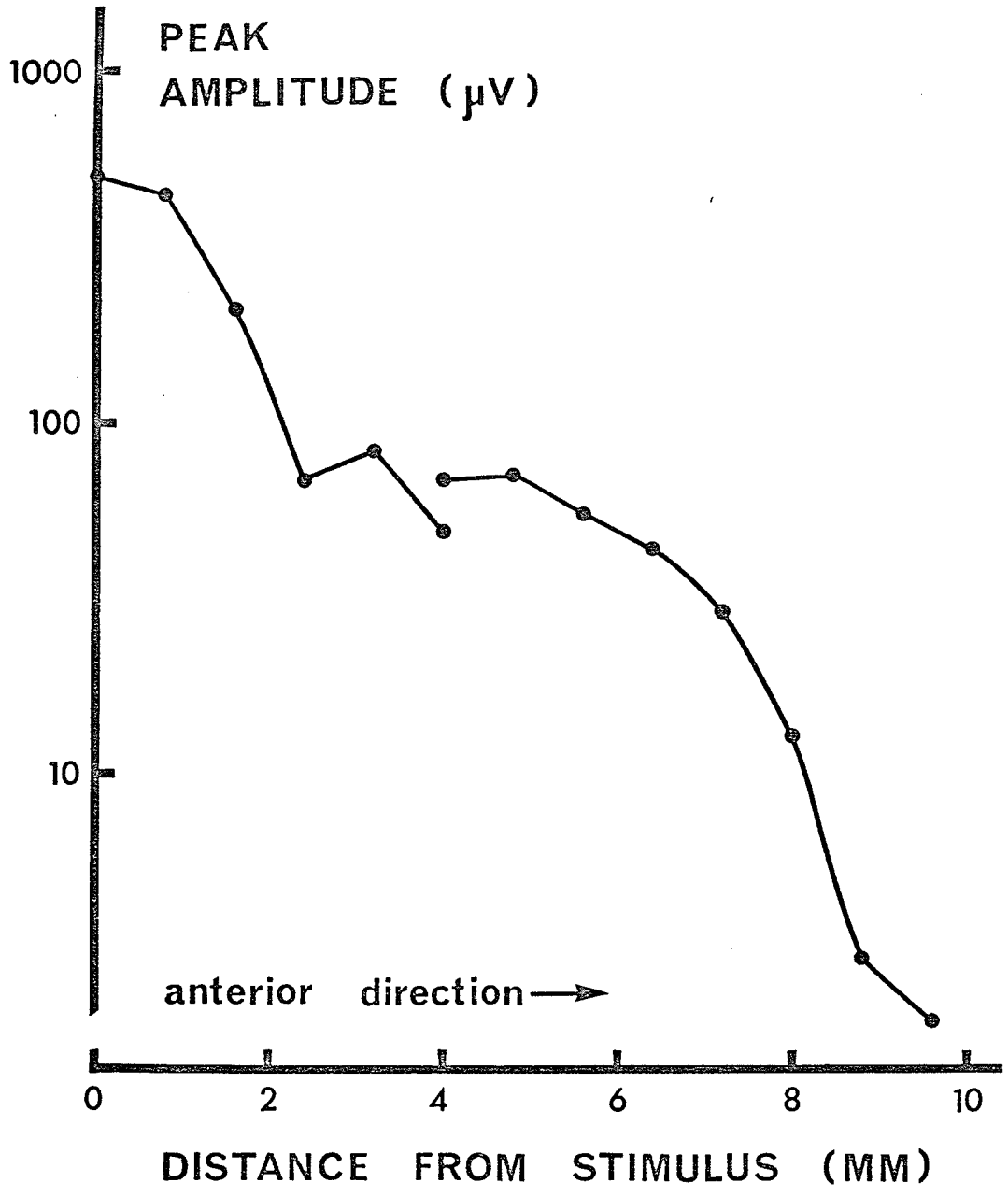


Fig. C-6. Trial showing very short region (about 0.8 mm) of slow rate of nDCR decline extending from site of stimulation. (Responses measured by Method #1.)

which should be considered in order to ascertain which might be the most probable:

1) Genetic differences and differences in environmental conditioning.

First of all, the variability might reflect the differences in the way each cat's brain had matured. These differences would be the result of variations not only in genetic inheritance but also in environmental factors such as the animal's learning experiences. It is very reasonable to assume some general pattern of cell development which would be maintained regardless of conditioning or normal variability in genetic inheritance. This would seem to be the case here since comparable patterns of nDCR spread have been recorded from several different cats. Also, there must be another source of variability more important than what could be expected from differences between individual animals since trials from different locations on the same gyrus or from the suprasylvian gyrus on the opposite hemisphere of the same cat show different patterns of nDCR spread.

2) Experimental conditions

Next, other experimental variables must be considered; these include temperature, time of trial, surgical trauma and stimulation strength. Brain temperature was kept within the limits of 34-37°C for all experiments and was generally maintained at a constant temperature from trial to trial within the same experiment. Trials done at exactly the same temperature but at different locations showed differences in the pattern of nDCR spread. Hence, these differences could not be due to temperature variation. Blood pressure

was also relatively constant from trial to trial although it generally slowly declined over a period of hours. At no time were the blood pressure at values low enough to cause inhibition of the nDCR (see Ochs, 1959).

Surgical trauma would have differed from cat to cat and may have been responsible for some of the variability between experiments. However, different patterns of nDCR spread were found within the same cat at different locations on the same gyrus. The general surgical procedures were the same for these trials. Hence, the only surgical procedure which could reasonably account for some of the variability within the same cat was the procedure for cutting the slab. The recording assembly was always centered along the slab so that stimulation and recording would be done on tissue which was a minimum of 2 mm away from the lengthwise-running sides of the slab. The cuts forming these sides of the slab would have some effect on any element which travels from the central regions of the slab to distances farther than these edges. Included in this category might be some axons in the molecular layer (Szentágothai, 1965 a and b; Jones and Powell, 1968). There is no prior reason to assume that one location along the center of the slab should have been affected very differently from any other by the cuts. However, since some of the elements must have been affected by the cuts, it will be profitable in future work to examine the spread of the nDCR on intact cortex with the technique of response averaging.

Next, there is the possibility that the state of the isolated cortex varies throughout the duration of the experiment. This is quite reasonable since the isolating procedure must cut many nerve

processes which subsequently go through different stages of degeneration as the experiment progresses. However, this possibility cannot explain all the variability seen between trials. Trials taken as long as three and a half hours apart at the same stimulus site show very little difference in the pattern of nDCR spread as characterized by amplitude measurements (see Fig. C-7).

One further important possible source of variability to be examined is the strength of stimulation. In experiments on 5 cats, different stimulation strengths were used for initiating responses at the same stimulus site. The pattern of nDCR spread as characterized by peak amplitude measurements was found to be very consistent regardless of a change in stimulus strength, even when one of the stimulation strengths used was slightly above threshold for the late positive wave (see Figs. C-8 and C-9). This was not the case, however, for peak latency measurements. When the stimulus strength was a little greater than the threshold for the late positive wave, the peak latency pattern of nDCR spread usually differed from that with a lower stimulus strength. For instance, in Fig. C-10, the peak latency of the nDCR increases very slowly between 2.4 and 4.0 mm for the lower stimulus strength while for the higher stimulus (above the threshold for the late positive wave) it increases markedly for the same region. Similarly, the peak latency for the lower strength is quite constant over the region from 6.4 to 8.0 mm and increases with distance from 8.0 to 10.4 mm while for the higher stimulus strength the situation is reversed. With the higher stimulus peak latency increases with distance from 6.4 to 8.0 mm and is relatively constant from 8.0 to 10.4 mm.

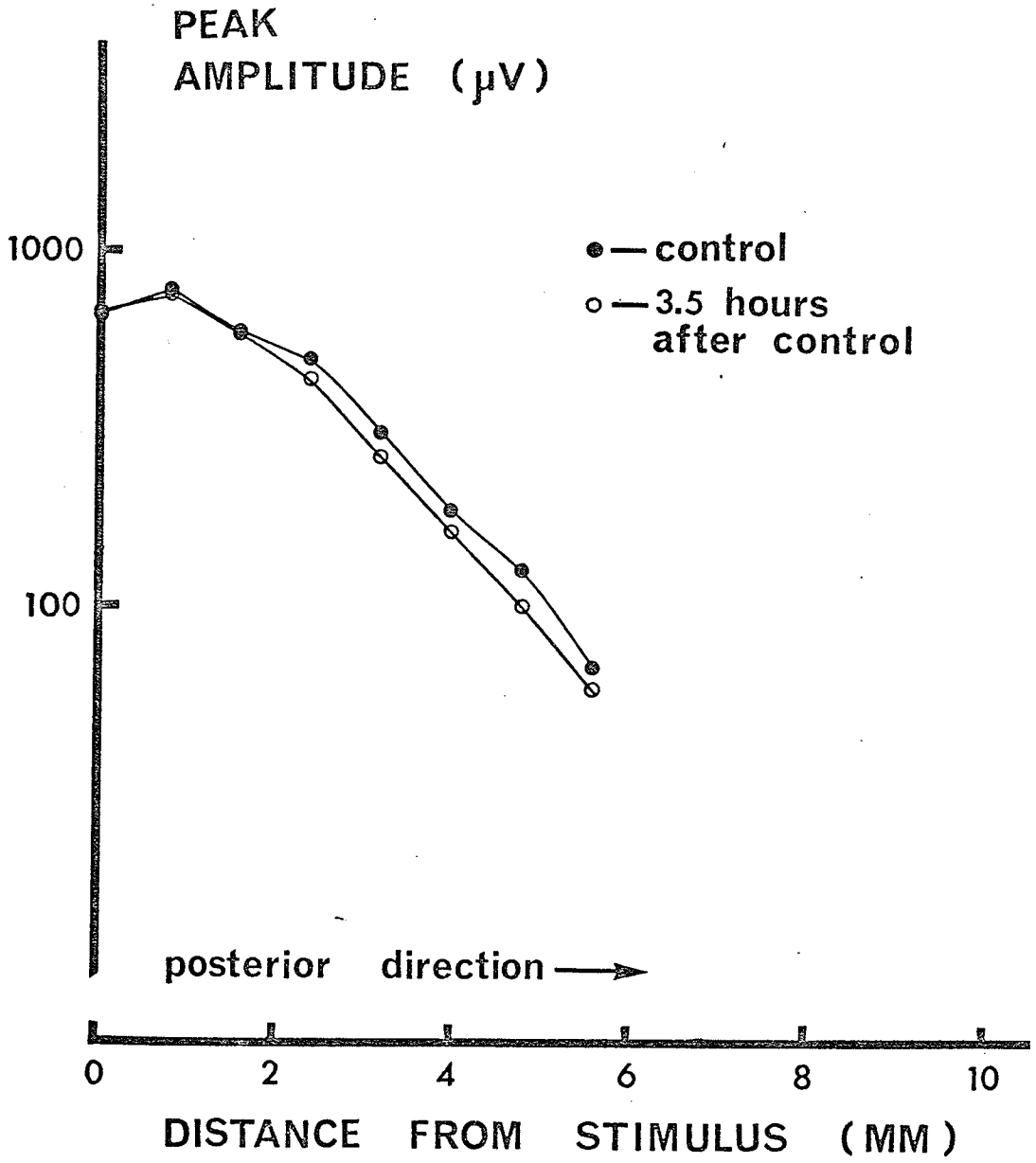


Fig. C-7. Comparison between trials taken 3.5 hours apart at the same site of stimulation. Only eight recording sites were used. (Responses measured by Method #2.)

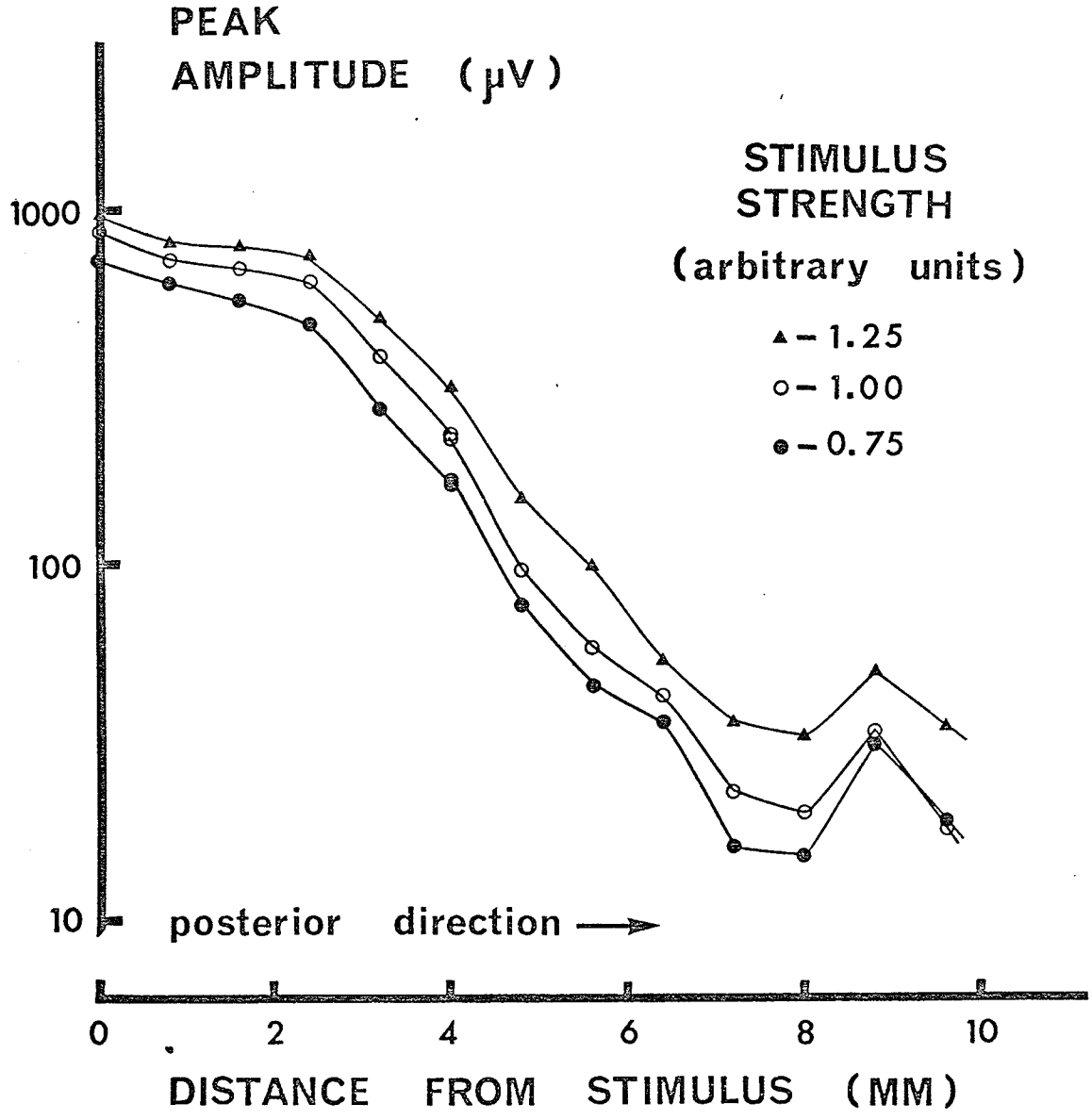


Fig. C-8. Comparison between trials taken at three different stimulation strengths below the threshold for the late positive wave. The general form of curve is unaffected by strength of stimulation. Note also unusually long region of fast rate of nDCR decline extending from 2.4 mm to 7.2 mm. (Responses measured by Method #2.)

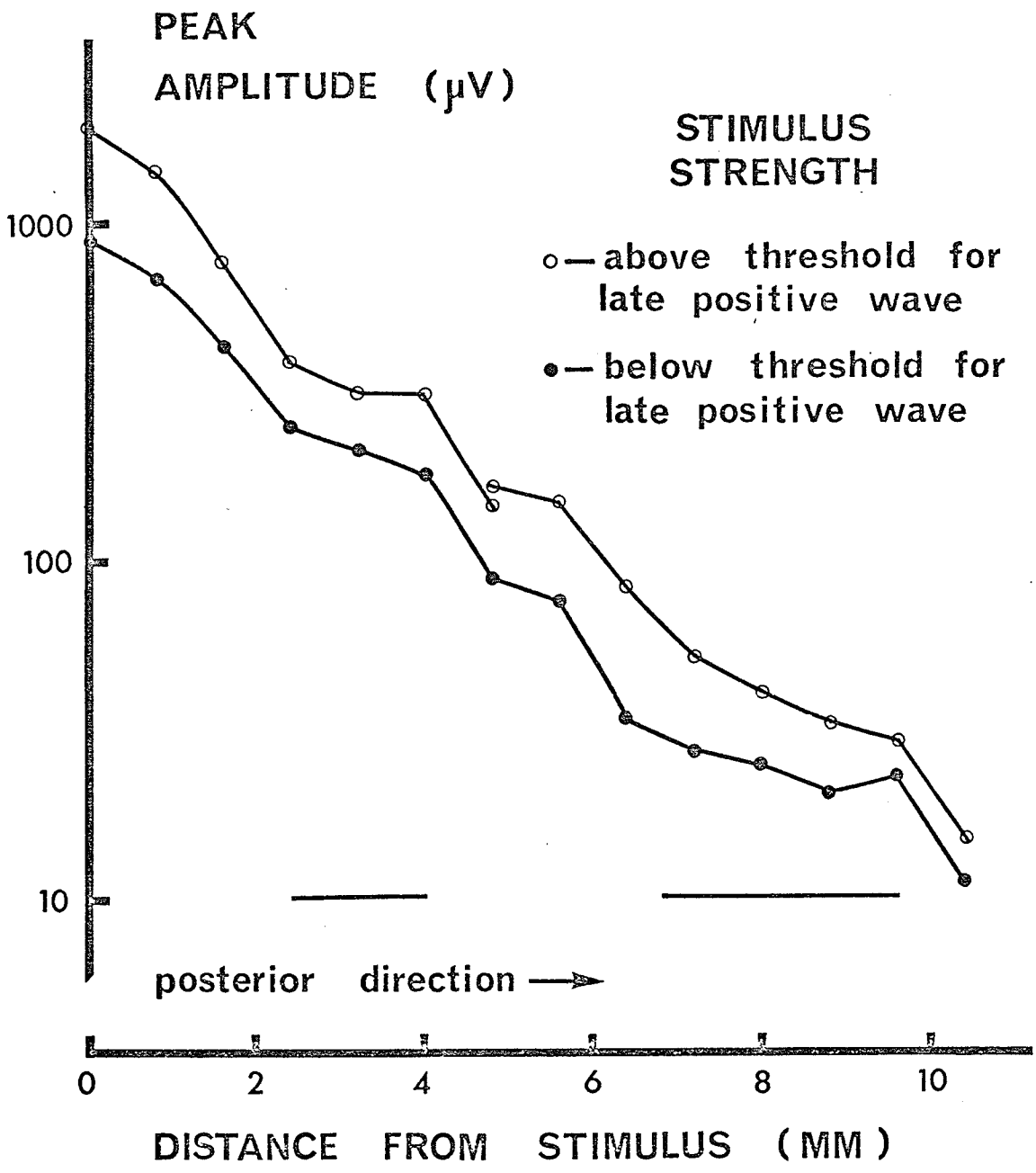


Fig. C-9. Comparison of trials taken above and below the threshold for the late positive wave. Very little difference in the pattern of nDCR spread occurs under these two stimulus conditions. Solid bars mark the location of reinforcement regions. (Responses measured by Method #1.)

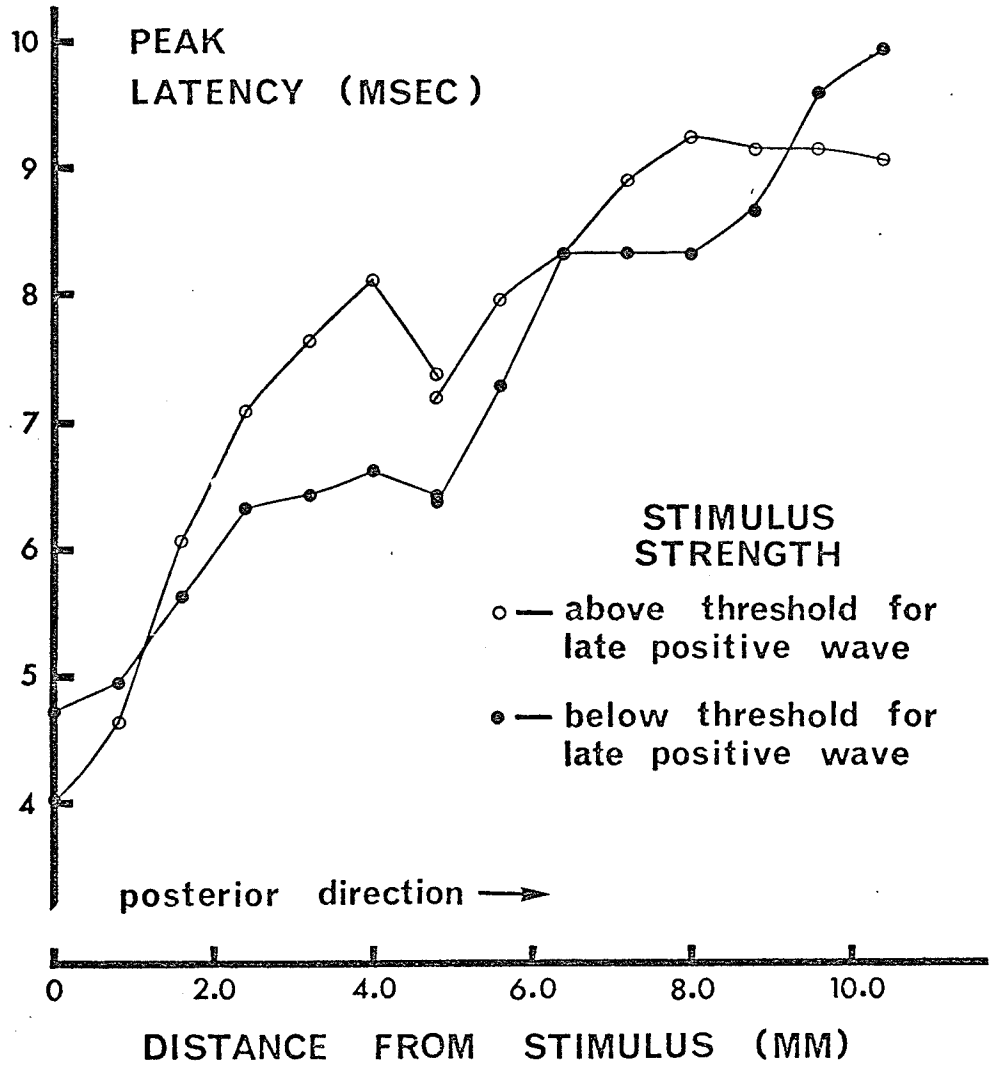


Fig. C-10. Comparison of peak latency patterns of nDCR spread from trials taken above and below threshold for late positive wave. Note differences in patterns of nDCR spread for these two stimulation strengths (see text). Trials are the same as those graphed in Fig. C-9. (Responses measured by Method #1.)

Even when the stimulus strength is below the threshold for the late positive wave, the pattern of nDCR spread as characterized by peak latency measurements shows minor fluctuations (Fig. C-11). This tends to make an analysis of the nDCR spread into various regions much less consistent and less reproducible than the analysis of peak amplitude data. Hence, although a certain degree of success had been obtained using peak-latency data (Beaubien and Pinsky, 1968), no attempt was made with the present data to analyze the spread of the nDCR directly in terms of peak-latency measurements.

Since the presence of a late positive wave can affect markedly the peak-latency pattern of nDCR spread, only stimulus strengths below (usually from 10 to 20%) that of the threshold for the late positive wave were chosen when comparing the nDCR spread from different stimulus sites (see Methods).

3) Location of the stimulus site on the gyrus.

This experimental variable is considered separately because of its great importance. As mentioned above, trials taken as long as three and a half hours apart from one another showed essentially the same pattern of nDCR spread as characterized by peak amplitude measurements (Fig. C-7). This occurred despite the fact that the stimulating electrodes had been moved to several different sites in the interim, before returning them as closely as possible to the original site in order to retest the nDCR spread from that location.

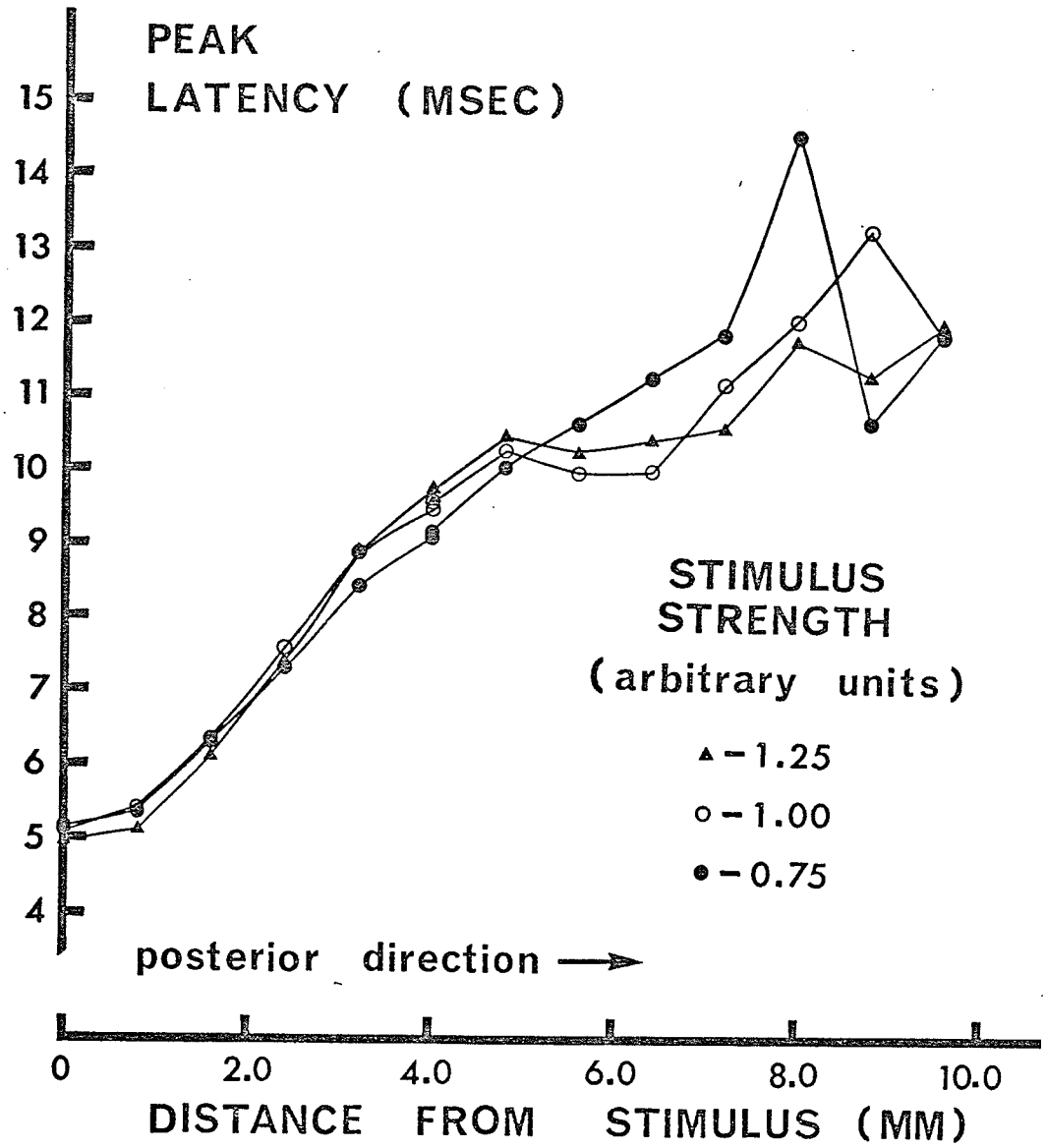


Fig. C-11. Comparison of peak latency patterns of nDCR spread from trials at three different stimulation strengths below threshold for the late positive wave. Although the curves do not differ significantly from one another, the minor variations which do occur (especially in the more distant regions) make it difficult to classify the pattern of nDCR spread. Trials are the same as those graphed in Fig. C-8. (Responses were measured by Method #2.)

However, the pattern of nDCR spread taken from different stimulus sites on the same gyrus is usually different for each location. The results shown in Figs. C-3 and C-6 have actually been obtained from the same gyrus but from two different stimulus sites. The second stimulus location (Fig. C-6) was 4.8 mm anterior to the first (Fig. C-3). After nDCR measurements had been made from the first site, the assembly was moved 4 mm anteriorly to enable measurement of the nDCR at further distances from the second stimulus site. It is quite obvious that the pattern of nDCR spread is very different with these two stimulus locations. The rate of decline in nDCR amplitude from the first stimulus site is relatively slow until about 3.2 mm from the stimulus site, and then is faster, until 7.2 mm, after which a region of reinforcement is observed (Fig. C-3). However, with the second stimulus location, the rate of decline of the nDCR amplitude is rapid beginning at only 0.8 mm distance from the stimulus site and a region of reinforcement begins after 2.4 mm. This reinforcement region then extends to about 7.2 mm after which the nDCR amplitude rapidly declines again (Fig. C-6).

e) Cortical areas of fast and slow rates of nDCR decline

The finding that the pattern of nDCR spread is often different and distinctive at different locations on the cortex, even within the same gyrus, is very consistent from experiment to experiment. In order to determine whether or not there is a pattern of cerebral organization giving rise to these differences, a series of experiments was conducted in which the cerebral cortex of the suprasylvian gyrus was scanned by the stimulating electrodes (see Methods).

These experiments showed that, along the line of the recording assembly, the reinforcement regions within a given gyrus tended to occur at the same location regardless of the position of the stimulating electrodes. A good example of this is the experiment presented in Fig. C-12 where log peak amplitude is plotted against location along the length of the gyrus for several different stimulus sites. For the purpose of illustration, the curves depicting nDCR spread from the various sites have been deliberately displaced from one another by a few units in the y-axis direction. The units on the y-axis have therefore been omitted since they would have had to be located differently for each curve. The arrows at the beginning of each curve indicate the location of the stimulating electrodes for that curve. The graph has been marked off into areas A and E by dotted lines which were drawn at points of inflection common to several of the curves (except for the line separating areas A and B which is based on the inflection point of the lowest curve only). Areas A, C and E are areas where the nDCR has a steep rate of amplitude decline, independent of the site of stimulation. Areas B and D are areas in which the nDCR declines at a slow rate; area B is a reinforcement region for the lowest curve, while area D is a reinforcement region for the rest of the curves. Another example of this is seen in Fig. C-13 where the reinforcement regions for the curves obtained from three different stimulus locations occur in area C. Similarly, when the amplitude data in Figs. C-3 and C-6 are plotted against location on the gyrus instead of against distance from stimulus, the reinforcement region is seen to occur at exactly the same location for both stimulus sites (Fig. C-14).

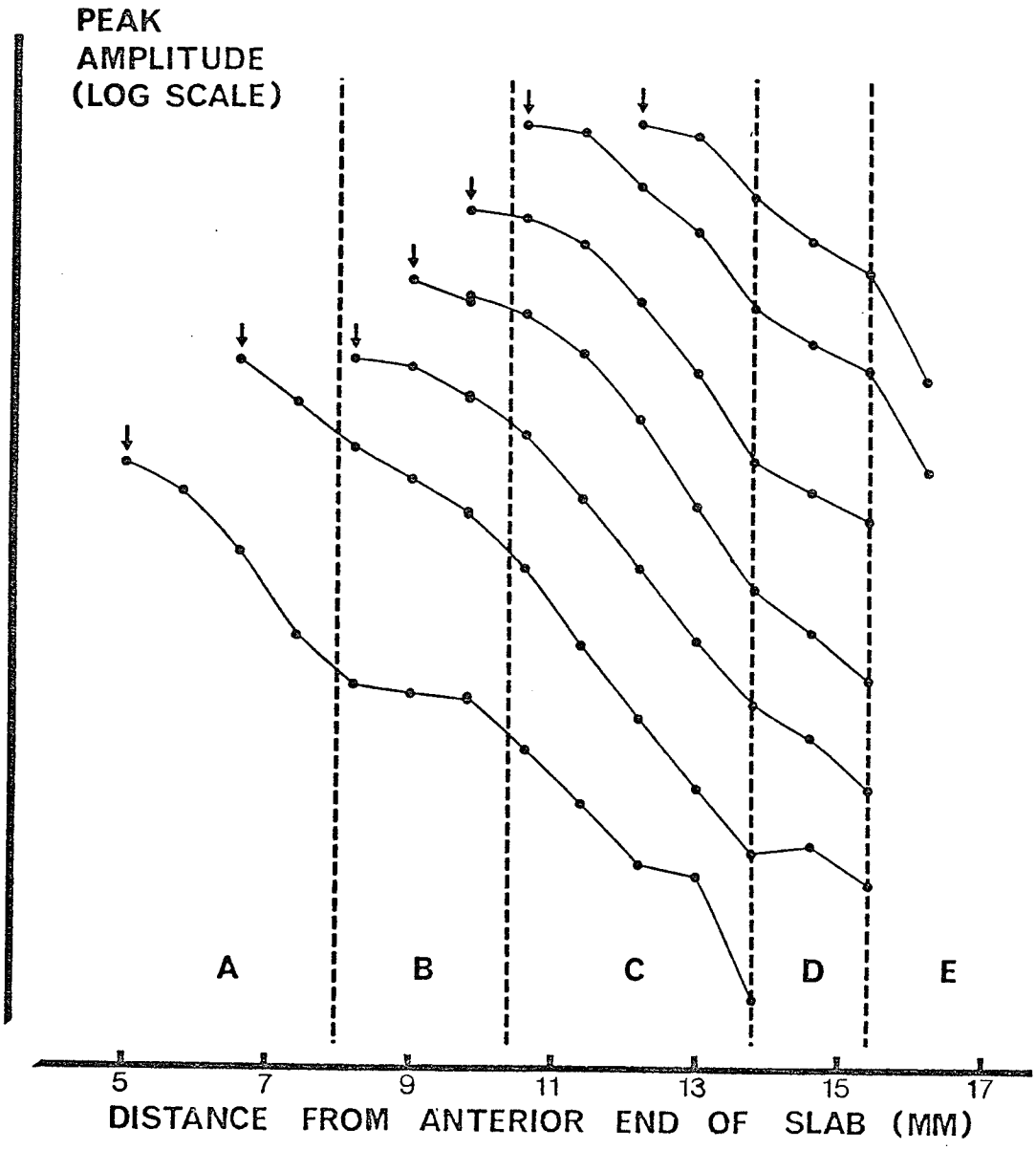


Fig. C-12. First example of an experiment showing separation of cortical slab into areas of fast and slow rates of nDCR decline (see text for explanation).

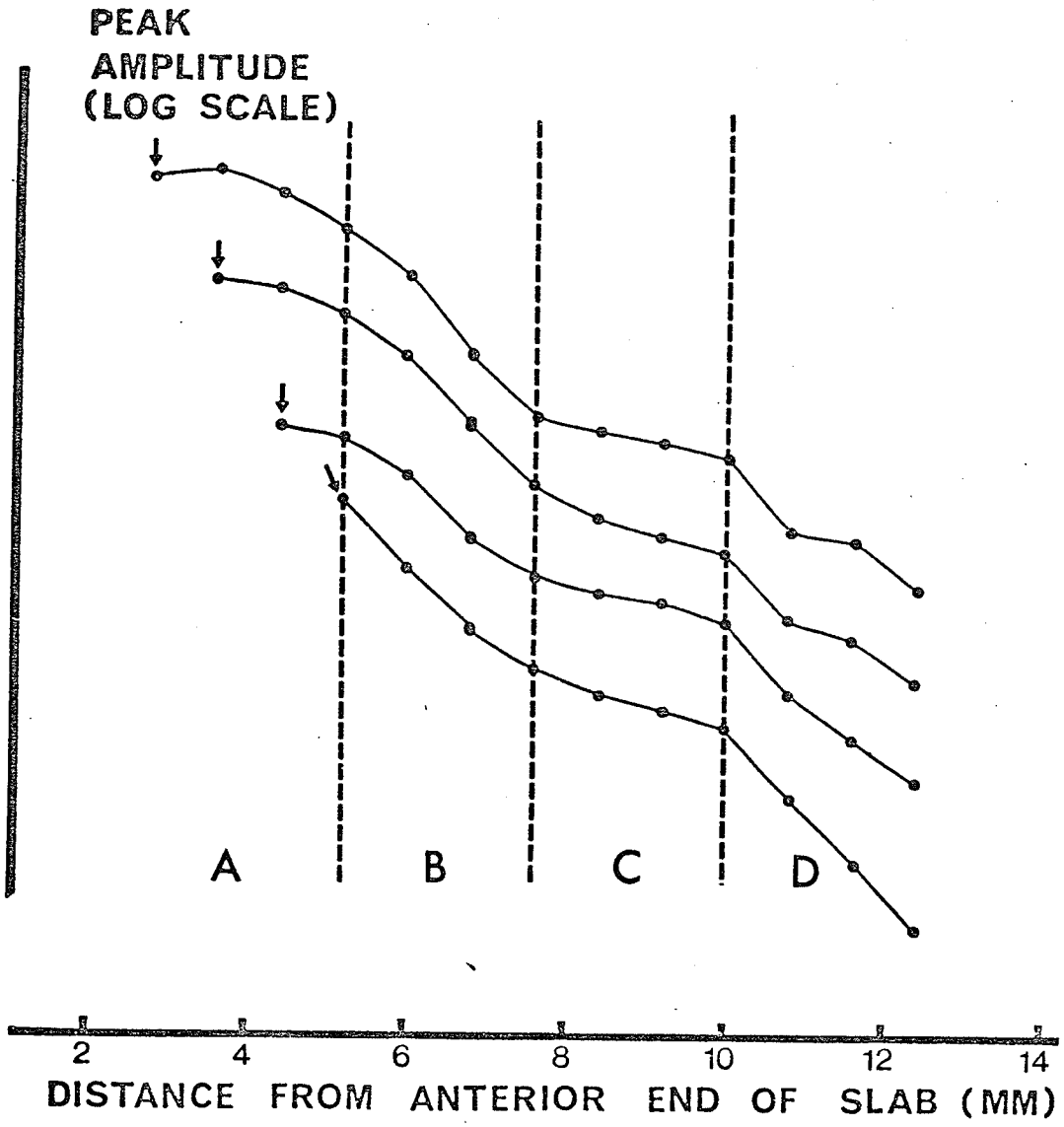


Fig. C-13. Second example of an experiment showing separation of cortical slab into areas of slow and fast rates of nDCR decline.

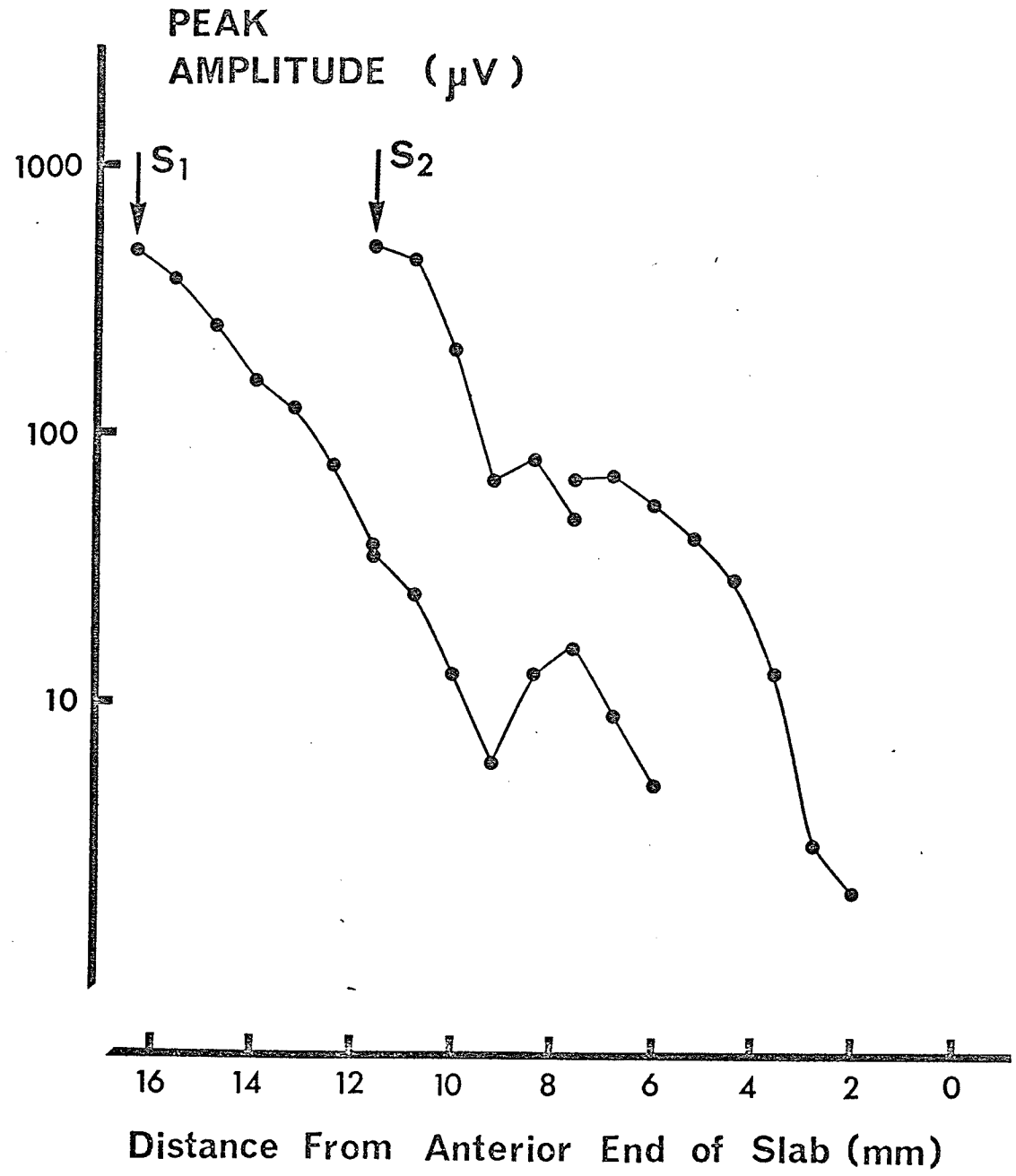


Fig. C-14. Plot of trials in Figs. C-3 and C-6 as a function of location on cortical slab instead of distance from stimulus site. The reinforcement regions occur at the same location for both trials.

The existence of two kinds of cortical areas, characterized by either a low or a high rate of nDCR decline, was observed in 10 out of 11 experiments in which the spread of the nDCR was measured from at least 2 different stimulus sites on the same gyrus. In 7 of these experiments, the nDCR spread was measured from a minimum of ten stimulus locations. The single experiment which did not correlate regions with location on the gyrus had data from 3 locations which were 5.6 mm apart from one another. Reinforcement regions might have been present in this gyrus and not discerned because of the large distances between stimulus sites.

II. Asymmetry of nDCR spread

The spread of the nDCR in the anterior and posterior directions was not symmetrical about the point of stimulation. There were 87 out of 100 trials, in 14 cats, which showed higher amplitudes in the posterior direction than in the anterior direction for equal distances from the site of stimulation. In the remainder of the trials (N = 23), higher amplitudes occurred in the direction anterior to the stimulus site. Fig. C-4 is an example of a trial in which the amplitude is higher in the posterior direction than in the anterior direction from the site of stimulation. It is also an example of a trial in which the asymmetry of the spread of the nDCR is so marked that the highest nDCR amplitude is not at the site of stimulation (arrow) but at an electrode posterior to it. This displacement of the maximum nDCR amplitude from the site of stimulation occurred in about 50% of the trials.

a) Average results showing a posterior orientation

The data from the trials which showed posterior orientation were fed into an IBM computer, aligned with respect to their stimulus

sites, and averaged. The results are shown in Fig. C-15. Peak amplitude is expressed on a log scale and peak latency on a linear scale. Closed circles represent n-wave DCR measurements in the posterior direction: the open triangles represent n-wave DCR measurements in the anterior direction. A paired t-test shows that both peak latency and peak amplitude measured posterior to the stimulus site differ significantly ($p<0.01$) from these variables anterior to the stimulus site. In the posterior direction, the peak amplitude of the response is significantly higher and the peak latency significantly shorter than in the anterior direction for equal distances from the site of stimulation. In this sense the nDCR may be said to have directionality in its longitudinal spread from the stimulus site.

b) Asymmetry as a function of distance from stimulus

In order to better characterize the elements responsible for nDCR asymmetry, the following procedure was used to locate the area where the asymmetry is produced with respect to the site of stimulation: The difference in amplitude between each pair of points in Fig. C-15 was calculated and then expressed as a percent of the amplitude at the anterior point. This difference was plotted as a function of distance from the site of stimulation, as shown in the upper graph of Fig. C-16.

From this figure it can be seen that the degree of asymmetry increases with distance for distances up to about 3 mm from the stimulus site and then asymptotes. The last point is the pooled result of the 5 most distant points. The lower graph of Fig. C-16

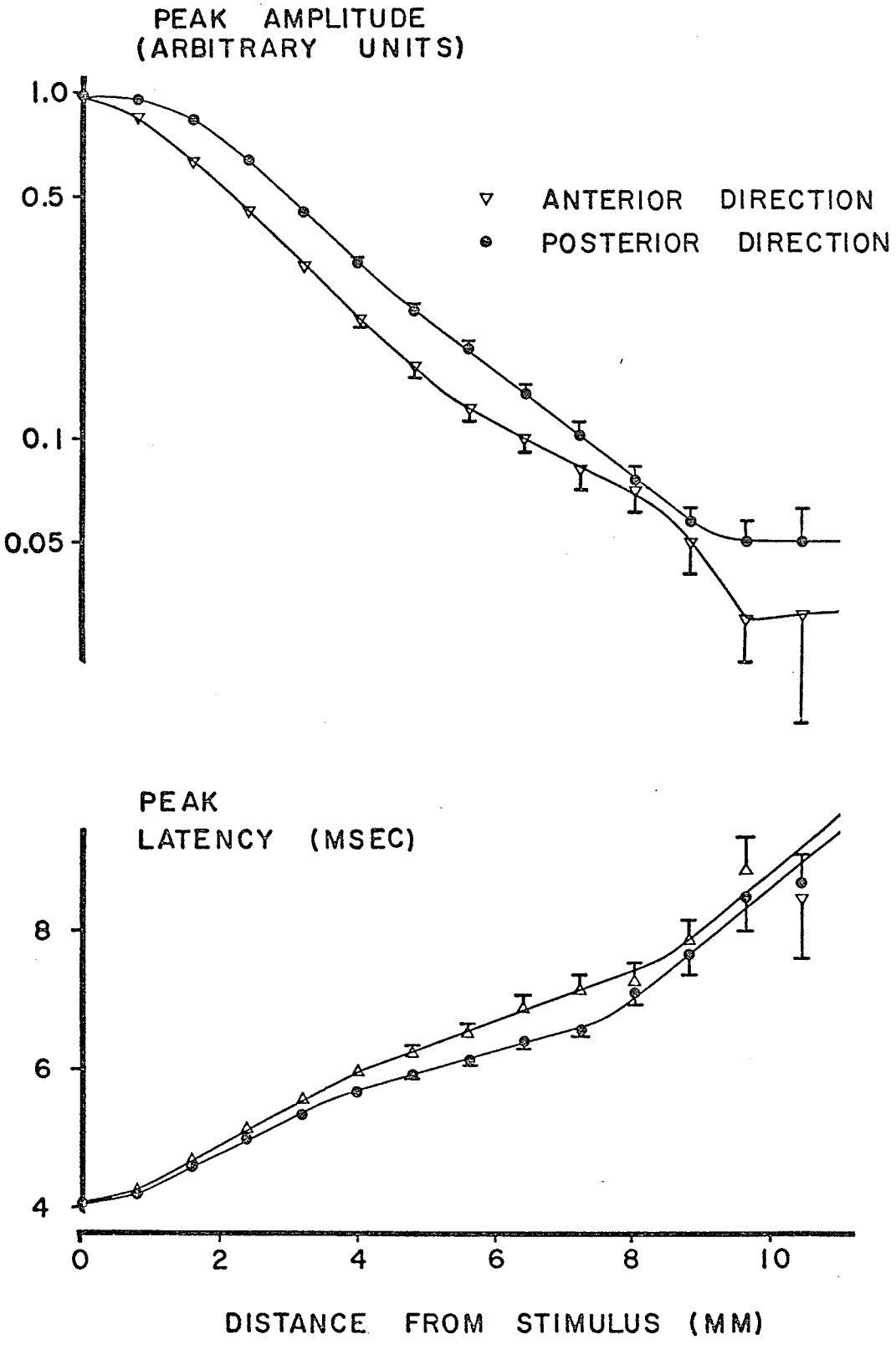


Fig. C-15. Comparison of the two directions of nDCR spread for posterior oriented curves. The nDCR is shown to travel with a higher amplitude and shorter peak latency in the posterior direction than in the anterior direction. The rate of nDCR decline for the posterior direction is about -0.4 log mV/mm (calculated from the region extending from 1.6 mm to 8.8 mm).

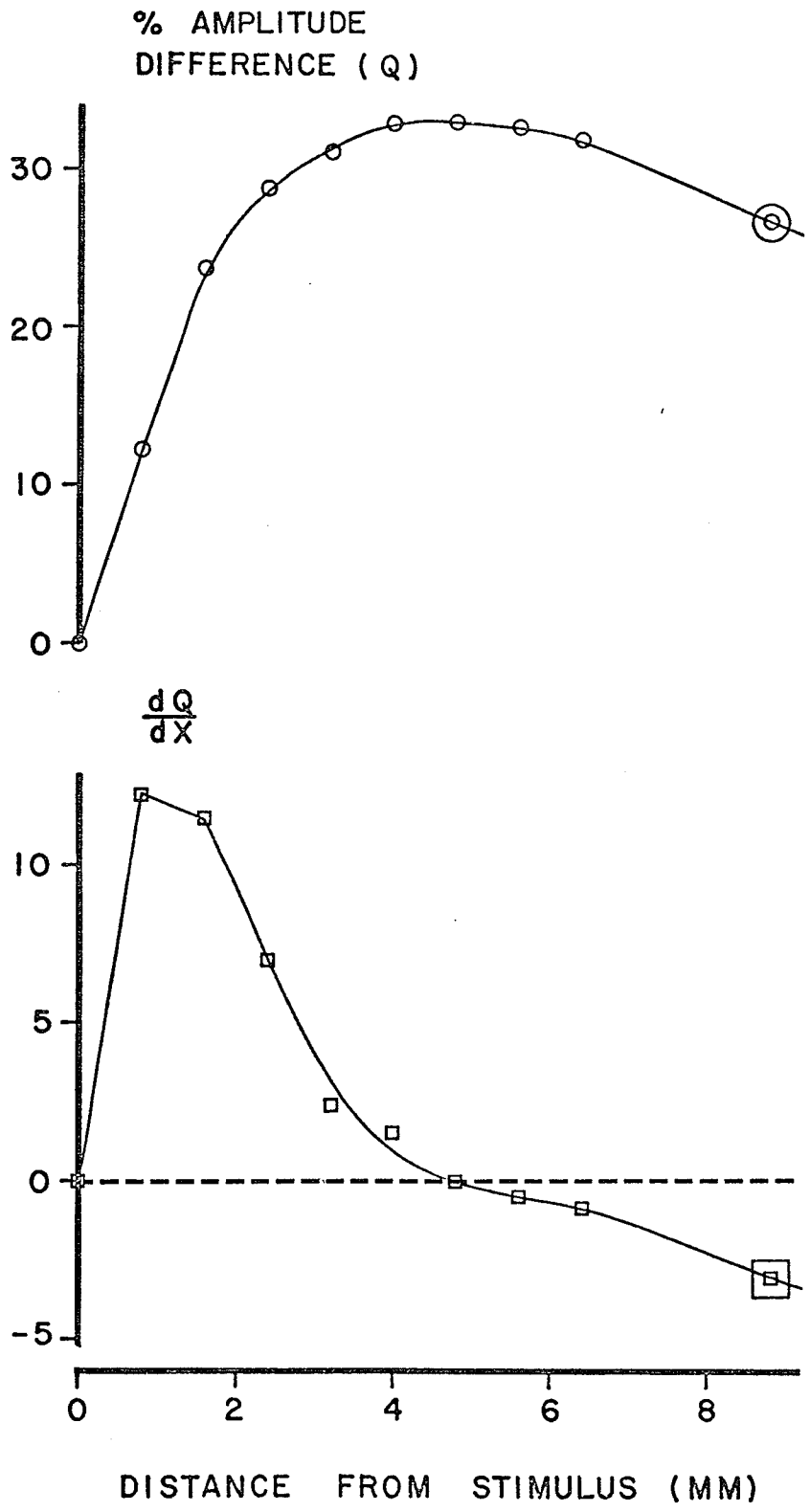


Fig. C-16. Locating area responsible for nDCR asymmetry (see text). Upper graph plots degree of asymmetry vs. distance from stimulus. Lower graph plots rate of increase of asymmetry vs. distance from stimulus.

is the first derivative of the upper graph. It expresses the rate at which the asymmetry increases with distance from the stimulus site. It shows that most of the increase in asymmetry occurs within 2-3 mm of the stimulus site and has a maximum increase at about 1 mm.

c) Relation of orientation of asymmetry to areas of the suprasylvian gyrus

In 23 out of 110 trials, the directionality of the n-wave DCR was anterior rather than posterior. Fig. C-17 shows the probability of occurrence of anterior directionality when the stimulus is applied at different locations along the length of the suprasylvian gyrus. The ordinate is the probability of occurrence of an n-wave DCR with a greater amplitude in the anterior direction than in the posterior direction. The abscissa gives the location of the stimulus site on the suprasylvian gyrus. Data for each location was obtained from 5-9 experiments. Anterior directionality occurred with about a 30% probability at either end of the gyrus but with a probability of 0% in the middle region. The probability of occurrence of posterior directionality is not plotted on this graph but it can be obtained by simply subtracting the anterior probability at each location from 100%. Thus, the probability of occurrence of posterior directionality is about 70% at either end of the gyrus and 100% in the middle region.

The regional distribution of occurrence of anterior directionality is similar to the distribution of visual evoked potentials observed in the suprasylvian gyrus by Buser and Borenstein (1959). They located two visual association areas in this gyrus, one at either end. They also located a somatic association area which covers

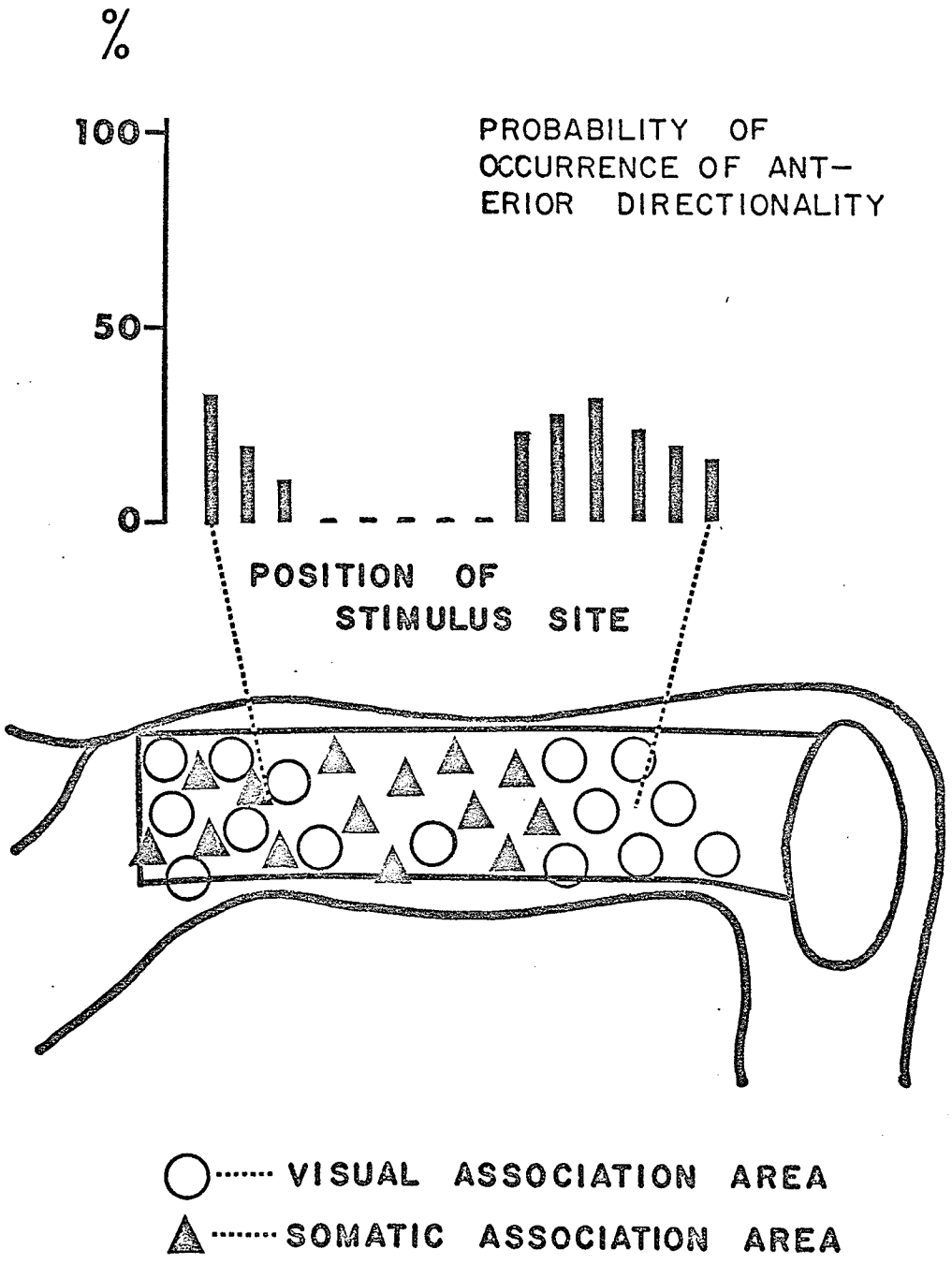


Fig. C-17. Relation of orientation of asymmetry to location on suprasylvian gyrus (see text).

the middle and anterior regions of the gyrus. These are depicted in the lower half of Fig. C-17. Anterior directionality of the n-wave DCR can be seen as occurring only where the visual association areas are prominent.

III. Effect of Cuts

Much uncertainty has existed over the extent to which cortical (and subcortical) cuts have to be made in order to block completely the spread of the nDCR in the tangential plane (Chang, 1951; Burns and Grafstein, 1952; Ochs, 1956; Ochs and Clark, 1968a; see Introduction). Since most of the controversy at one time centered on whether or not pathways existed in the lower cortical layers (or subcortically) as well as in the molecular layer of the cerebral cortex, it was decided to compare the extent of transmission block of a 0.5 mm cut with that of a cut ("complete" cut) which completely isolated one portion of the slab from the other except for the pial membrane.

a) Comparison of nDCR transmission across a complete cut with that across a cut 0.5 mm deep.

Each cut extended over the entire width of the slab, so that there would not be any pathways around the cut (see Methods). Theoretically, if any transmission occurred across the complete cut, it should only be due to volume conduction or spread of the stimulus current since both cortical and subcortical pathways had been severed. The difference, then, between the amount of transmission across a cut 0.5 mm deep and a cut completely severing all pathways would be the contribution of lower cortical or subcortical pathways to the transmission of the response in the tangential plane. This was

tested for several different distances (0.4, 1.2, 2.0, 3.6 and 5.2 mm) of the site of stimulation anterior and posterior to the cut since the lower pathways might be optimally activated only when the stimulus is applied at a specific distance from the cut.

Three experiments were done to test the 0.5 mm cuts and two experiments to test the complete cuts. Two trials, one on either side of the cut, were done at each distance of the stimulating electrodes from the cut. (Three other experiments were done with complete cuts but in these cases only one stimulus site 2.0 mm anterior to the cut was used.) The reason for not testing both cuts in the same animal was that the experiment would have lasted too long since each experiment was primarily conducted to obtain information on cortical domains (see above) before any cuts were made.

The results are graphed in Fig. C-18. The arrow points to the location of the cut in each graph. The data was normalized by expressing response amplitude as a percent of the amplitude of the response at the recording electrode 1.2 mm away from the cut (on the stimulated side of the cut). The response at this electrode was used as the standard rather than the response at the electrode 0.4 mm from the cut since it was feared that the latter would be too greatly affected by trauma from the cutting procedure. When the stimulus was applied 0.4 mm from the cut, however, the response at this closer electrode had to be used as the standard. Percent amplitude is plotted on a log scale in each graph.

The results shown in Fig. C-18 do not demonstrate any significant difference in the amount of transmission of the nDCR

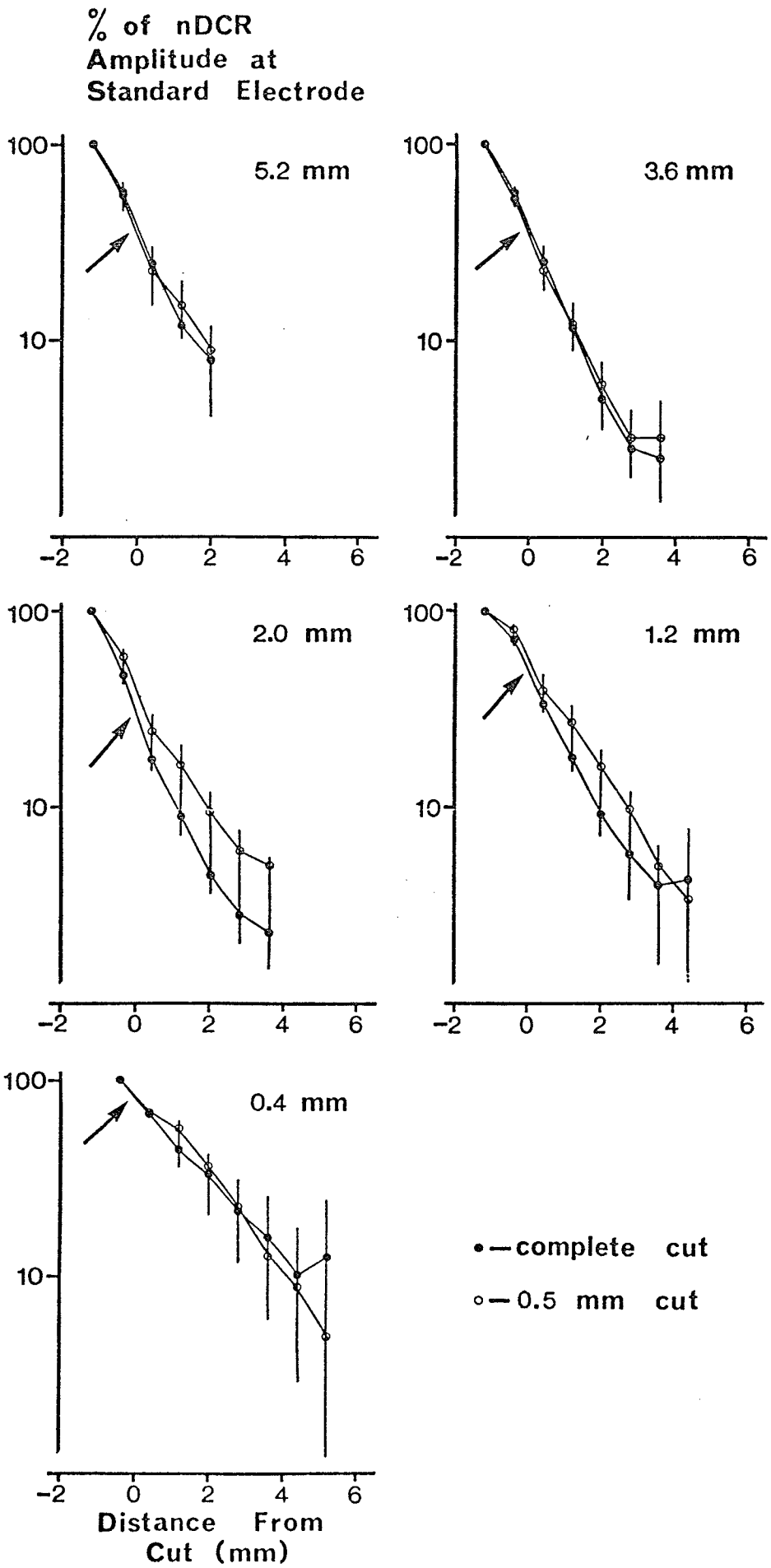


Fig. C-18. Comparison of nDCR transmission across both types of cuts for several distances (upper right corner of each graph) of the stimulating electrodes from the cut.

across a cut 0.5 mm deep and across a complete cut. This is true for all tested distances of the stimulus site from the cut.

b) Analysis of the nDCR which appears across a cut

The data show that, however unexpectedly, the nDCR did cross both types of cut. Figs. C-19 and C-20 show records of two separate experiments in which the nDCR could be recorded beyond a complete cut. Transmission across such cuts was a consistent finding occurring in 39 out of 42 trials in 8 cats. All 3 of the trials in which nDCR transmission did not occur across the cut were trials in which the stimulating electrodes were 5.2 mm from the cut.

1) Consideration of spread of stimulus current

One possible way in which the nDCR might be generated on the other side of a complete cut is by spread of the stimulus current across the cut. This possibility was examined in the following way: First of all, the results from the experiments with 0.5 mm cuts were pooled with those from the experiments with the complete cuts in order to increase the sample size. (This was considered to be justified on the basis that the results did not show any significant difference in the amount of nDCR transmission across the two types of cuts.) Next, it was considered highly unlikely that the nDCR could be generated across a cut by a stimulus (even if it were many times the strength normally used to elicit the nDCR) which was 5.2 mm from the cut. Hence, the results obtained using this distance of the stimulating electrodes from the cut were compared with those of the closer stimulus distances. The distance at which the stimulus current spreads across the cut should have a greater percentage of nDCR "transmission" across the cut.

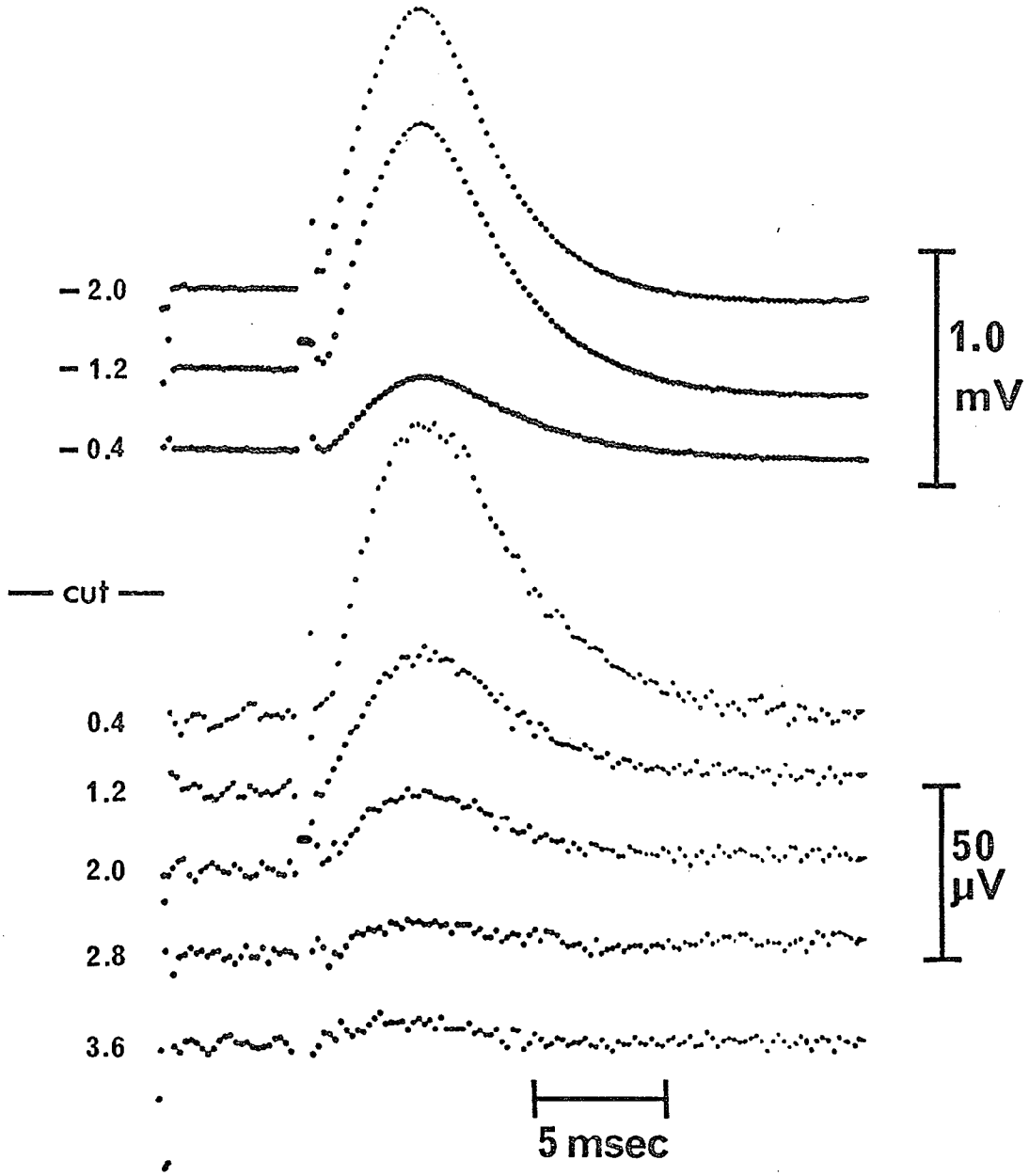


Fig. C-19. Trial showing constant-latency transmission across a complete cut.

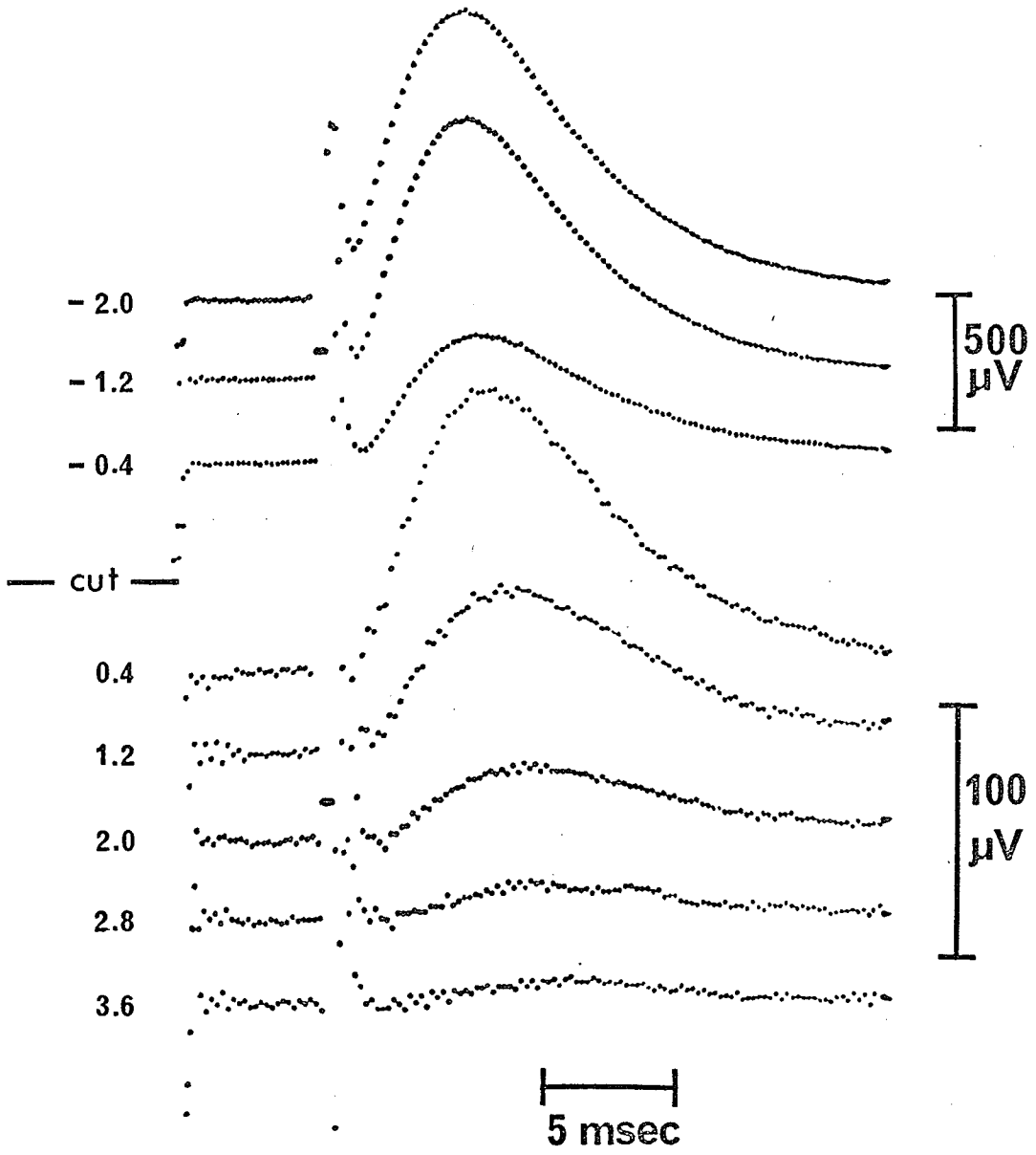


Fig. C-20. Trial showing increasing latency of nDCR peak for response transmitted across complete cut.

It was found that the percent transmission of the nDCR across the cut was not any greater with the stimulating electrodes as close as 2.0 mm to the cut than when they are 5.2 mm from the cut (see Fig. C-21). When the stimulating electrodes are placed 1.2 mm from the cut, it appears at first from Fig. C-21 that more transmission does occur across the cut from this distance of stimulation. However, the reason for this difference is that the response at the electrode 0.4 mm from the cut (on the stimulated side of the cut) has a significantly ($p<0.05$) greater amplitude when the stimulating electrodes are 1.2 mm from the cut than when they are further away. When the electrode which is 0.4 mm from the cut is used as the standard, no difference in percent transmission across the cut is found even when the stimulating electrodes are placed 1.2 mm from the cut (see Fig. C-22). A significant difference ($p<0.005$ for the first 4 electrodes on the non-stimulated side of the cut) is found, however, when the stimulating electrodes are only 0.4 mm from the cut. Hence, these results indicate that stimulus current does not spread across the cut when the stimulating electrodes are at least 1.2 mm or more away from the cut.

2) Two types of nDCR transmission across cuts

The response which is recorded across a cut most likely is not present there as a result of stimulus current spread, provided the stimulating electrodes were at least 1.2 mm from the cut. It might, therefore, be present there either as a volume-conducted response or else as an active response which has arisen as a result of neuronal activity on the stimulated side of the cut.

A very good way to determine whether or not the response is

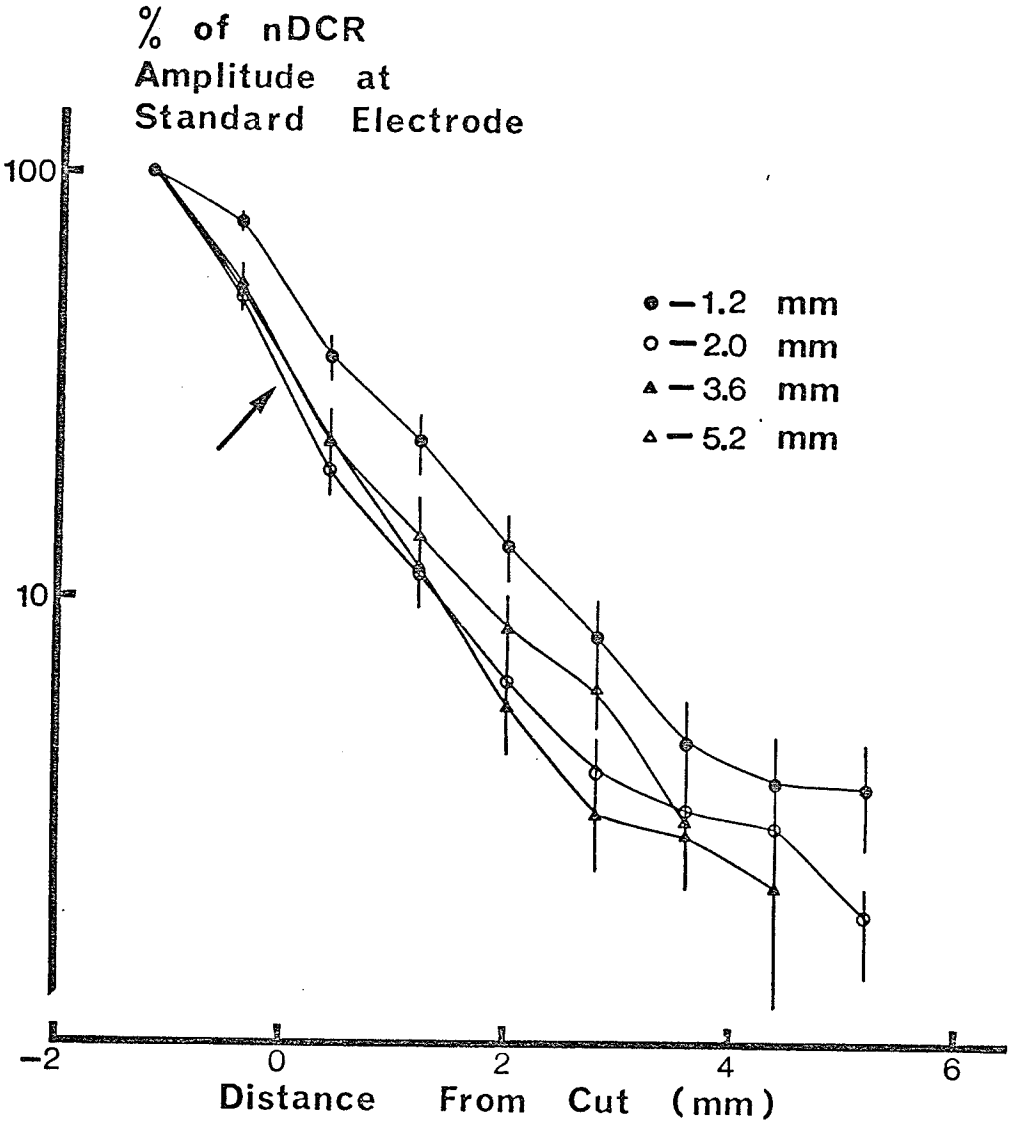


Fig. C-21. Comparison of percent transmission of nDCR across cuts for different distances of stimulating electrodes from cut: response 1.2 mm from cut used as standard.

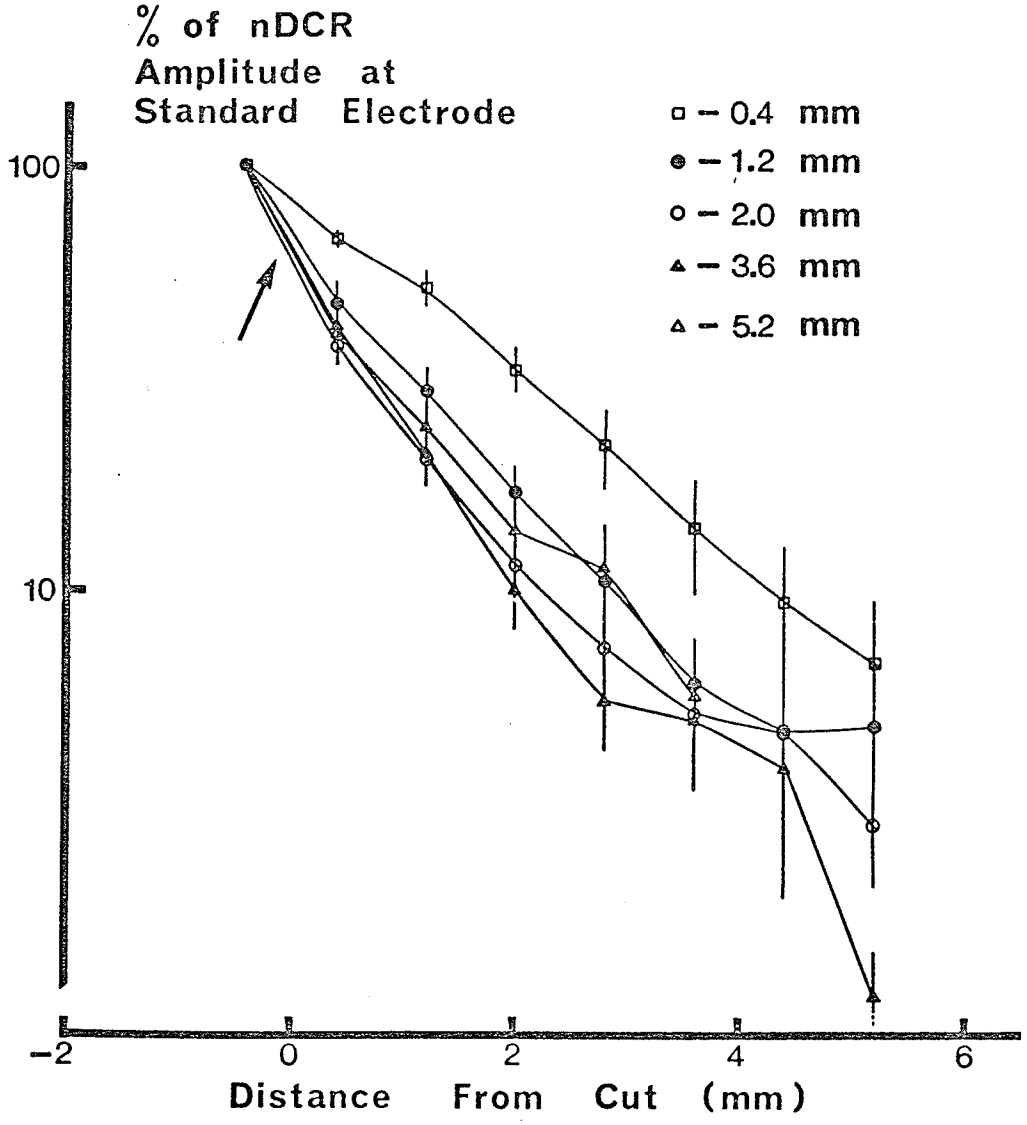


Fig. C-22. Comparison of percent transmission across cuts for different distances of stimulating electrodes from cut: response 0.4 mm from cut used as standard.

active across a cut is to examine the peak latency of the response at different distances perpendicular to the cut. If the peak-latency remains constant at all distances across a cut, then there is a good possibility that the response is present there as a result of volume conduction. However, if the response shows a change in peak latency at different distances from the cut, that response must be active. The waveform of a volume-conducted response would tend to resemble that of the nDCR on the stimulated side of the cut, especially the waveform of the nDCR which occurs closest to the edge of the cut. This waveform would be similar at all distances perpendicular to the cut since all of these points would "see" essentially the same dipoles which are created by the response on the stimulated side of the cut.

Two types of nDCR "transmission" were observed to occur across the cuts when the stimulus was applied at distances 1.2 mm or greater from the cut. One type ("constant-latency transmission") was characterized by having the same peak latency at all distances from the cut at which the response could be recorded (see Figs. C-19 and C-23). The other type of transmission ("latency-shifting transmission") was characterized by a peak latency which increased continually with distance (see Figs. C-20 and C-23). Both types of transmission occurred across cuts 0.5 mm deep and across complete cuts. However, the type of transmission which showed an increasing peak-latency with distance occurred with a much greater frequency across a 0.5 mm cut than across a complete cut. This latency-shifting transmission occurred in 64% of all the trials (N = 22) across a 0.5 mm cut but in only 29% of the trials (N = 21) across

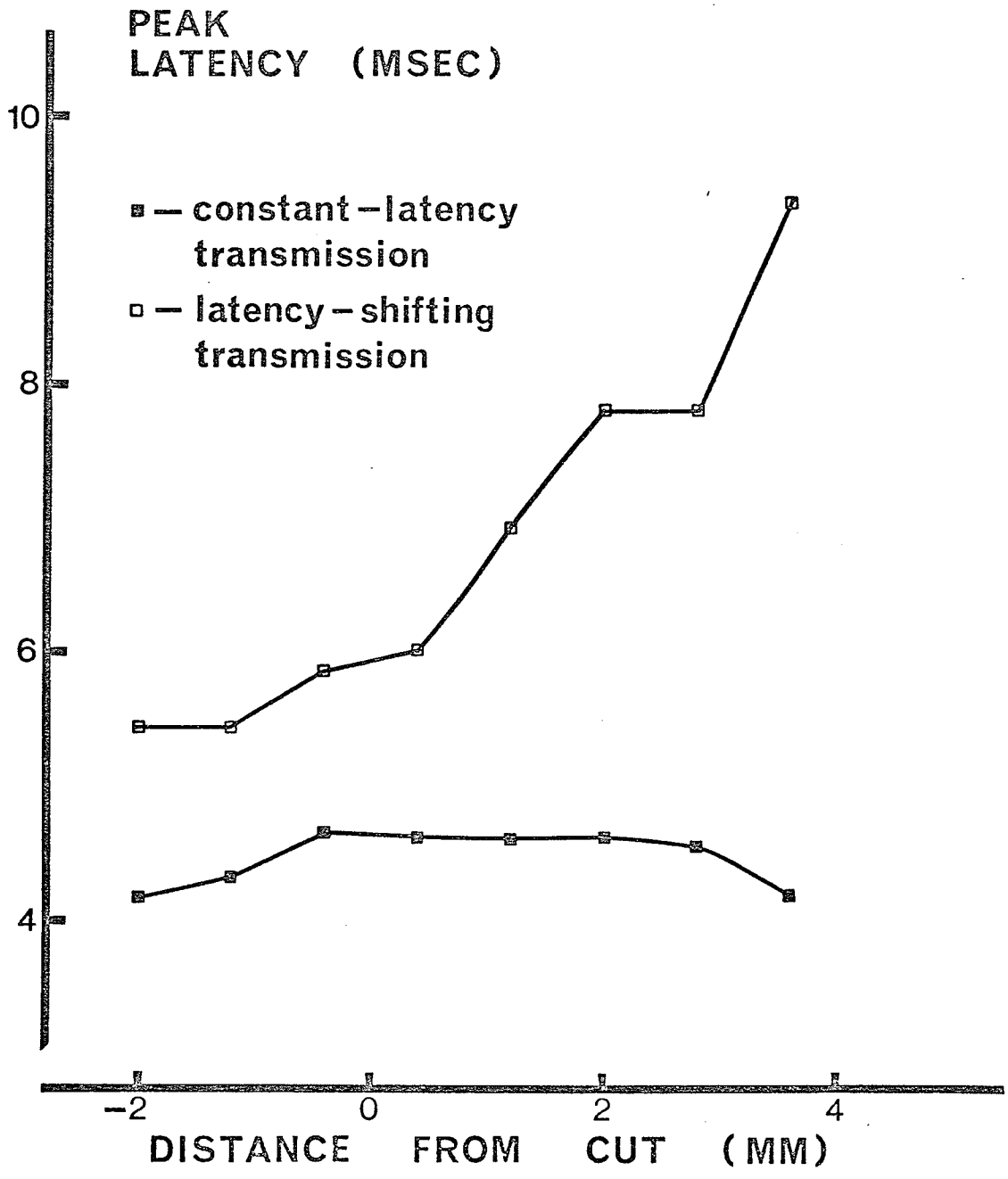


Fig. C-23. Plot of peak latency against distance for data in Fig. C-19 (example of constant-latency transmission) and Fig. C-20 (example of latency-shifting transmission). Responses measured by Method #1.

a complete cut (trials in which the stimulus was applied 0.4 mm from the cut are not included).

3) Comparison of the rates of nDCR decline for the two types of transmission across cuts

Since these two types of transmission differ in the way peak latency varies with distance, it was thought that perhaps their rates of nDCR decline with distance would also show a difference. The trials from each type of transmission were normalized by using the record at 0.4 mm from the cut (on the stimulated side) as the standard and then averaged. These averaged results are plotted in Fig. C-24. No statistically significant difference, either in the degree of transmission across the cuts or in the rate of nDCR decline on the non-stimulated side of the cuts, was found between the latency-shifting type of transmission and the constant-latency type of transmission.

The mean rate of nDCR decline ($\bar{s}$) on the non-stimulated side of the cut was also calculated for the two types of transmission. Expressed in units of log mV/mm, the mean rate of decline is -0.88, $\pm$ 0.06 for the latency-shifting type of transmission and -0.83, $\pm$ 0.06 for the constant-latency type of transmission. These rates are not statistically different from one another.

c) Reinforcement regions and cuts

Reinforcement regions were never found to occur beyond a complete cut and were observed in only 2 out of 22 trials beyond a cut 0.5 mm deep. These two trials were done on the same slab, one with the stimulating electrodes 1.2 mm from the cut and the other with the stimulating electrode 2.0 mm from the cut. In both these trials the reinforcement region occurred in the same region of the slab. Trials taken before the cut had been made revealed the presence

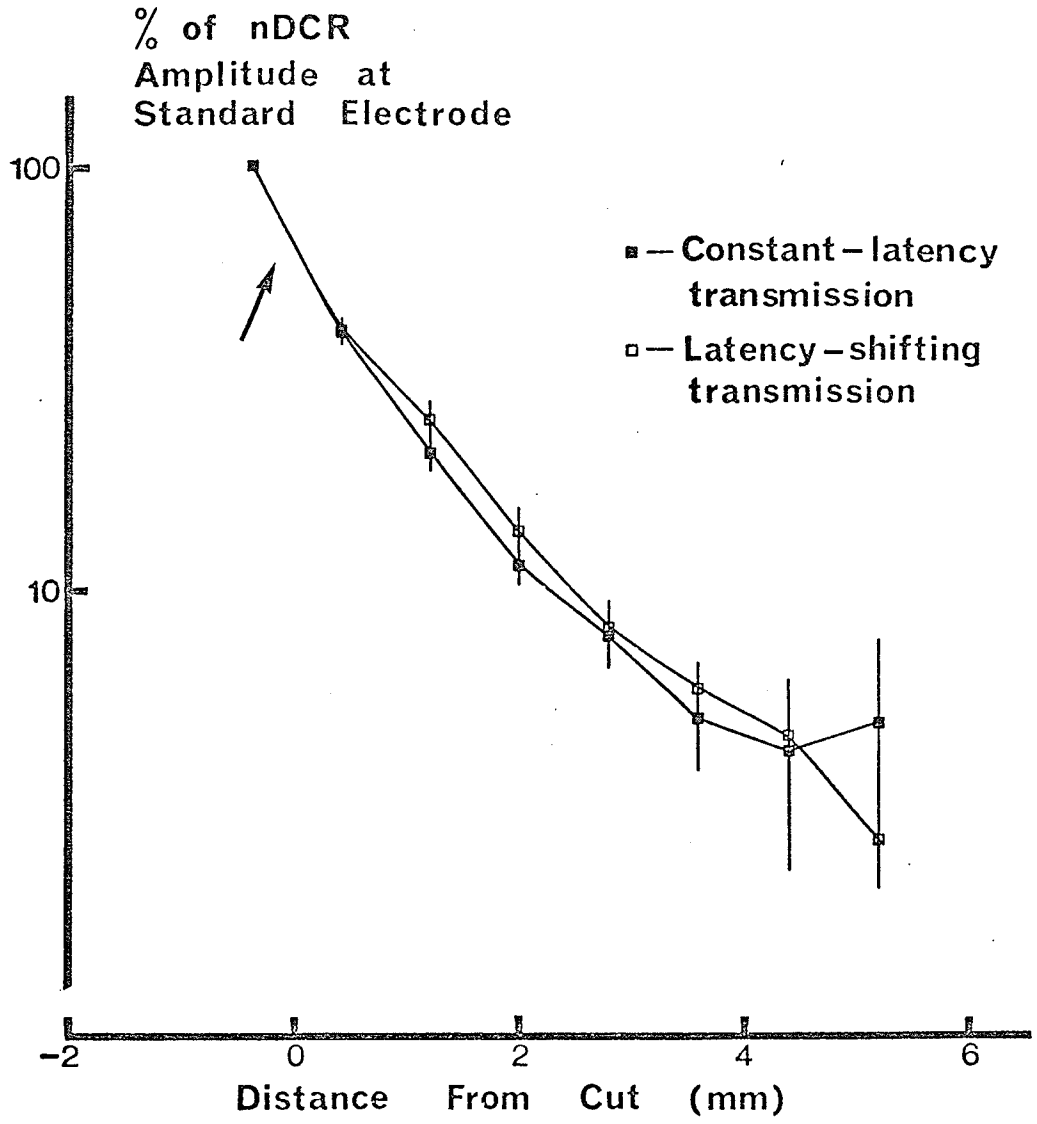


Fig. C-24. Comparison of amount of transmission across cuts between constant-latency and latency-shifting types of transmission. No significant difference in transmission is found.

of a reinforcement area whose location coincided with that observed after the cut except that the latter did not extend as far. Fig. C-25 shows the plot of log peak amplitude vs. distance for the same stimulus location before and after the 0.5 mm cut was made.

IV. Relationship between response size and stimulus strength at different distances from the stimulus site

Three experiments were done to test the relationship between response size and stimulus strength at different distances from the site of stimulation. The stimulus strengths used were those which lay between the threshold for the nDCR and the threshold for the late positive wave. All these experiments showed the response size to be an essentially linear function of the log of the stimulus strength. They also showed that the slope of the log stimulus-response curve is steepest at the site of stimulation and decreases in size as the distance from the stimulus site increases. Figs. C-26 and C-27 are graphs of the results of two of these experiments. They show the relationship between response size and log of the stimulus strength for distances up to 4.8 mm from the point of stimulation.

When the data shown in Figs. C-26 and C-27 are plotted on a log scale in the y-axis as well as in the x-axis, there is no longer a progressive decrease in the slope of the log stimulus-response curve with an increase in distance from the site of stimulation. This is shown in Figs. C-28 and C-29. The curves showing the relationship between log stimulus and log response are all approximately of the same slope for all distances tested and run quite paralled to one another. This indicates that a given increase

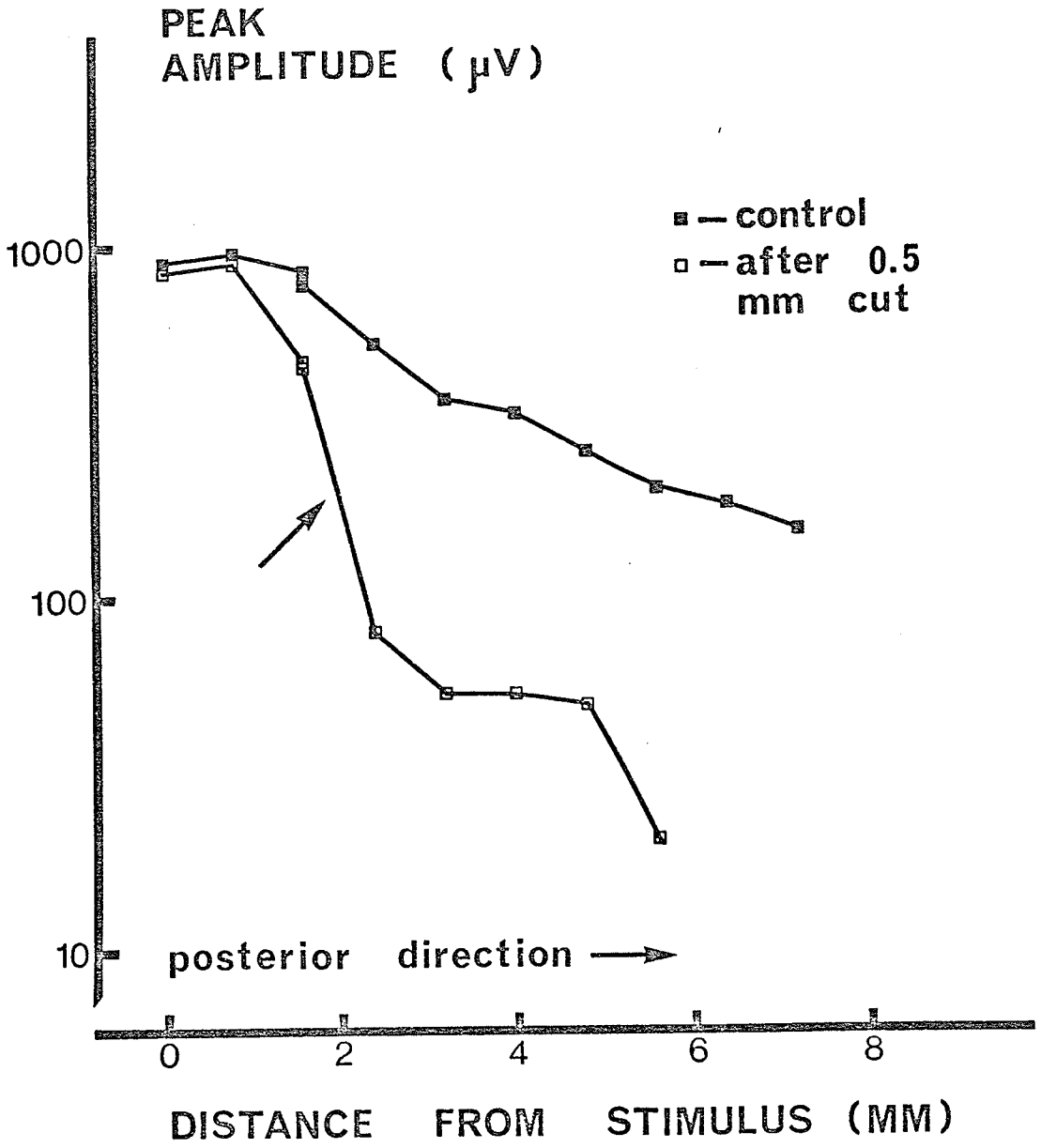


Fig. C-25. Trial showing presence of a reinforcement region beyond a cut 0.5 mm in depth. After the cut, the reinforcement region starts at the same location as control (i.e. at 3.2 mm) but extends only 1.6 mm.

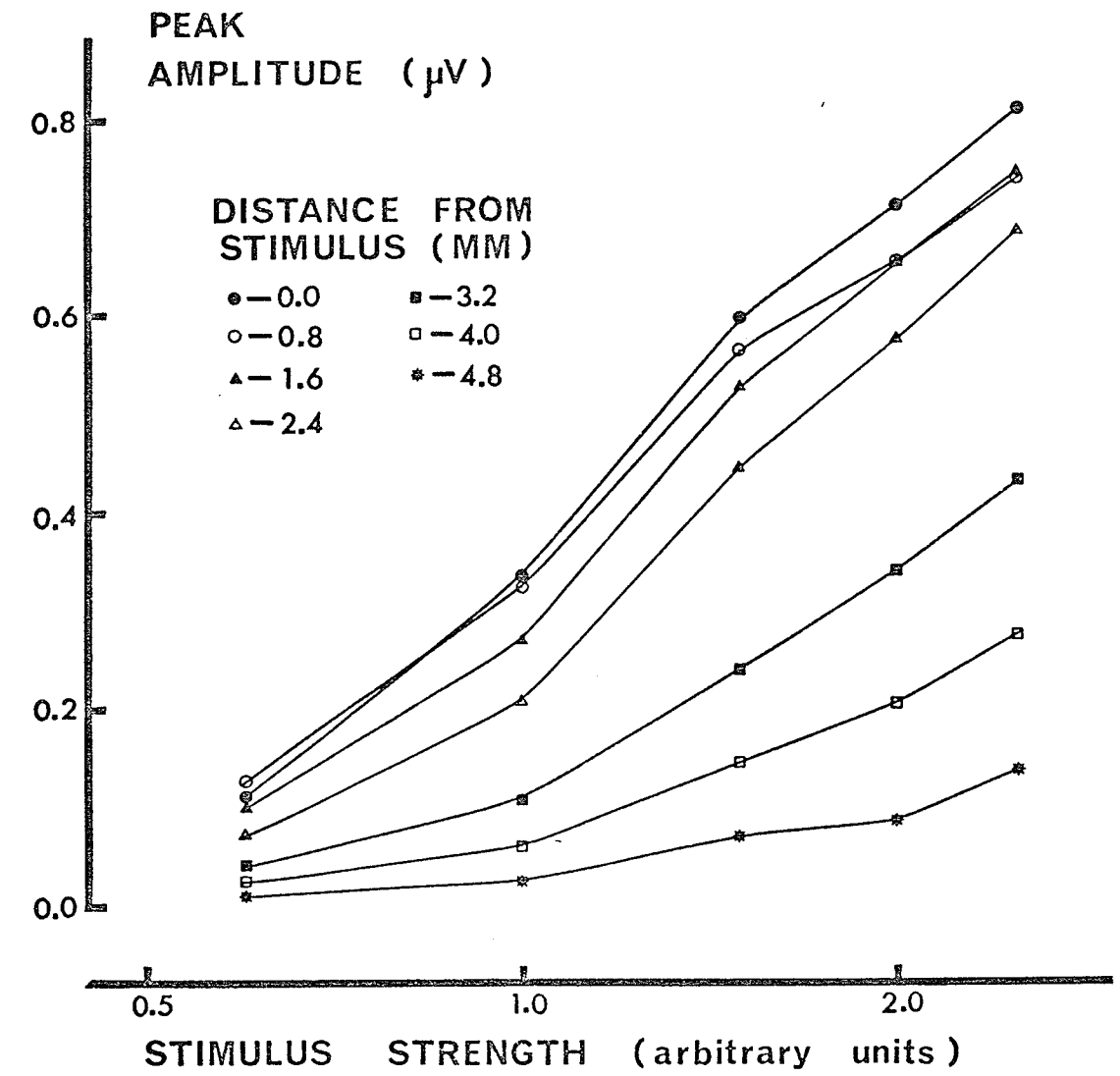


Fig. C-26. Example #1 of response size vs. log stimulus strength for several distances from the stimulus site.

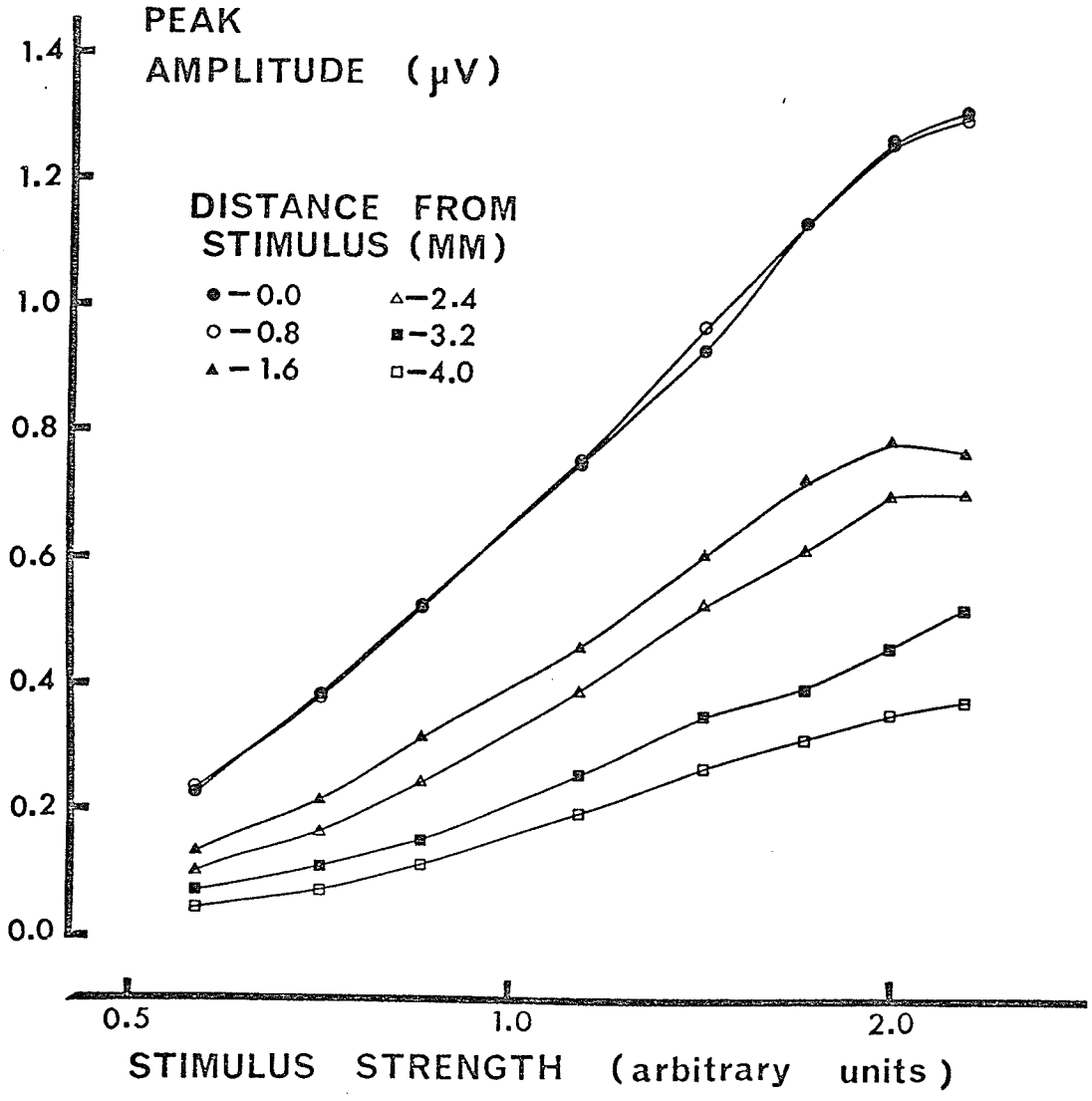


Fig. C-27. Example #2 of response size vs. log stimulus strength for several distances from the stimulus site.

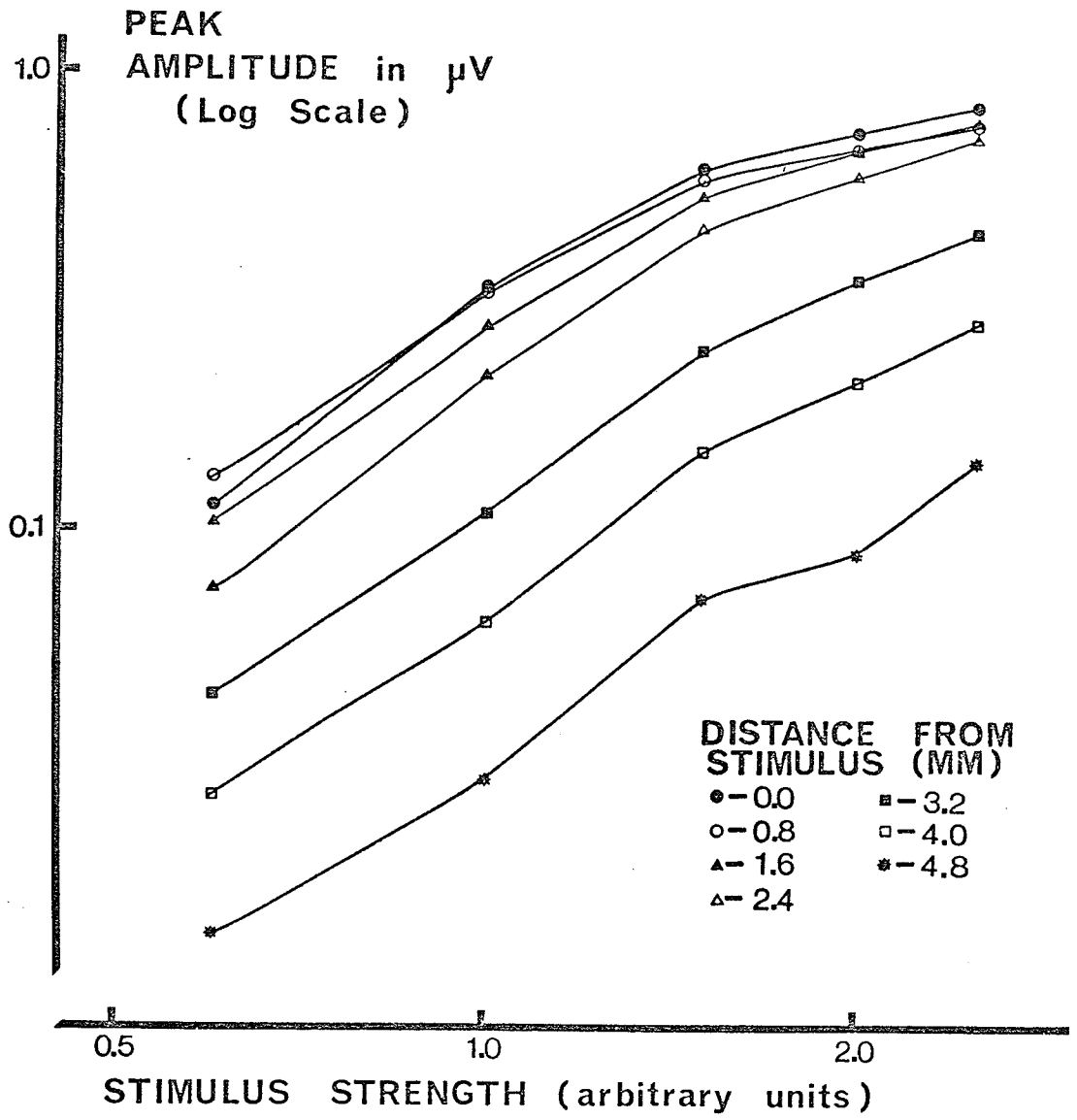


Fig. C-28. Example #1 of log response size vs. log stimulus strength for several distances from the stimulus site. (Data taken from same trials as those in Fig. C-26.)

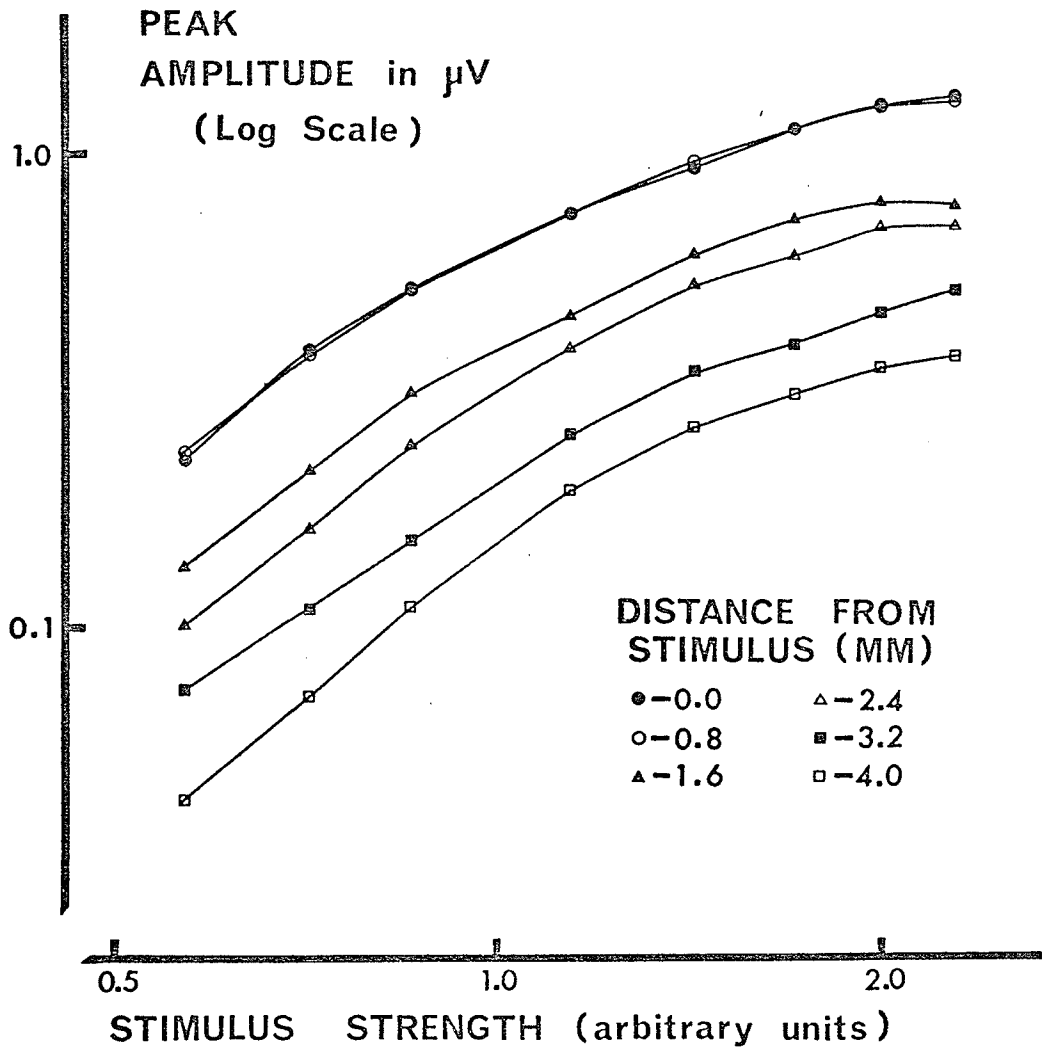


Fig. C-29. Example #2 of log response size vs. log stimulus strength for several distances from the stimulus site. (Data taken from same trials as those for Fig. C-27.)

in stimulus strength causes the response to increase in size in the same proportion at all distances (at least up to 4.8 mm) from the stimulus site. Hence, it can be deduced that the nDCR at all distances up to a minimum of 4.8 mm from the stimulus site is produced either by a single system, or by multiple systems each having the same functional relationship to the stimulus strength. If several systems are involved, they could be either systems which are stimulated in parallel or else systems arranged in series and coupled to one another by links which transmit activity in linear fashion from one system to the next. If the systems are in parallel, they must all respond with the same relationship to stimulus strength. If they are in series, the relationship of the directly stimulated system to stimulus strength will be the same as those of the indirectly activated systems since the relationship will be passed on unmodified to the systems which are activated by the directly stimulated system.

V. Paired-pulse experiments at long time intervals and the effect of tetanic stimulation on nDCR excitability

The technique of response averaging provided an excellent opportunity to determine how long it takes for the cerebral cortex to recover completely after a nDCR, because this technique enables the measurement of small differences between control and test responses with relative ease. Four experiments were performed to test the amount of recovery of nDCR excitability at intervals between 0.1 sec. to 10 sec. after a control response. The results are expressed as a percent depression of the test response compared to the control response (see Methods) and are plotted on a log scale in Fig. C-30.

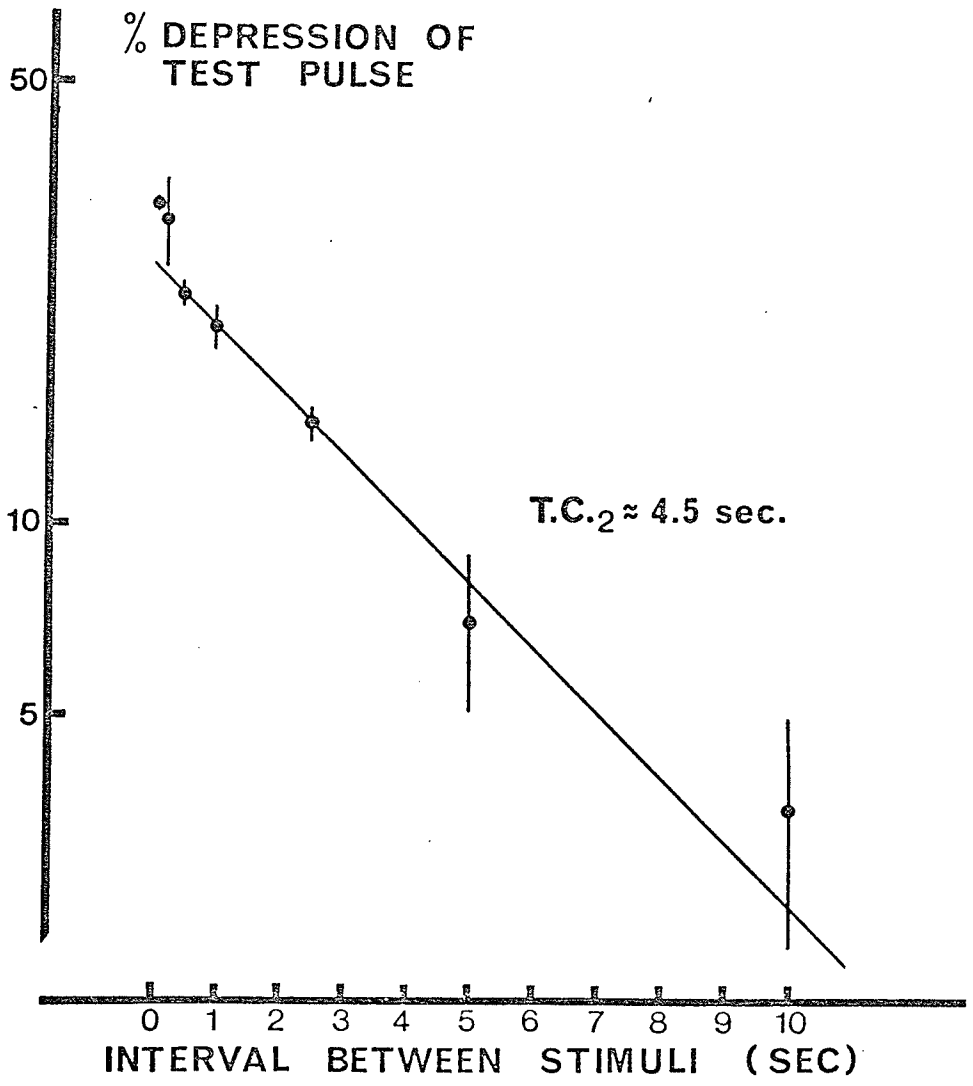


Fig. C-30. Test pulse depression (see text).

The curve in Fig. C-30 appears to be composed of two distinct components of test response depression, a steep component at the very short intervals (0.1 - 0.5 seconds), and a component of slow fall-off rate at the longer intervals. The straight lines through the points were fitted so as to make the lines pass through the standard error bars of each datum point. This gives an approximate measure of about 4 to 5 seconds for the time constant of the second component. Since there are only 2 datum points within the interval where the first component is active, subtracting the second component from the first would not help in calculating the time constant of the first component. However, since the first component appears to be finished by 500 msec., a reasonable estimate of its time constant would be approximately 100 to 200 msec.

The percent depression of the nDCR was also measured over the same intervals as above following a 10 per second tetany of 10 seconds duration. These results are plotted in the upper graph of Fig. C-31. There is no indication of any post-tetanic potentiation over the intervals measured. In fact, the entire curve shows more depression than that of Fig. C-30 for corresponding intervals between responses. The time constant of the second component is measured to be approximately 9 seconds. This component was subtracted from the datum points of the 4 smallest test intervals and the resulting values were plotted in the lower graph of Fig. C-31. This gives an approximate measure of the time constant of the first component of depression. It was found to be about 300 msec. Hence, the amount of tetanic stimulation used caused both time constants of the two components of depression to be approximately doubled in

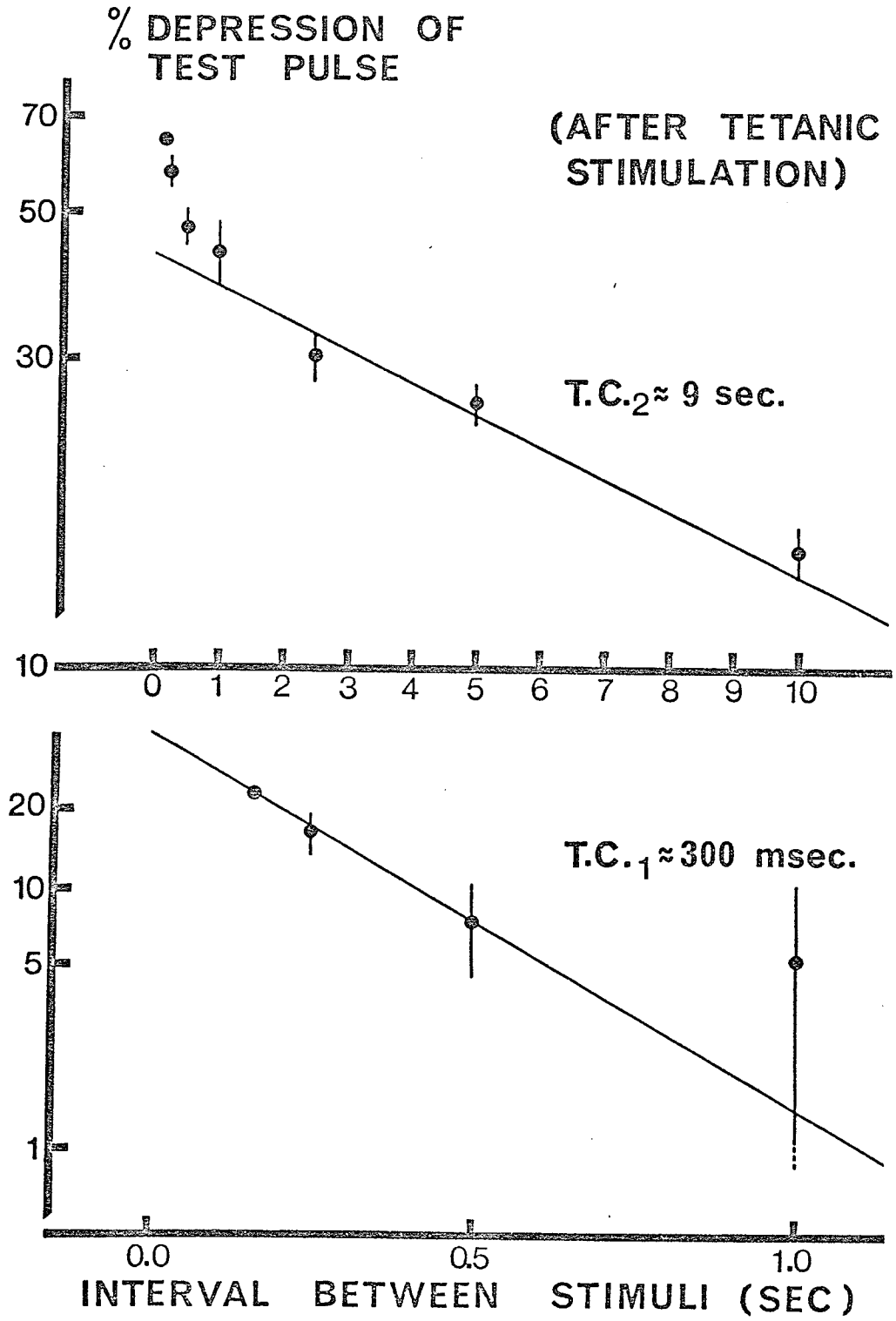


Fig. C-31. Test pulse depression after tetanic stimulation (see text).

length.

An increase in the magnitude of the two components of depression is what is to be expected if the depression is additive to any degree. Any interval following a tetany is really an integration of several intervals since the test response follows not one but several responses which occur very close together in time. However, the fact that the time constants of both components underwent definite changes in length indicates that the mechanisms underlying each component have been considerably altered by the repetitive stimulation of the tetany. If the properties of these mechanisms had remained unchanged by the tetany, only the magnitude of the depression would have been increased (due to summation of the effects of stimuli presented in rapid succession) and not the time constants of the components.

D. DISCUSSION

I. Attempt to correlate anatomical knowledge with the results

a) General discussion

The molecular layer of the mammalian cerebral cortex is composed of densely packed axons and dendrites. Both axonic and dendritic populations are composed of subpopulations which can be distinguished anatomically on the basis of cell origin. For instance, the dendritic population in the molecular layer derives chiefly from the apical dendrites of pyramidal cells, but these cells come from several different layers of the cortex. Each layer of these cells may be considered, at least on anatomical grounds, to contribute a subpopulation of dendrites to the molecular layer. Such a subpopulation may or may not perform a unique function which would distinguish it from the other subpopulations on physiological grounds as well. This issue will be decided only by future experiments.

Axons in the molecular may be categorized into subpopulations also on the basis of their anatomical origin. As discussed in the INTRODUCTION some of these are known to be (i) axons from the sixth layer fusiform cell, (ii) axons from corticocortical afferents, and (iii) axons from callosal afferents. Recurrent axons of pyramidal cells are believed to extend only to the apical arches of pyramidal cell dendrites (Szentágothai, 1965b; Scheibel and Scheibel, 1970). Hence they would mainly terminate in layer II, just below the molecular layer.

There is no anatomical proof of the extent of tangential spread in any one of these axonal subpopulations. However, it is

highly unlikely that they all travel for the same distance within the molecular layer. At least one of these subpopulations travels for distances up to 7 mm (Jones and Powell, 1968); other subpopulations travel much shorter distances than this.

Although Jones and Powell (1968) did not find any degenerating terminals beyond 7 mm from the edges of cortical lesions, it is quite possible that more advanced histological techniques may in future reveal the presence of even longer axons in the molecular layer. For this reason it is postulated that very long axons in the molecular layer are the transmitting elements for the nDCR at distances even beyond 7 mm from the site of stimulation.

b) Reinforcement regions

A reinforcement region is one where the rate of nDCR decline becomes less than in a region closer to the site of stimulation (see Results). Occasionally, the nDCR amplitude even increases with distance within this region (see Fig. C-3).

The reduction in rate of nDCR decline within a reinforcement region could be accounted for if there were a greater proliferation of axonal branches in such a region than in regions immediately preceding and following it. A large amount of branching would increase the synaptic density of the region, thus enabling a stronger response to be produced.

Brooks and Enger (1959) postulated that the reinforcement regions they observed may have been due to corticocortical axons, recurrent collaterals of pyramidal cells, axons of stellate cells, or a mixture of these. However, such explanations are unlikely for 2 reasons: (i) the long pathways which these explanations

require probably preclude the arrival of a reinforcing excitation early enough to account not only for a reduced rate of nDCR decline but also for an increased apparent "velocity", and (ii) excitation coming from lower regions of the cortex would likely cause the nDCR to be preceded by a positive wave within a reinforcement region, such as occurs in the response to pyramidal tract stimulation (Humphrey, 1968). As can be seen from Fig. C-1, no such preceding positive wave is needed for a reinforcement region to occur.

An unexpected finding in this research was that the reinforcement regions of nDCR spread were not a function of distance from the site of stimulation but were "locked" to specific locations on the gyrus itself (see Figs. C-12, C-13, and C-14). Reinforcement regions tended to occur at the same location on the gyrus independently of where the stimulating electrodes had been placed. Corollary to this, regions of steep rate of nDCR decline which immediately precede and follow a reinforcement region also occur at constant locations on the gyrus independent of the location of the site of stimulation.

Fig. C-12 shows two different locations of reinforcement regions (areas B and D) which are separated from one another by an area of steep rate of nDCR decline (area C). These results suggest a definite structural organization of the molecular layer of the cortex which consists of areas of extensive axonal branching separated from one another by areas of sparse axonal branching.

c) Asymmetry of nDCR spread

The finding that the nDCR does not spread symmetrically about the site of stimulation suggests the existance of a sub-population of elements which have a geometrical orientation in the molecular layer. This is not too surprising since examples of elements having a specific orientation have been found histologically in other regions of the cerebral cortex. For instance, Colonnier (1964) found that stellate cell dendritic trees in the cat have their long axes oriented mainly in the anteroposterior direction of the gyrus lateralis.

Although histological evidence is lacking, we may deduce something about the elements responsible for producing asymmetry of nDCR spread by locating the area (with respect to the site of stimulation) where the asymmetry appears to be produced. Examination of Fig. C-16 shows that almost all of the asymmetry is produced within the first 3 mm of the stimulus site, the greatest asymmetry being produced at about 1 mm from the stimulated point. Since apical dendrites in the molecular layer probably do not extend beyond a few hundred microns (Scholl, 1956) the elements which produce the asymmetry are likely to be axonal in nature. One such element might be an axon which bifurcates with an anterior-posterior orientation in the molecular layer into 2 unequal portions. The portion lying in the direction of greater response amplitude would run for about 2-3 mm and branch more extensively than the other portion. This element does not appear to be related to either reinforcement regions or to regions of fast rate of nDCR decline since asymmetry

of nDCR spread occurs in all trials regardless of the location of the stimulating electrodes.

The correlation between the location of the visual association areas and the location of regions of increased probability of occurrence of anterior directionality (see Results) suggests that at least one of the elements responsible for asymmetry of nDCR spread may be a cortical afferent which is linked to one or other sensory modality. This element might well be a cortical afferent either from the brainstem or from a primary sensory area of the cerebral cortex.

Regardless of what the postulated element might actually be, if histology could reveal the presence and identity of some structure in the molecular layer to account for the observed asymmetry in nDCR spread, this would furnish excellent evidence for linking, at least in part, the production or transmission of the nDCR to a specific neural element.

d) Response size as a function of stimulus strength

Three experiments were done in which the amplitude of the nDCR was determined as a function of stimulus strength at different distances from the site of stimulation (see Results). These experiments were done to see if a separation of systems generating the nDCR could be achieved on the basis of differences in their functional relationship to the stimulus strength. If these systems were present in different proportions at different distances from the site of stimulation, then a unique relationship of response size to stimulus strength would be expected at one or other distances from the stimulus site.

As was seen in Figs. C-28 and C-29 no such differences were found within the accuracy of the method. The curves relating the log of the response size to the log of the stimulus strength were essentially parallel to one another for all distances from the stimulus site up to 4.8 mm. These results seem to indicate that there is one major system involved in nDCR transmission which determines the relationship between response size and stimulus strength at distances up to 4.8 mm from the site of stimulation. This could mean that a system of long axons is the predominating element at all distances (even at short distances) from the site of stimulation.

Alternatively, if unlike systems give rise to the nDCR at different distances from the stimulus site, the difference in the functions which relate each of their responsiveness to stimulus strength might not be large enough to be noticed with the technique used in this study.

e) Cuts

There was no significant difference in the degree of nDCR transmission across a cut 0.5 mm deep as compared to a complete cut. This was true for all the distances (0.4, 1.2, 2.0, 3.6, and 5.2 mm) of the stimulating electrodes from the edge of the cut (see Fig. C-18). It would appear from this that there are no intracortical or subcortical pathways below 0.5 mm which are needed either for tangential spread of the nDCR or for reinforcement regions. The results in the experiment where a reinforcement region appeared beyond a cut (Fig. C-25) were likely due to very superficial axons which happened to be missed in the cutting procedure (see below).

The presence of a response on the unstimulated side of a cut, seen in 43 trials on 8 cats, requires an explanation. Such a response could be present there either as a volume-conducted response or as an active response. The latter could occur as a result of (i) stimulus current spread, (ii) uncut fibres, or (iii) ephaptic transmission.

In several trials (N = 23) with both 0.5 mm-deep cuts and with complete cuts, a response occurred across the cuts which did not show an increase in peak latency with distance from the cut (constant-latency transmission). Because of this and because the response had a very steep rate of decline, the nDCR on the unstimulated side of the cut was most likely present as a volume-conducted response. The rapid rate of decline ($\bar{s}$ = -0.83 log mV/mm) probably represents a good measurement of the space constant (= $-1/\bar{s}$) of the nDCR in the cerebral volume-conductor. This yields a value of about 1.2 mm which is in good agreement with that (1.5 mm) found by Brooks and Enger (1959) for the space constant of a stimulus artefact on the pial surface of cat brain.

The latency-shifting transmission (see Results) which occurred across cuts (both 0.5 mm deep and complete) is a little more difficult to explain. This response must have an active component since it undergoes an increase in peak latency with distance from the cut. Spread of stimulus current across the cut is unlikely to have caused this active component because there was evidence (see Results) that the stimulus current probably does not reach 1.2 mm beyond the stimulating electrodes. The trials showing latency-shifting transmission all had their stimulating electrode 1.2 mm or more from

the cut.

Because of the great difficulty in cutting the fibres which lie immediately under the pial membrane, it is very likely that some axons may have not been severed by the cutting procedure (see Methods). This would be true for both types of cuts but especially for the cuts 0.5 mm deep since the cutting surface of the flattened bead was more rounded than that of the edge-cutting knife. Also, it was more difficult to manipulate the bead since great care had to be taken so as not to cut lower cortical areas. Hence, the cause of the shift in latency for the latency-shifting transmission is most likely due to a very few superficial axons which did not get cut. This would be in accord with the observation of Jones and Powell (1968) that the axons which travelled up to 7 mm from a lesion, occurred in the most superficial part of the molecular layer. The failure to demonstrate a significant difference in the amount of nDCR transmission between the constant-latency and latency-shifting types of transmission across cuts (see Fig. C-24) indicates that the number of axons which might have survived must have been very small.

The possibility of ephaptic transmission across cuts cannot be entirely discounted, even though there is little evidence that this type of transmission is significant in normal cerebral activity. The size of the field currents generated by the elements producing the nDCR is much smaller than that generated by the stimulus pulse, and this latter does not seem to be effective in directly activating elements even as close as 1.2 mm away from the stimulating electrodes. On the other hand, the response on the stimulated side can travel right to the edge of the cut and therefore is in close proximity to

elements on the unstimulated side of the cut. If these elements were very sensitive to field currents, they might be affected by the responding elements on the stimulated side of the cut. For such reasons, the possibility of ephaptic transmission cannot be entirely discarded unless more evidence is made available to the contrary.

II. Theoretical Model of Cortical Organization

A hypothetical model of cortical structure (see Fig. D-1) has been proposed here in the light of the foregoing discussion. An attempt has been made in this model to account for the occurrence of reinforcement regions as well as for the regions of steep rate of nDCR decline. Included also are elements believed to be responsible for producing nDCR asymmetry.

Axon a_1 in Fig. D-1 is postulated in this model as the major element responsible for cortical organization leading to reinforcement regions. This axon is shown to arise from a fusiform cell in layer VI. Although still quite speculative, this is based on Szentágothai's observation that most of the axons in the molecular layer are derived intracortically from layers V and VI(Szentagothai,1965 a and b). The main feature of this axon is that it travels for very great lengths (up to 10 mm or more) and gives rise to extensive branching at one or more specific gyral locations along its course (2 areas of extensive branching are shown to arise from axon a_1 in Fig. D-1). Between these areas of profuse branching there are areas in which the amount of branching from axon a_1 is either absent or very sparse. The areas of extensive branching are postulated to give rise to reinforcement regions and the areas of sparse branching are postulated to give rise to the areas

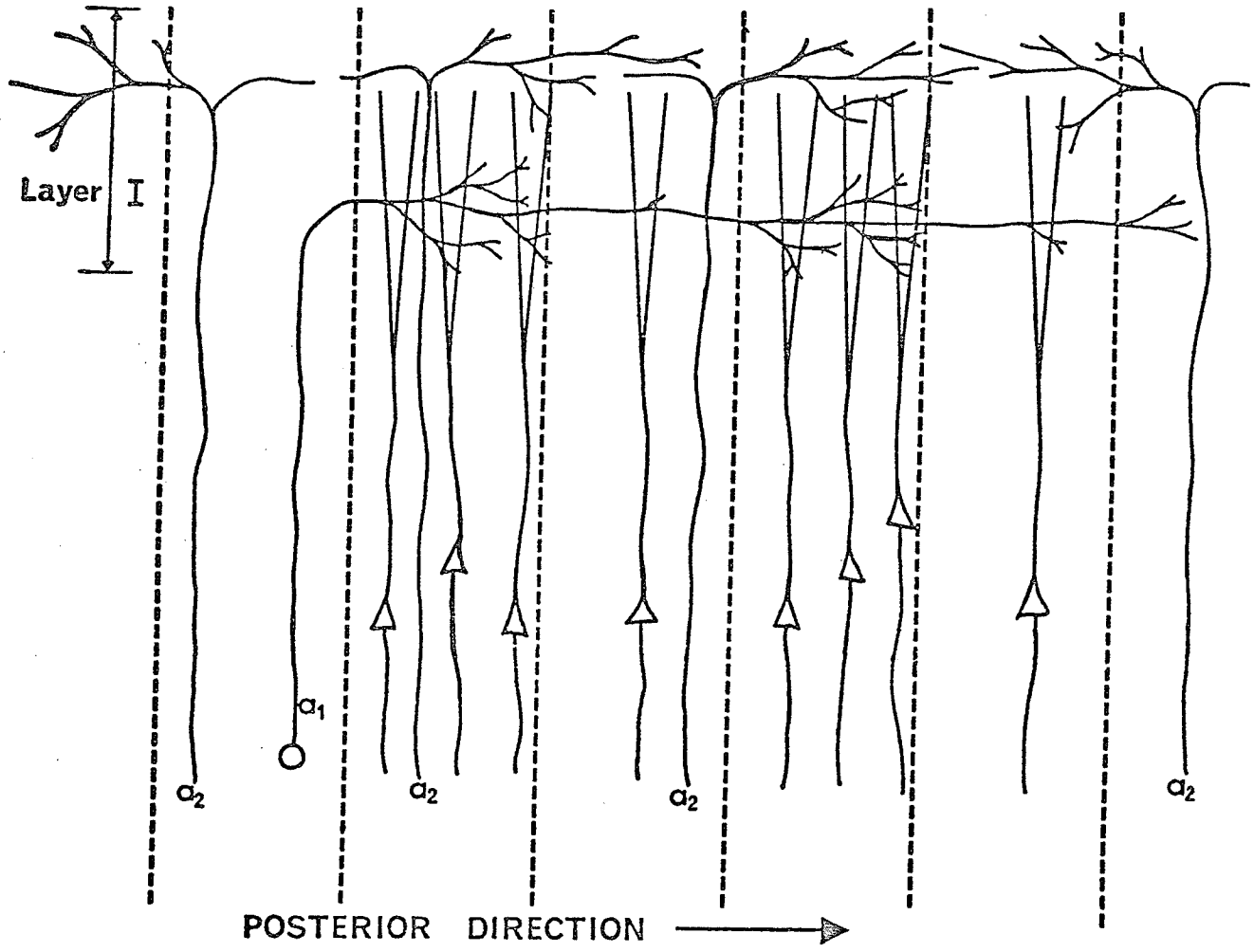


Fig. D-1. Theoretical model of cortical organization (see text for explanation).

of steep rate of nDCR decline which precede and follow reinforcement regions.

Because of its length, axon a_1 is postulated to be also the main element in the mechanism for the observed relationship between stimulus strength and response amplitude. This was seen to be the same for all distances up to at least 4.6 mm from the site of stimulation (see Results; Figs. C-28 and C-29). The nDCR amplitude depends, in this model, mainly on how many of these long axons have been activated by a given strength of stimulation.

More pyramidal cells are shown to be present in the reinforcement areas than in the areas of steep rate of nDCR decline. This was done simply to illustrate that the more extensive axonal branching in the reinforcement areas will lead to a greater number of activated apical dendrites than in the areas of steep rate of nDCR decline.

Finally, the elements responsible for asymmetry in the nDCR spread are represented by cortical afferent fibres (axons a_2). These ascend to the molecular layer where they bifurcate into two unequal portions which travel in opposite directions. One portion is 2-3 mm long and possesses a great number of branches; the other portion is branch-poor. These a_2 axons are present in all areas and are shown to have a posterior orientation in the middle regions of the diagram. At either end of the diagram they have been given an anterior orientation. This was done to illustrate the correspondence of posteriorly oriented asymmetry with the middle regions of the suprasylvian gyrus and the partial correspondence of anteriorly oriented asymmetry with the rostral and caudal portions of the isolated slab

(see Results).

III. Utilization of nDCR patterns of tangential spread

The non-homogeneity of the cerebral cortex has time and again been demonstrated histologically (cf. Colonnier and Rossignol, 1969). Certain aspects of this non-homogeneity have, in this thesis, now been demonstrated by the use of an electrophysiological technique, i.e., by examination of the spread of the nDCR from different sites of stimulation. The results of the experiments have shown that the cortex is organized into areas of nDCR reinforcement and areas of steep rate of nDCR decline. Reinforcement areas are separated from one another by areas of steep rate of nDCR decline.

These results suggest that it should be possible to create a "map" of the cerebral cortex based on the pattern which the spread of the nDCR reveals. The areas of reinforcement are very likely to be unequal in length in different regions of the cerebral cortex. Also, if the occurrence of reinforcement areas could be examined in two dimensions (i.e., over a portion of the surface area in the plane of the pial membrane surrounding the site of stimulation) then irregularities in the boundaries of the reinforcement areas surrouding a given stimulus location might prove unique to a given

region of cerebral cortex. Assuming that such differences would be found in the great majority of animals of a given species, this information could be used to characterize different areas of the cortex. Then a given region of cerebral cortex could be accurately localized in an individual member of the species on the basis of the pattern of occurrence of reinforcement areas characteristic of that region. A somewhat crude beginning towards locating cortical areas by electrophysiological techniques has already been achieved in this thesis; i.e., posterior directionality in the asymmetry of nDCR spread seems to be a constant characteristic of the somatic association area in the cat (see Results). The presence of an admixture of anterior directionality appears to characterize the visual association areas of this species.

The advantage of the techniques suggested in the foregoing would be to increase the accuracy of localization of cortical areas using minimal stimulation strengths. This ability would be invaluable for future research involving the cerebral cortex. Perhaps it may have a use in the more distant future also for delicate surgical operations on the cerebral cortex in man.

IV. Paired-pulse and experiments with repetative stimulation

a) Components of test response depression

There is a certain amount of disagreement in the literature

regarding the behaviour of the test response in paired-pulse experiments with the nDCR. Ochs and his co-workers (Ochs and Booker, 1961; Ochs and Clark, 1968b) find three separate periods in which the test response had a different excitability than the control response. The first of these was a period of facilitation lasting up to 5 msec after the control stimulus had been presented. This was followed by two periods of test response depression, the first of which lasted from 20 to 40 msec after the control stimulus. The second period of depression was seen only with strong stimulation and their records (Ochs and Clark, 1968b; their Fig. 1) show that this period of test response depression was maximal only with stimulation strengths large enough to elicit the prolonged second negative wave (cf. Chang, 1951). This second period of nDCR depression lasted 200-300 msec after the control stimulus (Ochs and Clark, 1968b). Other workers have had rather markedly different results. Kandel et al. (1958) found a period of test response augmentation lasting 10-150 msec after the control stimulus. Merlis (1965) found a period of augmentation of similar duration (0 to 5 msec after control stimulus) of that of Ochs and co-workers but found a period of depression lasting up to 500 msec. Part of the difference in results may be explained by the different anesthetic used by the various workers. However, there is some disagreement even here. Kandel et al. (1958) used succinylcholine and Merlis (1965) used curare to immobilize their animals. When they tested the effects of nembutal on the excitability of the test response, the former found a decrease in their period of augmentation (to about one-third control) and the latter found an increase in the period of augmentation. These workers also disagreed on the

effects of stimulus strength. Kandel et al. (1958) reported that higher stimulus strengths increased the degree of potentiation of the test pulse whereas Merlis (1965) reported that increasing the stimulus strength shortened the augmentation period.

The work of Berry and Hance (1965) may provide some answer to these peculiar differences. They found (with curarized cats) that the pattern of test response behaviour was a markéd function of the cortical area sampled. The medial suprasylvian gyrus showed a period of depression lasting about 30 msec followed by a short period of slight enhancement at about 40-50 msec after the control stimulus. The anterior sigmoid gyrus showed a period of depression lasting up to 25 msec followed by a period of marked enhancement lasting up to 400 msec after control stimulus. Long periods of enhancement (up to 400 msec) were also found for the lateral and ectosylvian gyri. These authors found that pentobarbital depressed the test response. In the medial suprasylvian gyrus, this anesthetic caused the period of slight enhancement to be replaced by a period of depression. Test response depression now had two components -- the first lasting about 30 msec, and the second lasting beyond 400 msec (from their Fig. 5). Neuronally isolated cortical slabs in the medial suprasylvian showed a similar two-component period of test response depression (from their Fig. 6). The first component lasted about 30 msec and the second lasted more than 400 msec (but less than 800 msec) after the control stimulus. Pentobarbital was not found to have any effect on test response excitability in the isolated islands.

The results presented in this thesis, done on decerebrate cats without anesthetic, show a two-component period of test response

depression. The first component has a time constant of about 100-200 msec and appears to be completed by 500 msec after the control stimulus. The second component has a much longer time constant of about 4 to 5 seconds and is still not completely finished even at 10 seconds after the control stimulus.

Because of its similar time course, the first component is likely to be equivalent to the second period of test-pulse depression observed by Ochs and Clark (1968b). These workers postulated this period of depression to be due to synaptic inhibition on the uppermost apical dendrites. They believe that the axons which cause this inhibition are present in the molecular layer since stimulation on the opposite side of a cut which leaves only the molecular layer intact, still produces this period of test response depression.

The second component of depression reported in this thesis has a time constant of 4 to 5 seconds and appears to be without parallel in previous work done with the nDCR. However, Nakahama (1959) noted that the long lasting positive-negative wave sequence (his A_1 and A_2 waves) which occurs in the somatic sensory area #2 in response to a stimulus in somatic sensory area #1 has a period of post-stimulus depression which does not fully disappear until about 20 seconds after stimulation. Whether or not similar elements are active in this response as in the nDCR is strictly a matter of conjecture right now. The mechanism of depression, however, is probably the same as the second component of nDCR depression since it has a similar time course.

It seems rather unlikely that synaptic activity (i.e., inhibitory synapses) could be responsible for this long-lasting

depression. It is unlikely that neuronal activity associated with the weak stimulus used here would extend beyond a second after stimulation. There is no field potential visible this long after the stimulus and no reports have been made of any activity detectable with a microelectrode in the post-stimulus period. A more likely explanation for the long-lasting depression would be one which is more biochemical in nature. For instance, the depression may be caused by depletion of readily-releasable transmitter, by inefficient removal of transmitter substance from the synaptic cleft, or by inability of the membrane pumps to restore ionic equilibrium in the molecular layer elements to its previous state. Both presynaptic and post-synaptic elements are of very fine diameter and have very dense packing in the molecular layer neuropil. The high packing density may lead to much competition for metabolites which cannot be supplied rapidly enough either by axoplasmic flow through narrow caliber fibres or from the molecular layer blood circulation.

Whatever the cause of the long-lasting depression, its long time course makes it tempting to consider its possible involvement in the process of short-term memory. A slight reduction in excitability in a given distribution of neurons may be interpreted as information by the cortex. The information would be present as long as the reduction in excitability persisted above a critical level.

b) Effects of tetanic stimulation

Tetanic stimulation had the effect of increasing the time constants of both components of test pulse depression. There are at least three possibilities which could explain this: (i) limited availability of necessary metabolites, (ii) buildup of byproducts

of axonic and dendritic activation, and (iii) buildup of inhibitory transmitter.

Certain constituents (e.g., adenosine triphosphate, synaptic transmitter) might be in short supply and become partially depleted by tetanic stimulation. In such instance, the increase in the time constants of the two components of test pulse depression might reflect the time constant of the restorative processes (e.g., synthesis, transport, and/or storage of necessary metabolites or precursors).

Byproducts of axonal and dendritic activation also might cause the increase in the time constants of test pulse depression. For example, repetitive stimulation might lead to an excess of K^+ in the extracellular space of the neuropil or to an excess of Na^+ within axonic and dendritic elements. Also, the extracellular concentration of synaptic transmitter may build up to a rate faster than that of the removal processes (e.g., uptake, metabolic breakdown, diffusion into the circulation) can handle it.

Buildup of inhibitory transmitter is also a possibility which cannot be ignored. Although the presence of inhibitory synapses has not yet been conclusively demonstrated in the molecular layer, these may be present in quantities sufficient to permit increases in extracellular concentration of inhibitory transmitter upon repetitive stimulation. The removal of this excess transmitter may be relatively slow.

The ultimate separation of the causes of component 1 and component 2 test pulse depression, and the enhancement of these by tetanic stimulation, will be accomplished only by further experimentation. Due to the close proximity of the molecular layer to the pial

surface, the nDCR could be an excellent model for studying the pharmacological and biochemical properties of neuropil in general. This response also has an advantage in being monophasic in form when the correct stimulation parameters are chosen.

E. CONCLUSIONS

The following conclusions may be drawn from the results obtained in this study:

(1) The nDCR travels in a tangential direction for distances of 10 mm or more from the site of stimulation.

(2) The logarithmic rate of nDCR decline with distance of the nDCR peak are non-uniform over the length of nDCR tangential spread. There occurred regions of slow and regions of fast rates of nDCR decline.

(3) Regions of slow rate of nDCR decline ("reinforcement" regions) tend to occur at the same location on the gyrus independent of the position of the stimulating electrodes. Corollary to this, regions of fast rate of nDCR decline which immediately precede or follow a reinforcement region also tend to be constant in their gyral locations, independent of the position of the stimulating electrodes. This suggests the existence of a functionally important spatial organization of the cerebral cortex extending beyond the limits of the vertical columns described by Mountcastle (1957) and Hubel and Weisel (1962).

(4) The tangential spread of the nDCR is asymmetrical about the point of stimulation. Responses have a greater amplitude and a shorter peak latency at electrodes located in one direction from the site of stimulation than at electrodes located the same distance away but in the opposite direction. In the suprasylvian gyrus, the directionality of asymmetry is mainly posterior (87 out of 110 trials).

(5) Almost all of the asymmetry in nDCR spread (with respect to peak amplitude) is produced within the first 3 mm of the stimulus

site. The greatest amount of asymmetry is produced at about 1 mm from the stimulus site.

(6) Directionality of asymmetry in nDCR spread is a function of stimulus location. In the middle regions of the suprasylvian gyrus (corresponding to the somatic association area) the asymmetry is always posteriorly directed. Regions nearer to either end of the gyrus (corresponding to visual association areas) show an admixture of anterior directionality.

(7) The tangential spread of the nDCR is along pathways in the upper 0.5 mm of the cerebral cortex.

(8) The transmission of the nDCR across cuts (both 0.5 mm deep and complete cuts) is mainly due to volume conduction of the response. For those trials in which nDCR peak latency increased with distance across either type of cut, part of the response transmission is believed due to very superficial axonal fibres which were missed in the cutting procedure.

(9) The logarithmic rate of decline of the volume conducted response is about -0.83 log mV/mm. This is about twice the average rate of decline for the physiologically conducted response.

(10) The relationship between response size and stimulus strength appears to be the same for all distances up to 4.8 mm from the stimulus site.

(11) The nDCR has at least 2 components of test pulse depression in an isolated slab of suprasylvian cortex: the first component has a time constant of about 100-200 msec and the second component has a time constant of about 4.5 seconds.

(12) Tetanic stimulation lengthens the time constants of both

components of test pulse depression. These changes are interpreted as alterations in each of the processes underlying the 2 components of test pulse depression.

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Note: The programs used in this thesis can be obtained either from the author or from Prof. E.W. Mazerall of the Dept. of Pharmacology and Therapeutics, Medical College, University of Manitoba, Winnipeg 3, Canada.