THE CURRENT-DISTANCE RELATION FOR REWARDING BRAIN STIMULATION

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ABSTRACT

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The ability to define the anatomical boundaries of brain regions that contain neural elements involved in the mediation of stimulation-induced behaviours has been impeded by the lack of information concerning the distance, from the electrode tip, over which electrical stimulation fires neurons. This experiment estimated the current-distance relation for rewarding brain stimulation through electrodes positioned in the rat lateral hypothalamus. Dual monopolar electrode assemblies were implanted in each rat; distances ranging from 0.30 to 0.66 mm separated the electrode tips in the lateral dimension. The rats were rewarded for lever depressions with 0.5 sec trains of cathodal pulse-pairs. The conditioning pulse was applied through one electrode and, after an adjustable delay, the test pulse was directed through the other electrode. In the low-amplitude assessments, all of the tested

delays between the pulses resulted in a constant level of excitation of the reward system, as measured by psychophysical scaling methods. In the high-amplitude tests, the effectiveness of the stimulation was greater at the long delays between the pulses than it was at the short delays. The highest tested amplitude that continued to yield constant levels of effectiveness across delays was taken as the current amplitude that resulted in adjacent stimulation fields with tangent boundaries. These amplitudes along with the corresponding interelectrode distances were used to estimate the constants of an equation which describes the current-distance relation. The obtained equation was: $I - 100\,\mu A = (1500\ \mu A/mm^2)r^2$, where I represents the amplitude in μA of a constant-current 0.1 msec pulse and r is the radius in mm of a circle, concentric with the electrode tip, that encloses the directly stimulated axons of neurons that participate in brain stimulation reward.

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Bill Mundl's expertise in matters electronic

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONT'D)

LIST OF FIGURES

LIST OF FIGURES (CONT'D)

INTRODUCTION

The first step towards the ultimate goal of understanding how mind and behaviour emerge from the the mass of cells called the brain is to document the brain-behaviour relations. Documenting brain-behaviour relations is conceptually straightforward; it amounts to specifying the neural circuitry that is responsible for the production or modification of any particular behaviour. In practice, the work is demanding; once the neuroscientist has selected the organism that he or she wishes to study, the task is to conduct experiments with the aim of learning the locations, inputs, connections, outputs, and functional inter-dynamics of the neurons that compose the behaviourally relevant circuitry.

One of the most powerful tools, especially useful in the attempt to determine the location of circuit elements, is the direct excitation of central nervous tissue with electrical pulses applied through an electrode permanently residing in the brain. The technique was introduced by Hess (1931) and it has enjoyed many applications in the last half-century. The method involves chronically positioning the uninsulated tip of a fine wire into a brain region

that is suspected to contain members of the neural circuit under investigation. Attempts are then made to elicit, or inhibit, the target behaviour by the electrical activation of the cells in the vicinity of the electrode site. By testing a range of placements, some idea is formed about the spatial organization of different elements belonging to the behaviourally important circuitry.

At present, mapping the brain for the location of circuit elements is not as fine a technique as it could be; indeed, the strategy used resembles the approach taken in oil-finding expeditions. Once a site is found to contain oil—or, in the case of brain stimulation, to modify the desired behaviour—a three-dimensional survey of the surrounding region ensues with the aim of delimiting its boundaries. The disadvantage suffered by the neuroscientist is that the relative resolution offered by the strategy is poor compared to that needed by the geologist. The poor resolution is not the fault of any difficulty in finding the location of an electrode that has been lodged in the brain for some time. Typically, the brain is removed and serial sections of frozen or impregnated tissue are examined for the damage produced by the presence of the electrode.

The tip, or deepest extent, of the damage is taken as the central location of cells which, when excited by stimulation through the electrode, are early links in the chain of neural events leading to the altered behaviour.

Instead, the poor resolution largely stems from the inability to specify the size of the region, around the electrode tip, that contains cells which are not only successfully depolarized by the stimulation but also involved in the production of the target behaviour. Because we do not know how far from an electrode a behaviourally relevant cell or cell group must be situated in order to fail to be excited by stimulation at some particular intensity, the attempts to locate small brain areas containing critical circuitry are necessarily crude. Undoubtedly, the power of mapping studies would be greatly enhanced if the researcher were able to specify the size of the stimulation field resulting from any given set of stimulation values.

The idea of a "stimulation field", established around the tip of a monopolar electrode, must be clarified. The term is used as an abbreviation for the brain region containing neurons that generate action potentials as a direct consequence of one

applied stimulation pulse. Any cell membrane that is brought beyond the threshold potential for firing is said to lie within the field; cells only partially, or not at all, depolarized are said to lie beyond it.

The word field may conjure up a two-dimensional image. Yet, the current radiates to the cathodal electrode tip in the three dimensions. Recall that with monopolar stimulation the indifferent, or reference, electrode is distant from, and has a large surface area relative to, the stimulating electrode. Might it not be preferable to use a term, such as stimulation space, to properly reflect the fact that the points at which action potentials are generated lie within a three-dimensional enclosure?

The answer to that question, based upon the following consideration, is no. In many, perhaps in the majority of, applications of brain stimulation methodology, researchers examine cell groups which share a common trajectory of axonal processes. Imagine that the tip of an electrode is embedded in a bundle of parallel axons. An excitatory pulse delivered through the electrode radiates inwards from all directions. The density of current flow is highest at points closest to the electrode and the current

density falls off, or thins out, at points more distant from the tip. An axon that is situated near to the electrode may experience a suprathreshold depolarization over a relatively long portion of its length. One that is more distant may undergo suprathreshold excitation over a shorter length of membrane. The point at which an action potential is initially propogated is the farthest one from the electrode along the suprathreshold portion of the membrane. Indeed, for any single axon there are two such points, one proximal to the synapse where the orthodromic action potential is initiated and one distal where the antidromic one originates. Thus, the action potentials produced by an applied pulse are conceived to be spatially arranged as a shell, not necessarily of spherical shape, surrounding the monopolar electrode tip.

With a rectangular cathodal pulse of short duration, one orthodromically conducted action potential will be conveyed to the synapse in each of the axons reached by the stimulation. Because the fibers are organized in a parallel arrangement within a bundle, an adequate description of the set of excited cells may be developed by collapsing, or projecting, the half-shell which represents the

sites of origin of the orthodromic action potentials onto a two-dimensional plane orthogonal to the fibers' axes and containing the electrode tip. Thus, despite the fact that action potentials are generated in a complex space around the tip, an analysis of the size of the enclosed region may begin with the simpler concept of a two-dimensional stimulation field. Note also that it is not strictly necessary for the fibers to be perfectly parallel. The same simplification may correctly be applied for axons that are fanning away, or converging towards, each other as consecutive transverse planes are examined. The important requirement is that they maintain relatively constant positions within the expanding, unchanging, or converging bundle.

A final note on the idea of a stimulation field addresses the implicit notion that a single circle drawn around the electrode tip is an accurate representation of the elements excited by a stimulation pulse. I do not take issue with the view that the stimulation field is circular; its shape is discussed later. The point that is challenged, here, is that a single figure is sufficient.

Different cells have different current thresholds for excitation; the current threshold is inversely

related to fiber diameter (Erlanger & Gasser, 1937; Ranck, 1975; this point is discussed is detail below). A large myelinated cell quite far from the source of the stimulus might be excited whereas, in response to the same pulse, a small unmyelinated cell nearer to the electrode tip might receive only subthreshold depolarization. The effective spread of excitation cannot realistically be represented with a single boundary if the surrounding tissue contains fibers of various size and myelination. A single pulse may result in several stimulation fields, differing in size, depending upon the heterogeneity of fiber type in the electrode's neighbourhood.

The aim of this dissertation is to measure the size of the stimulation field for fibers, coursing through the lateral hypothalamus of the rat, that upon electrical activation support self-stimulation behaviour (first discovered by Olds & Milner, 1954). The thesis goes a little further in that an attempt is made to specify the parameters of an equation that describes the current-distance relation for rewarding brain stimulation. Specifically, the goal is to arrive at an expression that relates the current amplitude, or intensity, of a 0.1 msec cathodal pulse to the distance, from the electrode tip, over which the

pulse fires reward neurons. Note that the term relation is in the singular form; the initial working assumption is that the reward-relevant fibers are similar enough to each other to be considered as a single type.

The present endeavor does not stand alone nor is it the first attempt to determine a current-distance relation. Three different approaches have been taken towards the solution of such relations and each has provided valuable information upon which this work is based. These approaches and the essence of the information derived from them are briefly introduced at this point in order to place this thesis in perspective. The experiments within each approach are subsequently reviewed in detail and, finally, the logic behind the experiments of this dissertation is explained.

The first approach to the solution of current-distance relations has been taken by workers in the neurophysiological domain. The strategy that has been customarily employed features a moveable stimulating electrode, often a microelectrode, that is used to excite a targeted cell. Thresholds for excitation are documented for each of several electrode positions and the relation is derived

from these intensity-position data pairs. Because the successful activation of the target is detected by intra- or extra-cellular recording, this line of investigation has produced current-distance relations that are specific to cells either with particular conduction velocities or which are members of well-known neural pathways. The results of these studies are in remarkable agreement in supporting a quadratic form for the relation; the current required to excite a cell is a function of the square of the distance between the cell and the electrode tip.

The second approach has been to use the radiolabelled 2-deoxy-d-glucose method of selectively marking cells driven directly by a stimulating macroelectrode. Autoradiographic material is examined for heavily labelled tissue surrounding the locus of the electrode tip. This method allows one to determine the greatest reach of the current for all of the cells in the vicinity of the tip. Because any particular intensity excites large cells farther from the source than small cells, this technique would over-estimate the size of the stimulation field if the cells of interest were the smaller ones. Nonetheless, information pertinent to this thesis is revealed by an inspection of the material; the shape of the

stimulation field around a monopolar electrode is roughly circular.

The third approach is of special interest to the student of brain-behaviour relations. Here, estimates of current spread are based upon the behavioural output of the organism. Because of this, assurance is provided that the estimates reveal the effective spread for the cells that are active participants in the behaviour under study.

Current-distance relations derived with this third strategy are most useful to the psychologist. Not often armed with a priori knowledge about the relative size or conduction velocity of the behaviourally relevant neurons, the behavioural scientist cannot extract, from the neurophysiological work, an estimate of the stimulus spread for his or her own preparation. The estimates of current spread obtained with this approach are specific, not to cells of a particular size or with membership in defined neural pathways, but rather to cells distinguished by their participation in a particular behaviour. The present experiments follow in this tradition. The current-distance relation is developed for lateral hypothalamic neurons involved in brain stimulation reward.

Neurophysiological Experiments: Form of the Equation Relating Current and Distance

As has been explained by BeMent and Ranck (1969b; Ranck, 1975), and is repeated here, working out the current-distance relation, even with neurophysiological techniques, is not as simple as it may first appear to be with a cursory consideration of the problem. True, all that is needed is a measure of the closest distance to the target cell from the stimulating electrode and a record of the stimulation parameters that are just sufficient to excite it. With a straight axon running at a right angle to the line between the stimulating and recording electrodes, the distance could be calculated from the co-ordinates used to position the electrodes.

Practical problems, however, preclude the possibility of simultaneously stimulating a neuron and monitoring its local response. The delivery of a stimulation pulse produces a recorded shock artifact with an amplitude that is at least an order of magnitude greater than that of the recorded cellular response. Combined stimulation and recording is usually accomplished by spatially displacing, along the length of the axon, the positions of the electrodes. The shock delivered through the

stimulating electrode is still strongly registered by the recording electrode but, because of the temporal delay resulting from the propagation of the action potential from the stimulation to the recording site, the neuron's response is detected long after the shock artifact has dissipated. The problem of trying to detect a millivolt signal against a volt background artifact is solved, but the solution exacts a cost; the distance between the stimulating electrode and the first depolarized region of the neuron is no longer known. Somehow, an estimate of this distance must be obtained.

The methods that are used to judge this distance vary from report to report. However, the basic strategy that is used to collect the data is identical. A recording microelectrode is employed to directly or indirectly monitor the success of attempts to activate a target neuron by pulses applied through a moveable stimulating electrode. The position of the stimulating electrode is varied either along straight lines over the surface of the CNS or down vertical penetrations. Current thresholds, values of intensity that are just sufficient to produce some criterion response by the cell, are determined for each position. Surface-stimulation studies are

discussed first; the obvious advantage of exciting cells from the surface of the brain or spinal cord is that the tissue underneath is minimally distorted by the procedure.

Landgren, Phillips, and Porter (1962) studied the excitability of large pyramidal cells in the baboon. They used a ball-tipped silver-silver chloride electrode to deliver anodal pulses to the surface of the motor cortex and the responses of single axons were detected with a recording microelectrode entering the spinal cord at the fifth or sixth cervical level. Once a cell was found to respond with short, invariant latencies, the position of the surface anode was varied in the attempt to find a point on the cortex which required the least intensity to elicit an action potential. Presumably, this lowest-threshold point corresponded to the shortest distance between the electrode and the first-depolarized region of the cell; the excited element must have been directly below the electrode. From this lowest-threshold point, the anode was systematically repositioned along lines parallel and perpendicular to the central fissure and the current threshold was measured at each point. The procedure continued until ranges of ±10 mm were tested from

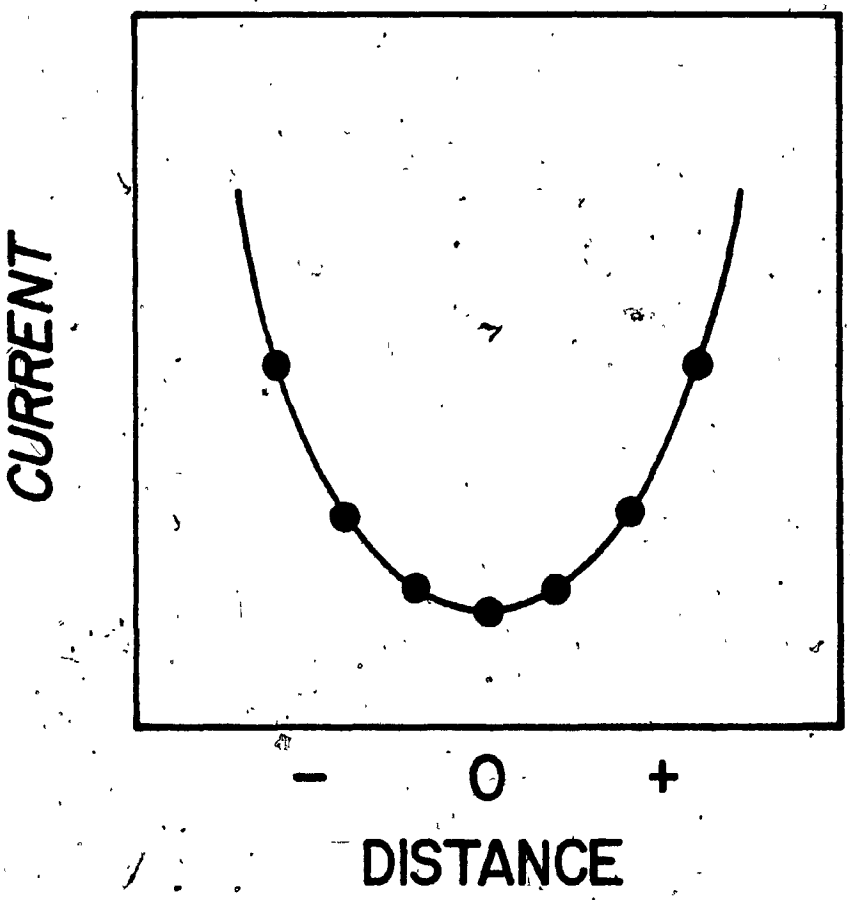
the original lowest-threshold point.

A sketch which reflects the essentials of their findings without the burden of quantitative detail is presented in Figure 1. (I promise to burden the reader later in the text.) The lowest-threshold point is centered along the abscissa and is labelled "0" on this distance scale. Linear departures from this point are arbitrarily indicated as plus and minus signs. The ordinate linearly scales the current intensity necessary to register a response. As would be expected, it takes more current to excite a cell if the electrode is located some distance away from the cell. The important finding here is that the relation, apparently, is not linear. A displacement of the electrode from the lowest-threshold point requires a modest increase in current to promote an action potential; a similar change in position when the electrode is distant from the lowest-threshold point requires a large change in intensity. The parabolic shape of these results suggests that the current required to excite a cell increases as a power function of the distance.

Using cathodal excitation of the distal branches of cat climbing fibers of the cerebellum, Armstrong, Harvey, and Schild (1973) documented a similar

Figure 1

Illustration of neurophysiological mapping data. The abscissa represents the relative position of a moveable electrode and the ordinate represents the current intensity needed to activate the target.

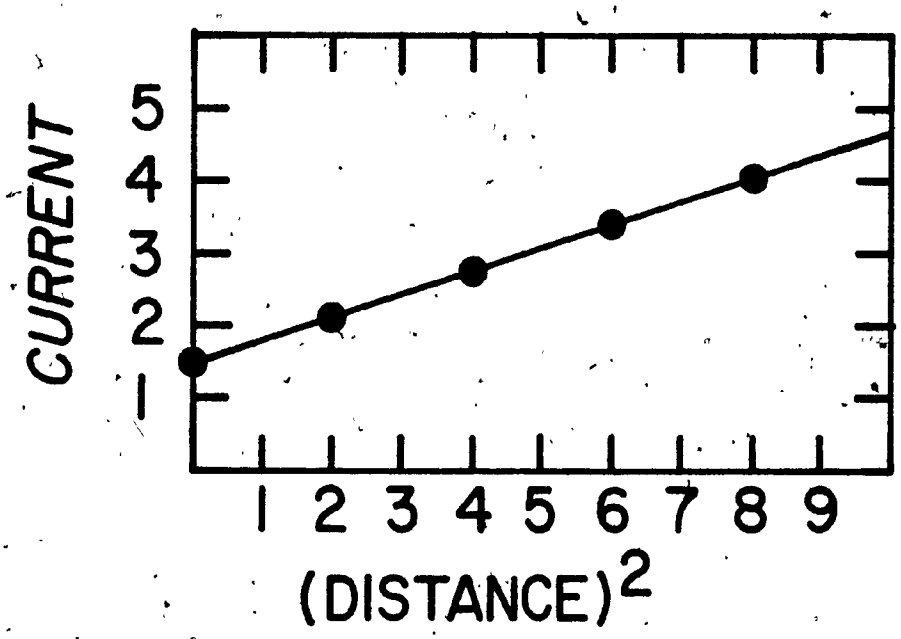

CURRENT
−
0
+
DISTANCE

relationship between current and distance. In their preparation, excitation was thought to be antidromically conducted towards the junction of the electrically activated branch and a companion collateral. The arrival of the action potential at the junction would generate an orthodromically conducted disturbance in the companion branch which, in turn, projected to a Purkinje cell. Thus, a successful surface-cathodal activation of one branch of a climbing fiber was revealed by an excitatory post-synaptic potential in the intra-cellular record of a distant Purkinje cell. Once a lowest-threshold point was found, the excitability of the branch was examined along two tracks, one parallel to, the other perpendicular to, the folial axis. In both cases, the data fit the relation depicted by Figure 1.

These researchers took their data one step further by transforming the distance scale to its square and re-plotting the results for five cells. Figure 2 illustrates, with arbitrary scaling, the resulting graph for one cell. Their points now fell along straight lines; this finding led to their conclusion that the current density at any point was inversely related to the square of the distance. Stated another way, the "inverse-square" law recommends

Figure 2

Transformation of the data depicted in Figure 1.


CURRENT
1
2
3
4
5
1 2 3 4 5 6 7 8 9
(DISTANCE)2

that the current required to excite a neuron is proportional to the square of the distance between the electrode and its target.

Additional support for the quadratic nature of the relation is derived from the work of BeMent and Ranck (1969b). These researchers solved the problem of estimating the true distance between a surface electrode and the neuronal target. This was achieved largely by a careful selection of the test preparation. The long axons of the medial part of the dorsal columns hold a constant position, relative to the midline and to the dorsal surface of the spinal cord, over several cord segments. BeMent and Ranck employed cathodal surface stimulation to excite the cells at one cord level and they recorded the responses of single axons with deep microelectrodes entering at another, as much as 20 mm away. If the axon kept its relative position in the coronal plane at the levels of both the stimulating and recording electrodes, then the co-ordinates that were used to find the cell with the recording electrode indexed the distance between the surface cathode and the first depolarized region of the cell. Once a responsive cell was found, current thresholds were determined for several positions of the stimulating electrode. Here,

the mapping was confined to points lying in the transverse plane.

Two of their findings are of special interest. Germane to the issue at hand is the discovery that the current required increases by greater amounts as the distance increases. Because this work contained accurate estimates of the real electrode-to-target distances, these data offer strong support for the non-linear nature of the current-distance relation. Of pertinence to a second topic is their finding that cells with fast conduction velocities had lower current thresholds than those which conducted slowly. Relying upon Hursh's (1939) rule that the conduction velocity in meters per second is six times the fiber diameter in micra, these authors concluded that small cells require more current than do large ones.

Complementary information, towards the solution of the form of the current-distance relation, comes from the deep-stimulation studies. As was the case in experiments with surface stimulation, the successful activation of a neuronal element is revealed by unit records through a distant microelectrode fixed in position. The main methodological difference is that now a stimulating microelectrode, usually measuring only a few micra in diameter, penetrates the CNS.

Current thresholds are noted as the electrode occupies different positions in the vertical dimension. The electrode approaches the target from some point above, passes nearby, and proceeds to points below the cell. Conceptually, there is no difference between the deep-stimulation experiments and those with surface stimulation.

Porter (1963) lowered microelectrodes towards the cell bodies of the hypoglossal nerve in the cat. The cell's response was detected by electromyographically monitoring single motor unit responses in the tongue. As the stimulating electrode was lowered, a parabolic fall in threshold current was witnessed; presumably, this corresponded to the electrode approaching the level of the first excited region of the cell. Further descent of the electrode was not, however, accompanied by a symmetrical rise in the required current. Rather, departures from the lowest-threshold level resulted in a more gradual increase in threshold.

Porter accounted for the asymmetry by considering the orientation of the target cell. The somata of the hypoglossal nerve fibers have dendritic trees fanning up towards the surface. The axon hillock points down; it gives rise to a myelinated axon

that runs downwards as it veers away. As long as the cell were approached from above, excitation would occur at the axon hillock (Ranck, 1975). As the electrode was lowered beyond the level of the hillock, the possibility arose that the region first excited by the stimulation would pass from the hillock to the first node of Ranvier. Effectively, the result is that the locus of excitation would follow the electrode; the changes in electrode-to-element distance would therefore not be as great below the level of the axon hillock as they would be above it.

Similar methods were used and a like problem was encountered in a study of the excitability of branches of the lateral vestibulospinal tract cells in the cat (Abzug, Maeda, Peterson, & Wilson, 1974). The stimulating electrode was lowered through a lumbar segment of the spinal cord and current thresholds were noted at each position. The antidromic responses were recorded extracellularly in Dieter's nucleus, a sub-cerebellar aggregation of the somas of the cells. On the approach side, a parabolic fall in current thresholds was seen but, as the electrode passed beyond the lowest-threshold point, the curve was somewhat flatter; at times,

kinks appeared in the curve. Again, a changing locus of excitation could account for the asymmetry of the results but, because the branches lie in horizontal planes, the jumps in locus could not have been from node to node. More likely, the explanation is that a second branch, perhaps even the main trunk, of the same recorded cell was now brought within the reach of the stimulation. The second branch would now be approached from above; the predicted outcome is that additional lowest-threshold points would appear in the data. An inspection of the records of some of the cells suggests that this indeed was the case. Although it was not mentioned earlier, the same observation was made by Armstrong et al. in their surface maps. Their conclusion was that additional lowest-threshold points reflect the activation of other branches of the same cell.

Obviously, the part of the curve that is telling is the initial portion which corresponds to the electrode's approach to a single, unchanging locus of excitation. Both in the case of Porter's approach to hypoglossal somas, and in that of the Abzug et al. descent towards spinal branches, the initial portions resemble the downward leg of a parabola (e.g. Figure 1; negative side to zero).

Instead of simply relying upon a visual appraisal of the general shape of the results, Marcus, Zarzecki, and Asanuma (1979) chose to submit some data to a curve-fitting assessment. They analysed the data that had been collected in an experiment which mapped the numerous branches of monkey pyramidal axons (Shinoda, Zarzecki, & Asanuma, 1979). Moveable microelectrode penetrations had been made through several cervical levels and the antidromically conducted impulses had been detected through recording electrodes fixed in the motor cortex. At each of the positions of the moveable stimulating electrode, current thresholds had been noted. The results of twelve penetrations were fit to two models, both of which incorporated the idea that the true distances between electrode and target followed the Pythagorean rule. In one model, the current required to excite the cell was proposed to be a linear function of the distance and in the other, the required intensity was stated to increase as the square of the distance. With lower least-square residuals as their criterion, Marcus et al. selected the parabolic relation as the better account.

Jankowska and Roberts (1972) tracked the current-distance relation in cat spinal interneurons.

Fine stimulating microelectrodes penetrated the sixth or seventh lumbar segments of the cord, aimed at the terminal branches of interneurons in the ventral horn. Extracellular recording electrodes were positioned rostrally near the cell bodies. In the attempt to ascertain the exact distance between the stimulating electrode and its target, they used cells which demonstrated lowest-threshold values of less than one tenth of a microampere. They argued that when the threshold was that low, the electrode must lie within 30 micra of the first depolarized region of the cell. Given the relatively long length of the penetrating track (±200 μm), the 30 μm distance was considered negligible and the vertical co-ordinate was taken as a good approximation of the electrode-to-element distance.

A parabolic relation best describes their data. They, as had done BeMent and Ranck (1969b), also examined the relation between conduction velocity and threshold and found that rapidly conducting (large) cells were more easily excited than were slower (smaller) axons. Except for the relation between rate of conduction and threshold, the same techniques were applied to, the same rationale was used in, and a similar, parabolic-shaped, current-distance relation

emerged from a study of Renshaw cells (Jankowska & Smith, 1973).

Bagshaw and Evans (1976) approached the problem by making several vertical penetrations around a single target cell. Their preparation involved the stimulation of cat and rabbit dorsal column axons and the antidromic action potentials were detected in single surviving axons of the dissected and frayed sural nerve. They determined the location of the target by transforming the data of several penetrations into concentric "iso-intensity" circles. These appear as a series of differentially valued rings surrounding the position of the cell. It resembles an archery target except that the values (current thresholds) are higher in the peripheral rings. The bull's eye, of course, represents the location of the electrically activated axon. Their conclusion was that the relation developed from the inverse-square law, that is, the current required to excite a cell is proportional to the square of the distance, was correct for monopolar stimulation.

In that study, Bagshaw and Evans had approached the locus of excitation not with a single track but rather with several penetrations per cell. The award for thoroughness was almost theirs; it goes, however,

to Roberts and Smith (1973). Roberts and Smith used dozens of carefully planned penetrations for each axon with the aim of getting the stimulating electrode as close to the nodes of Ranvier as possible. They worked with fibers belonging to the dorsal spinocerebellar tract in the cat. Stimulating electrode entries were made at the first or second lumbar level; records of single-cell responses were taken in the third or fourth lumbar segment. The initial few penetrations were lined longitudinally, parallel to the fiber under investigation, in the attempt to locate the low threshold points that presumably corresponded to the sites of the nodes of Ranvier. Once two or more nodes were found, additional penetrations were aligned in the transverse plane at these low threshold points; now the aim was to approach, as closely as possible, the nodes. Their findings support the two main ideas carried here. First, the current-distance relation is curvilinear and, second, the current required to excite a cell varies inversely with conduction velocity.

Up to this point, I have surveyed the reports of workers who have determined the locus of excitation, and thus the critical distance between the stimulating electrode and the first depolarized region of the

cell, largely by argument. We have seen the rationale that a current threshold of 0.1 μA indicates close proximity (Jankowska & Roberts, 1972), the notion that the target can be found by constructing circular iso-intensity profiles (Bagshaw & Evans, 1976), and the idea that with many penetrations the cell may almost be touched (Roberts & Smith, 1973). Each of these arguments is compelling. Yet, there is one current-distance study that stands out from all the others. This experiment is distinguished by the implementation of a very clever strategy, a strategy which eliminated the need to argue about the distance between electrode and target. With their method, Stoney, Thompson, and Asanuma (1968) were able to measure the distance directly.

These investigators examined the excitability of cat pyramidal tract fibers. A large stimulating electrode was used to excite the axons at the level of the medulla but this electrode was fixed in place and was not employed in the mapping per se. Mapping the relation was accomplished with a more complicated arrangement comprising two parallel microelectrodes that together entered the cerebral cortex. One of the two microelectrodes was always used as a recording electrode. The other, here referred to as the

stimulating/recording electrode, was used to deliver a pulse and, immediately after, was electronically switched to a recording mode. The microelectrode pair was mounted on a special manipulator that permitted conjoint positioning of the pair and independent driving of the stimulating/recording electrode. The distance between the recording and the stimulating/recording electrodes could at all times be determined from the co-ordinates used to position them. The pair would be lowered into the cortex and initially the recording electrode was deeper in position than was the stimulating/recording companion. Together, they were driven in small steps until a cell was found, by the recording electrode, to respond to shocks delivered through the fixed medullar electrode. An intensity for medullar stimulation was selected which would reliably activate the cell.

Now the experiment began. A cortical pulse through the stimulating/recording member of the pair could, depending upon the intensity, excite the cell tapped locally by the recording electrode. Whether or not the cell responded could not be immediately known because of the overwhelming shock artifact. Soon after the cortical pulse, a second pulse was

administered, this time through the medullar electrode.

Consider the events. Suppose that the cortical pulse, the first one, were sub-threshold for excitation. No orthodromic action potential would be generated. The subsequent excitation at the medulla would produce an antidromic action potential that would make its way, unimpeded, to the cortical recording electrode and a spike would appear on the record. Now assume that the intensity of the first, cortical, pulse is sufficient to activate the neuron. An orthodromic disturbance would begin to be conducted towards the medulla. The following medullar stimulus would again initiate an antidromic impulse but now the antidromically travelling impulse would collide with the orthodromic one. No action potential would be seen at the recording electrode. Thus, the successful activation of the cell by the cortical, stimulating/recording electrode was revealed to these investigators by the absence of the reliable spike originating from the medulla.

The procedure was repeated at several intensities of stimulation through the cortical electrode; the point of transition, as intensity increased, between the presence and absence of the antidromic spike was

deemed the current threshold. The independently manipulable stimulating/recording electrode would be lowered to a new position and a new current threshold would be determined. Recall that, at all times, the distance between the stimulating/recording electrode and the recording electrode (where the first depolarized region of the cell is located) could be determined directly from the position co-ordinates.

No comment has yet been made concerning the recording aspect of the stimulating/recording electrode. After a pulse was passed through this electrode it was switched to a recording mode. If the pulse had failed to locally excite the cell, and if this electrode were close enough to the target neuron, then it too would detect the antidromic action potential. Of course, the amplitude of this record would vary (inversely) as a function of the distance to the cell. The amplitude would attain its peak when the stimulating/recording electrode was at its closest approach to the cell. Conversely, the current required to excite the cell would be minimal at this point; together, maximal recorded amplitude and minimal current threshold served to define the closest approach to the cell by the stimulating/recording electrode.

The results of this study offer the strongest empirical support for the idea that the parabolic form best describes the current-distance relation. A happy coincidence is that the parabolic, or quadratic, function is also predicted theoretically. The theoretical argument may be found in several reports (Asanuma & Sakata, 1967; Bean, 1974; BeMent & Ranck, 1969a); the development that is presented here most closely follows the one put forth by Stoney et al. (1968).

Neurons display stable current thresholds. At threshold, the voltage gradient ($\Delta V/\Delta r$) across any two points on the cell membrane is therefore constant. The term ΔV is the difference in external potential established during the application of a constant current pulse and Δr is the difference in the distance from the two points to the source of the stimulation.

In a volume conductor, the voltage, V, at any distance, r, is given by:

$$V = Is/4\pi r$$

where I is the current amplitude and s represents the specific resistance of the medium. For any particular kind of preparation, the specific resistance is considered to be constant throughout the tissue.

The voltage gradient, $\Delta V/\Delta r$, is changed to its point-slope form, dV/dr, and the first derivative of voltage with respect to distance gives:

$$\Delta V/\Delta r = dV/dr = -Is/4\pi r^2$$

It is understood that the voltage gradient at threshold is a constant, as are s, the specific resistance, and the values 4 and π. These are together expressed as a single constant, k, and the relation between current and distance becomes:

$$I = kr^2$$

In the process of deriving the relation, the resistance of the medium was included in k; the constant is thus sensitive to this factor. The constant is also affected by other factors such as the duration and polarity of the applied pulse and the stimulation configuration, for example, surface vs. deep, monopolar vs. bipolar, etc. (The reader is referred to Ranck, 1975 for an excellent description of how these factors influence the current-distance relation.)

With the above variables held constant, k is exquisitely sensitive to the size of the target axon. Stoney et al. (1968) determined the value

of k and measured the conduction time of 12 of their tested units. The correlation between these measures was 0.67, indicating that k increases as conduction time increases. The implication is that the effective spread of current, or the size of the stimulation field, is greater for large axons than it is for small ones. If the relation between conduction time and fiber size is linear, then close to half of the variation in k is explained by cell diameter.

That the empirical grounds and the theoretical derivation converge in recommending the quadratic form for the relation is reassuring but an important practical consequence emerges from this result. In order to specify the current-distance relation for a particular preparation, it is sufficient to offer a value for the constant k. The objective of this thesis is to obtain an estimate of this constant.

The 2-Deoxy-d-Glucose Method: Direct Visual Inspection of the Shape of the Stimulation Field

Except in cases of extreme starvation, the brain derives its energy by intracellular metabolism of glucose (Friedman & Stricker, 1976). The demand on energy, and thus on glucose, is greater when a cell is firing frequently than when it is relatively quiet. Accordingly, glucose uptake into the cell is enhanced as the firing rate increases.

A structural analog of glucose, 2-deoxy-d-glucose (2-DG), is similar enough to the glucose molecule to be incorporated into the cell through the glucose uptake mechanism. The analog is not, however, similar enough to participate in subsequent metabolic events. Consequently, if 2-DG is systemically introduced, it competes for uptake with glucose and becomes concentrated in the neuron. Rather high concentrations of 2-DG would be achieved if the cell were firing at a rate well above baseline (Sokoloff, 1977).

Gallistel, Karreman, and Reivich (1977) injected a radio-actively labelled form of 2-DG into rats that were self-administering trains of rewarding brain stimulation through unilateral electrodes placed in the lateral hypothalamus. They reasoned that the

neurons driven directly by the stimulation would fire at exceptionally high rates; the activity of these cells would certainly exceed that of their unstimulated counterparts in the opposite hemisphere. Hence, it was expected that these driven cells would take up much more of the 2-DG label.

Following a bout of self-stimulation, the rats were sacrificed and serial coronal sections of brain tissue were examined autoradiographically. The material revealed a dark (heavily labelled, high uptake of the 2-DG marker) region surrounding the tip of the electrode track. Indeed, serial sections anterior and posterior to the electrode's position contained similar dark regions, relative to the unstimulated side, that accurately followed the course of the medial forebrain bundle. Quite apart from the obvious utility of the method in tracing the trajectory of a directly stimulated bundle of axons, and its potential for marking the neural consequences of any stimulus presented to an intact organism (cf. Kennedy, Des Rosiers, Sakurada, Shinohara, Reivich, Jehle, & Sokoloff, 1976), the pertinence of the Gallistel et al. study to this thesis concerns the effective spread of current from a monopolar source.

The procedure could be repeated using

experimenter-delivered stimulation trains fixed in duration and in pulse frequency. Each animal would receive a different current amplitude and the current-distance relation would be worked out from an inspection of the extent of labelled material surrounding the electrodes. Such an experiment would not cast light on the effective size of the stimulation field for behaviourally relevant cells; all of the neurons near the tip, including those that do not actively participate in the behaviour of interest, will have been excited. If the target behaviour were one mediated by smaller cells, for which stimulus spread is less extensive, then a measure of the spread based upon a visual inspection of the autoradiographic results would over-estimate the true, behaviourally relevant, reach of the stimulation. Nonetheless, such an experiment would define the greatest extent of current spread in the selected brain region; it would serve to place an upper bound on the stimulus' reach.

The finding of greatest bearing here is that of the shape of the stimulation field. The sections were taken in the coronal plane and this plane cuts the medial forebrain bundle, at the level of the stimulating electrode, transversely. By recalling

the comments on an adequate, two-dimensional, representation of the set of fibers excited by a pulse, one realizes that a coronal section at the level of the electrode tip shows the shape of the stimulation field. The shape surrounding a monopolar macroelectrode is circular. True, the circular shape results from the excitation of the largest fibers in the bundle but there is no reason to believe that a different picture would be seen if the effects of the stimulation could somehow be restricted to the smaller axons.

The rationale for the present experiment, which is presented in the section after the next, might have opened by making the assumption that the shape of the stimulation field is circular. The idea follows naturally out of a consideration of how current radiates from a point source. Furthermore, the notion is implicit in the neurophysiological work discussed earlier. Unfortunately, the electrodes used in behavioural work are very large. To treat them as points is to push the argument too far. Thanks to the efforts of Gallistel et al., the present work is relieved of the need to posit the circular shape as an assumption.

Behavioural Output Techniques: How far does Stimulation Reach for Behaviourally Interesting Neurons?

The theme running through this text emphasizes the need for current spread estimates for neurons that are marked by their participation in a particular behaviour. In order to conclude, with confidence, that obtained data reflect the spread for behaviourally relevant cells, estimates should be based upon the effect of the stimulation on the behavioural output of the organism. The stimulation's influence on all of the cells in the vicinity of the electrode tip becomes transparent when behaviourally based determinations are made; the approach sees through the excitation of non-participating elements and effectively focuses the assessment to the influence of the stimulation on the neurons critical to the selected behaviour. The four experiments described here employed the behavioural output approach.

Wetzel (1970) prepared cats with chronically indwelling electrode arrays, with closely spaced tips, aimed at the lateral hypothalamic region. Each electrode was tested individually, that is, stimulation was referred to an indifferent return, and a short train of 200 Hz, 0.2 msec, rectangular pulses was delivered following the depression of a

pedal by the cat. Although the amplitude of the rewarding stimulation was not rigidly fixed, the range of variation (400 - 500 μA) was narrow.

Her main finding was that immediately adjacent electrodes often gave rise to impressive differences with respect to their ability to support self-stimulation responding. The implication is that current spread, for fibers of the lateral hypothalamus involved in brain stimulation reward, was fairly restricted in extent. Wetzel herself does not offer a numerical assessment of the effective spread; my belief is that she was keenly aware of the quantitative limitations imposed by the missing information on the distribution of reward-relevant fibers around the electrode tips. A crude estimate, ventured on the basis of an inspection of her histology, is that the reach was well within one millimeter. This ball-park figure is in perfect agreement with a similarly made appraisal of the stimulus spread revealed by the 2-DG method (Gallistel et al., 1977), adjusting for differences in pulse width.

Wetzel's experiment has recently been repeated in rats (Steiner, Bodnar, Nelson, Ackermann, & Ellman, 1978) and the authors of this report claimed to have

dispelled the myth that effective current encompasses the rat cerebral hemisphere.

Wise (1972) lowered moveable stimulating electrodes (Wise, 1976) towards, through, and beyond rat lateral hypothalamic regions which, upon electrical activation, give rise to eating and to drinking behaviour. At each position in the series of small advances, the current thresholds for the elicitation of eating and drinking were noted. It should be immediately apparent that this strategy is identical to that employed in the neurophysiological approaches towards single axons by vertical microelectrode penetrations. The chief, and important, difference was that now the effect of the stimulation was monitored behaviourally.

Because the behavioural outcome was thought to depend upon the activation of many cells distributed over a finite region, a perfect, parabolic shaped, relation between current and electrode travel was not predicted. Wise constructed several possible arrangements of distribution of behaviourally relevant cells. He showed, by argument, that as long as the electrode were enclosed by a region containing an even distribution of eating (or drinking) fibers, a fair length of the electrode's excursion would

witness repeated lowest-threshold points (see Case I, Figure 2, page 547 of that paper).

Four of the six animals showed this pattern. A sudden drop in threshold was observed in one move of the electrode and this low threshold was seen over several subsequent descents. As suddenly as it had dropped, the threshold rose with the next move. The abrupt changes in threshold and the large number of consecutive lowest-threshold points are consistent with the ideas that, for the fibers involved in eating and in drinking, first, the size of the stimulation field is small compared to the size of the steps (0.13 mm) and, second, it is also small relative to the size of the region that contains these behaviourally relevant cells. On the basis of these data, Wise suggested that 25 μA of sine wave current (rms) excites cells within about 1/8 mm and 100 μA reaches cells 1/4 to 1/2 mm away.

Using an acute preparation, Potegal, Blau, and Miller (1980) lowered macroelectrode arrays towards the motor nucleus of the facial nerve in the rat. Stimulation pulses were passed through the electrodes at each of the one-millimeter-spaced positions in the track and the current thresholds for the production of just noticeable vibration of one or more

vibrissae were recorded. The steps were large and the target was small; the resulting data show U-shaped functions of current threshold against extent of electrode travel. The average lowest-threshold was 71 μA and at a point 2 mm higher 365 μA, on average, was needed to produce vibrissal vibration. (I discuss here, as was done for the paper by Wetzel, only the results obtained in their monopolar configuration tests. The fixed stimulation values were: 0.1 msec cathodal pulse duration, 50 Hz pulse frequency, and 2 sec train duration.)

These authors did not offer a quantitative assessment of the current-distance relation. Instead, they suggested in their discussion that current might be effective as much as 4 mm from the electrode tip. I have taken this to mean that a target as distant as 4 mm from the tip could be excited if one were to raise the current intensity to a high value. Doubtless, lower current levels would spread less.

Their reluctance to venture strong conclusions about their data was based upon a thoughtful, and correct, analysis of the factors involved in assessing current thresholds for a behavioural consequence. It was mentioned before that moving a macroelectrode towards a neural target, the excitation of which

yields a behavioural change, is a situation analogous to the neurophysiological penetration towards single axons. The analogy stops at that conceptual level. The current thresholds measured in the neurophysiological experiments were thresholds for the elicitation of an action potential in a neuron following a single pulse. In the behavioural work, it is the threshold for the production of a particular response by the organism that is measured. The key problem stems from the fact that behavioural outcomes are rarely obtained with single pulses. A train of pulses must be delivered and the presentation of a train is accompanied by two additional stimulation variables, pulse frequency and train duration. The strength of a behavioural response, at least in the case of self-stimulation, is known to be monotonically related to these two parameters (Edmonds, Stellar, & Gallistel, 1974; Gallistel, Stellar, & Bubis, 1974); presumably, all stimulation-induced behaviours are mediated by neural circuits that integrate excitation over time. The result, mentioned by Potegal et al., is that current thresholds for the elicitation of a behavioural response are not absolute; low current thresholds are observed at high pulse frequencies and high current thresholds are witnessed at low

frequency stimulation (see Animal #3, Figure 2, Page 771 of the Potegal et al. paper for a concrete example).

Stated succinctly, very little has been learned about the current-distance relation from experiments on stimulation-produced behaviour. Even the method holding the greatest promise, that of moving the electrode through a brain region that supports stimulation-induced behavioural changes, is beset by too many unknowns. Before using the method, one must have an accurate picture of the spatial organization of the target cells, both in terms of boundary and density. Even with these known, the method still cannot offer absolute measurements without some estimate of the number of elements that must be excited in order to see a behavioural change. At best, that method can offer only relative assessments. Some other technique is required.

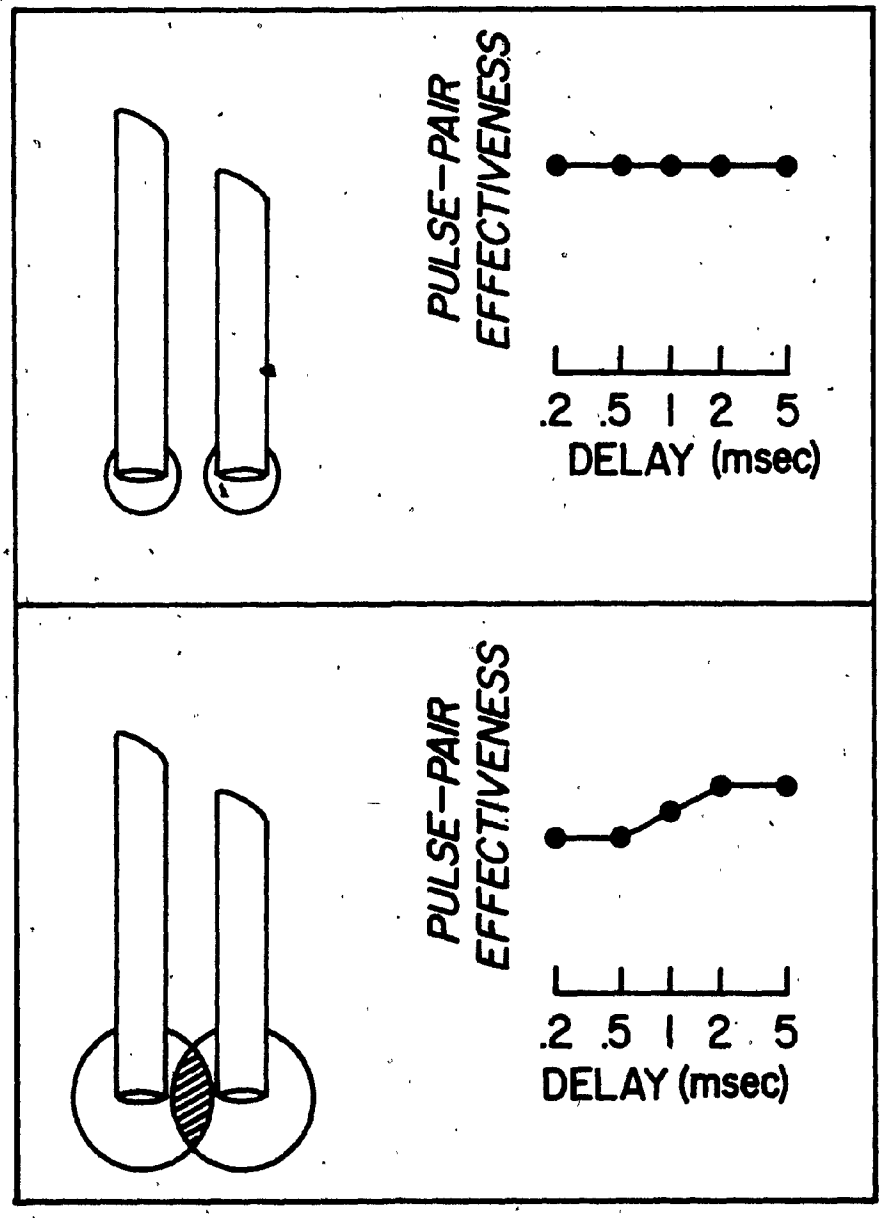
Rationale

Overview. The aim of this research is to determine the current-distance relation for the excitation of neurons, coursing through the rat lateral hypothalamus, that participate in brain stimulation reward. The work follows in the tradition of behavioural-output studies; that the relation is specific to reward cells is assured because the dependent measures are derived from self-stimulation responding. The rationale for the experiment is presented in the following six paragraphs and the reader is urged to inspect Figure 3 while working through them.

Two monopolar stimulating electrodes, prepared and fixed together so that the distance between their tips is known, are positioned into a brain region that supports self-stimulation. The electrodes are oriented so that the imaginary line joining their tips lies in a plane orthogonal to axonal traffic. The preparation is conceived to establish two adjacent stimulation fields which may, if the stimulation amplitude is of sufficient strength, overlap. In other words, at relatively high intensities there are some neurons, lying in the intersection of the two circular stimulation fields, which may be excited by a pulse delivered through either of the two

Figure 3

Effectiveness of pulse-pair stimulation as a function of test intensity and delay. Left: representation of electrodes and stimulation fields. Right: anticipated results. Top: low intensity with no overlap in stimulation fields. Bottom: high intensity with some overlap in stimulation fields.


PULSE-PAIR EFFECTIVENESS
.2 .5 1 2 5
DELAY (msec)
PULSE-PAIR EFFECTIVENESS
.2 .5 1 2 5
DELAY (msec)

electrodes. For these cells, a pulse applied through one electrode will temporarily render them refractory to stimulation passed through the other.

Consider the events associated with the presentation of two equal-in-current-intensity pulses, each with its own electrode destination, with a delay interposed between them. Following the application of the first pulse, all of the cells within the first field, by definition, will be fired including, of course, the cells lying in the intersection of the two fields. If the delay between the pulses exceeds the refractory period of the cells common to both fields, the second pulse, delivered through the adjacent electrode, will successfully fire all of the cells within its proper field. If, however, the delay is too brief, the second pulse will find the common cells refractory to its influence. As a result, it will be less effective in contributing to the rewarding effect of brain stimulation. The cells common to both stimulation fields will have fired one volley per pulse-pair at short inter-pulse intervals whereas they will have supplied two sets of discharge at the longer delays. Thus, in this hypothetical situation in which we have established large stimulation fields with sufficient overlap, the delay between the pulses

is a critical factor in determining the reward efficacy of the pulse-pair.

Consider now the events at lower current intensities, intensities that produce two small stimulation fields with no overlap between them. No neuron is excited by pulses through both electrodes; excitation delivered through one electrode does not influence the excitability of cells lying within the field of its companion. Now, no matter what delay is interposed between the two, differentially destined, pulses, the same total number of firings is achieved with each pulse-pair. With this imagined situation of small, non-overlapping, stimulation fields, the delay between the pulses ceases to be a determining factor in the reward efficacy of the pulse-pair.

The basic plan is to perform a series of modified refractory period determinations. The modification is that whereas conventional tests employ the presentation of pulse-pairs to the same electrode, the critical tests of this thesis feature the splitting of the pairs to different electrodes. An intensity is selected and the relative effectiveness of the pulse-pairs in producing reward is determined for a range of delays. If the function relating effectiveness to delay is found to possess a

rising step as delay is increased, then the stimulation fields are deemed to be overlapping. If, at a lower intensity, the function is flat, then the stimulation fields must be independent. By inspecting the effectiveness-vs.-delay functions over a systematically selected family of test intensities, two groupings should emerge: First, at the low intensities there should be a set of flat effectiveness-vs.-delay functions. Second, at the higher test intensities the functions should posses a step.

Such an outcome is anticipated by considering that, beginning with the low intensites, small, non-overlapping stimulation fields exist. As higher and higher intensities are tested, the boundaries of the stimulation fields approach, touch, and finally intersect each other. The point of transition, along the intensity scale, passing from a flat to a step-like function is then taken as the current intensity at which the boundaries of the two stimulation fields are just touching each other; with equally sized stimulation fields, the point at which they meet is half-way between the electrode tips.

Recall that the interelectrode distance is known. The value of current intensity that

separates the flat and step-like functions is therefore the intensity that just reaches reward cells half the interelectrode distance away from the electrode tips. These values form one co-ordinate pair towards the plot relating current and distance. Additional points are obtained by testing additional preparations; an attempt is made to provide each rat with a different interelectrode distance.

Two final topics need elaboration. First, in order to get an accurate estimate of the current-distance relation, the preparation calls for the positioning of the electrodes so that the line between the tips is aligned at a right angle to the longitudinal axes of the axons responsible for the behaviour. The orientation of these fibers must be identified. Second, because the data have as an ultimate basis the behavioural output of the organism, some comments must be made concerning the scaling methods employed in this research. Since this work features both conventional and modified refractory period determinations, the comments on scaling are included in a brief survey of behaviourally based refractory period determinations.

Orientation of the reward fibers. There is virtually no disagreement concerning the alignment

of directly stimulated reward-relevant axons at the mid-hypothalamic level in the rat. The consensus is that the fibers lie along the longitudinal body axis. Olds and Olds (1963) provided the earliest data on this question by showing that electrodes located in or near to the trajectory of the medial forebrain bundle support high rates of self-stimulation at rather low current intensities. Similar data were obtained when the intent was to examine the role of the nigro-striatal bundle (Prado-Alcala, Kent, & Reid, 1975), one of the many constituent pathways of the medial forebrain bundle (Lindvall & Bjorklund, 1974; Ungerstedt, 1971).

Additional support for a rostrocaudal (or caudorostral) orientation comes from investigations designed to determine the actual direction of conduction. Was the important information conveyed rostrally or caudally? Electrolytic lesions disrupt self-stimulation responding when they are made either posterior or anterior to the location of a stimulating electrode (Valenstein, 1966) and the same equivocal result is obtained with reversable lesions accomplished by the infusion of the local anaesthetics procaine (Nakajima, 1972) and xylocaine (Stein, 1968, 1969).

Szabo, Nad, and Szabo (1972) aligned the tips of bipolar electrodes parasaggitally and examined the effect of reversing the polarity of the stimulation. They obtained parallel rate-intensity curves with the cathode-anterior condition displaced towards the lower current intensities. This finding is consistent with the idea that the stimulated axons send their impulses rostrally. However, the opposite view, that the directly stimulated fibers convey information caudally, was forwarded by German and Holloway (1973). They measured the rate of response for trains of rewarding pulse-pairs, the members of which were split between two electrodes located in opposite hemispheres and at different anterior-posterior levels. Their assumptions were that the simultaneous arrival of rewarding impulses at some common integrator produced the highest reward value and that the highest reward would yield the highest rate of response. Their finding was that the highest rates were obtained when the more anterior electrode was given the first pulse; their conclusion was that the important direction of conduction was caudal (see Yeomans and Koopmans, 1974 for a critical appraisal of this study). Whether the stimulated axons send information to synapses located rostrally or caudally from a

mid-hypothalamic electrode site is still uncertain but the direction of impulses is not important for the present work. The important point, over which there is no controversy, is that the axons lie along a rostrocaudal line.

By far, the strongest support favouring the rostrocaudal axonal line stems from the experiments performed by Bielajew and Shizgal (1980; Shizgal, Bielajew, Corbett, Skelton, & Yeomans, 1980). These investigators implanted rats with two electrodes, one aimed at the ventral tegmental level of the medial forebrain bundle and the other aimed at a more rostral point, about 3 mm distant, at the level of the ventromedial nucleus. Trains of pulse-pairs were used to reward lever depressions by the rat and the two pulses in each pair were delivered to different electrode destinations. If the trajectory of the reward fibers were such that some axons passed through both stimulation fields, then, at short delays between the pulses, the orthodromically conducted volley of action potentials generated at the proximal electrode would collide with the antidromic set initiated at the distal probe. For fibers within both fields, one set of action potentials would arrive at the synapses. With longer delays, which would permit the action

potentials and their associated refractory wakes to pass the second electrode before the application of the second pulse, two volleys of action potentials would reach the synapses.

Unambiguous evidence for collision was obtained in these experiments. At the shorter delays, a constant low-valued effectiveness of the stimulation was seen. When the delay was increased beyond 1.2 msec (Mdn.) an abrupt rise in effectiveness occurred and further increases in delay continued to yield this constant, high-valued, level of effectiveness. That the rise occurred as late as it did rules out the possibility that refractoriness was being measured; recovery from refractoriness in self-stimulation tests is first seen at a delay of 0.4 msec and it is complete by about 1.2 msec (Miliaressis & Rompré, 1980; Rompré & Miliaressis, 1980; Shizgal & Skelton, 1980; Yeomans, 1975). Instead, this step in effectiveness was taken to reflect a true collision of ortho- and antidromic impulses in an uninterrupted, reward-relevant, collection of axons.

Taken together, the results of these studies unanimously support the idea that the directly stimulated reward cells are aligned parallel to the longitudinal axis. In order to use the

interelectrode distance as a direct measure of the separation between the centers of the two adjacent stimulation fields, the electrode pair must be positioned so that the two tips lie in the same coronal plane.

Behaviourally based inference of refractoriness. The study of the excitability cycle of a single axon involves a fairly direct procedure. Two electrodes, one stimulating and the other recording, are placed at different points along the axon's length. A pair of stimulating pulses is applied. The first pulse is termed the conditioning (or C-) pulse; it is fixed at a just-suprathreshold amplitude for the production of an action potential. The second stimulus is called the test (T-) pulse; it is presented after a variable delay (C-T interval) following the onset of the C-pulse. At any particular C-T interval, attempts are made to obtain two recorded action potentials per pulse-pair by varying the amplitude of the T-pulse.

The phases of excitability in an axon following the application of a C-pulse have been reviewed by Yeomans (1979) and they are outlined here as well. Two stages are well known but as many as four successive phases have been identified in some cells (Waxman & Swadlow, 1977). The earliest is termed

the absolute refractory period which lasts approximately the duration of the action potential. During this period, the cell does not fire in response to a T-pulse no matter what intensity is set. The next phase is called the relative refractory period during which the cell is excitable but the amplitude of the T-pulse must exceed that of the C-pulse in order to be effective. A supernormal period follows in some cells. At this stage the axon is excitable not only by T-pulses equal in amplitude to the C-pulse but also by somewhat less intense T-pulses. Finally, a long-lasting subnormal period may be seen. A T-pulse equal in amplitude to the C-pulse may fail to excite the membrane; an increase in intensity of about 5% is required for an action potential to occur.

In 1964, Deutsch introduced the idea that the behavioural output of a self-stimulating rat might reflect the post-stimulation excitability characteristics of the directly stimulated neurons that participate in brain stimulation reward. In other words, the behaviour of the subject was used as the barometer indicating the degree of recovery of these reward cells. In the first experiment, rats were rewarded with trains of pulse-pairs and only the C-T interval was systematically varied. Deutsch

reasoned that as the C-T interval was gradually increased, the T-pulse would find progressively fewer cells refractory to its stimulating effect. The resulting increase in the total number of fifings per stimulation train is analogous, from the rat's viewpoint, to the neural effects of increasing the frequency of single, evenly timed, pulses. Over a narrow range of pulse frequency, increases in this variable enhance response rates; accordingly, Deutsch predicted that as the T-pulse was delayed by greater and greater intervals, the rates of response would climb.

This prediction was borne out. Self-stimulation rates surpassed operant levels at a delay of 0.9 msec and maximal rates were obtained between 1.1 and 1.3 msec intervals. A preference test was also performed. Here, rats were offered the opportunity to select between stimulation trains with variable C-T intervals and a standard train with 0.7 msec delays. A shift in preference away from the standard was first seen when the alternative was set at 0.5 to 0.6 msec and, beyond 0.7 msec, the selection was random. In a third experiment, Deutsch assessed refractoriness by using a voltage-scaling procedure. A drop in the voltage required to sustain criterion performance

began at delays of 0.5 - 0.7 msec and it reached a low plateau at C-T intervals of 0.7 - 0.8 msec.

From a qualitative stand, the results of these experiments support the idea that the output of the organism might index the physiological events occurring in the behaviourally important neurons that are excited by the stimulation. From the quantitative vantage, however, the disconcerting aspect is that the ranges of C-T intervals, spanned by the changes in behaviour which presumably reflect recovery from refractoriness, differed from experiment to experiment. The rising portion of the rate changes occurred over the interval 0.9 - 1.3 msec, preferences shifted over the range 0.5 - 0.7 msec, and voltage thresholds changed between 0.5 and 0.8 msec C-T intervals. In each case, one or another form of self--stimulation was used as the dependent measure. Why were different ranges of refractory period seen?

Yeomans (1975) supplies the answer. In addition to documenting the response rates at different C-T intervals, he also examined the influence of varying the C-C interval (period of the waveform, equivalent to the reciprocal of pulse-pair frequency). The 56 to 95 different combinations of C-T by C-C intervals were tested in a random order and Yeomans assessed the results in two ways.

In the first method, the data (rates of response) were arranged as if a series of rate vs. C-T interval experiments were performed with each experiment conducted against a different background C-C interval (or frequency). At long C-C intervals (low frequency of pulse-pairs), the entire range of tested C-T intervals produced no responding. In a like fashion, variation of C-T interval against a high-frequency background of pulse-pairs failed to influence the rates of responding; now the rates were maximal at all delays. When the effects of varying the C-T interval were assessed at intermediate frequencies, the rates of response followed the pattern seen by Deutsch; in general, the rates were low at short C-T intervals and they were high at the longer delays. Moreover, a clear trend emerged over these intermediate-frequency tests. The range of C-T intervals containing the rising portion of the rate vs. C-T curves systematically drifted towards the lower C-T intervals as the frequency of pulse-pairs increased. The important implication of this finding is that different refractory period estimates are obtained depending upon the background test frequency employed. This result weakens the value of refractory determinations based entirely upon response rates.

In the second method, Yeomans re-arranged the data and now presented them as if each C-T interval were held constant while the frequency of pulse-pairs varied. This generated a family of roughly parallel ogives on the rate vs. frequency plot. The single-pulse condition, in which the T-pulses are absent, was located farthest to the right (indicating that highest frequencies were required for performance by the rat). The double-frequency condition, in which the T-pulses were temporally situated midway between the C-pulses, was positioned farthest to the left (lowest frequencies were needed to sustain the rat's interest). The rate-frequency functions for the different C-T interval conditions lay between these extremes.

For its heuristic value, I mention here that this method of arranging the data bears a conceptual similarity to the method used in pharmacology for the assessment of dose-response effects. Drugs that possess a modest efficacy are represented by ogives displaced to the right in the percent-effect vs. log-dose graphs. The more potent drugs' ogives are found in the left-hand region of the graph. In order to compare potencies of different drugs, the pharmacologist derives point-estimates of dose.

Graphically, this is accomplished with a horizontal line, arbitrarily positioned at, for example, fifty percent effect. This line cuts each drug's ogive and the abscissa at the cut point is termed the ED_{50} (Effective Dose needed to produce a 50% effect). It is presumed that when doses of different compounds produce the same degree of effect, provided that the ogives are parallel to each other, then the proportion of the receptors occupied by the different agents is constant.

In the same way that constant percent-effects are inferred by the pharmacologist to reflect the same proportion of occupied receptors, Yeomans proposed the following. He began with the idea that the vigour of behaviour (rate of responding, running speed, percent preference, etc.) is monotonically related to the excitation delivered to the nervous system. He then proposed that when one observes the same, intermediate-between-zero-and-maximal, level of performance then this level must have been the result of the same degree of excitation delivered to the nervous system. In more graphic terms, the idea is that a horizontal line across the family of rate-frequency ogives cuts these curves at equivalent-stimuli points. The points are

equivalent in the sense that they each supply the same degree of neural excitation.

The method used to scale the effectiveness of the T-pulse, relative to that of the C-pulse, was derived by considering how electrical stimulation affects a bundle of axons. Both pulses are applied at the same intensity; the same stimulation field would be produced by either pulse presented alone. The field contains a constant number of excited reward cells and this Number of axons is represented by N. Each C-pulse in the single-pulse condition fires these cells once and so the total excitation produced by the train is given by the product NC where C represents the number of C-pulses. When the train contains both C- and T-pulses, some of the cells that fire in response to the C-pulse will fail to respond to the T-pulse. That only a subset of the N cells fire a second time is represented by an Effectiveness term, E, which is applied as a proportion scalar against N, the number of axons fired by the C-pulse. The total excitation delivered towards ultimate post-synaptic integration in a train of pulse-pairs is given by NC + ENT where T stands for the number of T-pulses. The number of applied T-pulses always equals the number of administered C-pulses so the term may be

rewritten: NC + ENC.

Recall that when different stimulation configurations produce the same level of behaviour, it is assumed that the net neural excitation must be the same. Suppose that one were to cut, at the arbitrarily selected level of half-maximal response rate, the rate-frequency ogives obtained in the single-pulse (SP) condition and in one of the pulse-pair conditions (C-T). Perpendicular lines are drawn, from the cut points to the abscissa, and point-estimates of the frequencies required to produce half-maximal response rates with each condition are thereby obtained. Because the excitation produced at these values is presumed to be the same, the following equality is asserted:

$$NC_{SP} = NC_{C-T} + ENC_{C-T}$$

where the subscripts SP and C-T refer to the number of pulses, and pulse-pairs, delivered in the single-pulse, and pulse-pair, conditions respectively. Both sides of the equality are divided by N and it is re-arranged to solve for E:

$$E = (C_{SP} - C_{C-T})/C_{C-T} = (C_{SP}/C_{C-T}) - 1.$$

The quantities on the right-hand side are all obtained from the data. The terms C_{SP} and C_{C-T} respectively are the number of single-pulses and the number of pulse-pairs required to sustain a half-maximal rate of responding by the rat. With trains of stimulation fixed in length, pulse frequency may be readily used instead:

$$E = (F_{SP}/F_{C-T}) - 1$$

where F_{SP} and F_{C-T} are the single-pulse and pulse-pair frequencies, interpolated between obtained data points, which would support half-maximal responding.

The degree to which cells have recovered from refractoriness is scaled by E. When it is equal to zero, none of the cells excited by the C-pulse is activated a second time by the T-pulse. When E is equal to one, every one of the neurons fires a second time. Values of E intermediate between zero and one reflect proportional degrees of effect of the T-pulse relative to the power of the C-pulse. When Yeomans applied this method, the data from six different electrodes (five rats) converged in demonstrating earliest recovery at 0.4 msec and a plateau achieved at 1.2 msec. The use of this method in other laboratories has yielded the same estimates

(Miliaressis & Rompré, 1980; Rompré & Miliaressis, 1980; Skelton & Shizgal, 1980).

Yeomans' scaling method is used in the secondary experiment of this thesis. Individual refractory determinations are made for each electrode of the implanted pairs and at each of the current amplitudes used in the primary experiment. These estimates do not contribute directly to the current-distance relation; rather, their inclusion offers a check on the interpretation of data from the main experiment. At the higher tested intensities, steps are expected to appear in the effectiveness vs. delay functions. These steps are thought to reflect recovery from refractoriness in cells that lie between the two electrodes. If so, then the ranges of C-T interval spanned by these steps must match the refractory period estimates in the individual tests. The secondary experiment is designed to supply these estimates.

The assessment of effectiveness in the primary experiment is accomplished with a modified version of the E calculation also proposed by Yeomans (1979). The modification was recommended for the situation in which the C- and T-pulses were presented through the same electrode but at different current

amplitudes, thus producing stimulation fields of unequal size. In the present case the amplitudes are the same but the pulses are destined to different electrodes. Although the field sizes are imagined to be the same, one field may contain more behaviourally related cells than the other. The modified effectiveness determination takes this potential discrepancy into account.

How it does so is perhaps best described by starting with a rephrasing of the task performed by the conventional computation. When equal-in-amplitude stimuli are passed through the same electrode, both the C-pulse and the T-pulse attack the same stimulation field. The calculation of E is viewed to answer the question: On a scale of 0 to 1, how much of a rewarding effect does the T-pulse contribute relative to that offered by the C-pulse? When equal amplitude pulses are directed through different electrodes, the C-pulse may find more (or fewer) behaviourally relevant neurons within its reach than does the T-pulse. Seen in this light, the question answered by the modified version, presented below, is: On a scale of 0 to 1, how much, relative to the maximum that it could offer, does the pulse applied to the poorer field contribute to the rewarding effect

established by the pulse sent to the richer? (Shizgal, Note 1).

In operational terms, one field is deemed to be richer than its partner if the targeted behaviour is obtained with a lower single-pulse frequency. The single-pulse frequencies needed to yield criterion performance are denoted with the subscripts SPLo (Lower) and SPHi (Higher) and respectively relate to the richer and poorer stimulation fields. The modified calculation is:

$$E_{PP} = ((F_{SPLo}/F_{C-T}) - 1)/(F_{SPLo}/F_{SPHi})$$

The numerator on the right-hand side is the conventional effectiveness calculation for any particular C-T interval assessed against the single-pulse condition applied to the richer field. The denominator acts as a correction factor adjusting for the fact that each pulse delivered to the poorer field carries less hedonic impact that does a stimulus applied to the richer field. The subscripts PP (Pulse-Pair) are added as a reminder that this effectiveness does not always refer to the power of the T-pulse.

To summarize, the plan is to perform a series of double-pulse tests. In the main experiment, the pulse-pairs are split between two adjacent electrodes

in order to detect whether some of the rewarding effect of the brain stimulation is being conveyed via cells enclosed by both stimulation fields. Evidence for such overlap in the stimulation fields should be obtained in the high-intensity tests and it should be absent in the low-intensity tests. The intensity that separates the tested range into two portions, one with and one without evidence for overlap, is taken as the intensity at which the stimulation fields just meet each other. That value of intensity and the corresponding half-interelectrode distance form a co-ordinate pair towards the solution of the current-distance relation for rewarding brain stimulation.

METHOD

Subjects and Surgery

Twenty-three male hooded rats, ranging in weight from 370 to 590 g, were surgically prepared for this investigation. The rats were housed individually in stainless-steel cages and they had food and water available at all times. Circadian lighting was adjusted to a 12/12 hr cycle with light onset at 07:00 hrs.

The rats were anaesthetized with Nembutal (Abbott Laboratories, 60 mg/kg intraperitoneally) and they were injected subcutaneously with 0.9 mg atropine sulphate to limit the accumulation of fluid in air passages. The head was fixed in a stereotaxic instrument with the upper incisor bar set 5 mm above the center of the ear bars. The co-ordinates used to direct the electrode assembly to the lateral hypothalamic region were: 1.0 mm behind bregma, 1.4 to 1.7 mm left of the mid-saggital suture, and 8.0 mm below the cortical surface. The lateral co-ordinate, which varied from rat to rat, refers to a point mid-way between the two electrode tips. Four or five #0 stainless-steel machine screws served as anchors for the dental acrylic and a length of bare stainless-steel

wire, leading to the indifferent connector, was wrapped around at least three of them.

Electrodes

The electrode assemblies were constructed from short lengths of straightened stainless-steel wire (254 µm dia.) soldered to Reli-a-tac plugs (Amphenol) and insulated with three to five coats of Formvar (General Electric). Each assembly consisted of a pair of electrodes fixed together such that the electrode shafts were parallel and equal in length.

This was achieved by placing two right-angle bends close to the connector in one member of each pair. The two electrode shafts were then trapped beside each other between small blocks cut from a pencil eraser and a drop of epoxy was used to fix them. While the epoxy was setting, small adjustments in the distance between the tips were made by hand.

Once the epoxy had set, the electrode pairs were cut to length (about 10 mm) and the tips were ground flat with a polishing stone and polished with a piece of ground glass. The finished assemblies were inspected with the aid of a dissecting microscope and were then tested for insulation and continuity. This procedure produced electrode pairs with intertip distances ranging from 0.3 to 0.7 mm (center to

center) and with exposed surface areas that were uniformly 0.05 mm^2.

Apparatus

The test cage was made of three white plywood walls and one plexiglas wall which permitted the experimenter to observe the rat. The walls were 38 cm high and the dimensions of the 13 mm grid floor were 36 by 30 cm. Two Lehigh Valley rodent manipulanda were located opposite each other, centered on the narrower wooden walls. Both levers extended 25 mm into the chamber and their top surfaces were 4 cm above the floor. Throughout the experiment, only one lever was used.

Stimulation was provided by a dual constant-current source (Mundl, 1980) which was continuously adjustable to 1000 µA. The two channels were cross-linked such that when one channel was delivering a pulse the other was forced into a high-impedance state. This feature assured that the current return was always through the indifferent electrode. Although the stimulation was always monophasic, another feature of the constant-current unit prevented the temporal accumulation of charge at the electrode tips. When neither channel was called upon to deliver a pulse, both electrodes were shunted to ground through 560 ohm resistors. The stimulation was continuously monitored

by reading the voltage drop across a 1 kohm resistor in series with the current return.

Procedure

The Paradigm. The paradigm employed was designed for the rapid accumulation of rate-frequency data. Each depression of the lever produced a 0.5 sec train of 0.1 msec cathodal pulses as long as the response was made while no train was being delivered. The control equipment automatically advanced through a descending sequence and each frequency in the sequence was made available for eight stimulation trains. The steps from one frequency to the next were roughly 0.1 log unit in size; each presented frequency was about 80% of the previous one. For example, the first eight stimulations were delivered at 104 Hz, the next 8 at 80 Hz, 8 at 64 Hz, then 52, 40, 32, and so on. The range of test frequencies presented was selected on an individual basis depending upon the rat and the values of the other stimulation parameters. The selection was guided by the attempt to obtain maximal rates of response at least at the first two, and preferably over the first three, presented frequencies.

It had been found that with this sequence, and with attempts to obtain ceiling performance at the

three initial test frequencies, rats working for lateral hypothalamic stimulation would take about one-half min to progress through to a frequency that they would not self-administer. Accordingly, an automatic timer, which controlled resets to the initial frequency of the series, was adjusted to a 60 sec interval. Thus, resets to the inital frequency occurred at one min intervals irrespective of the rat's behavior. This reset, which except in early training sessions always occurred at a time when the rat had quit responding and was lying quietly in the test cage, was loudly signalled by the concurrent resetting of a Gerbrands cumulative recorder.

From the rat's perspective, here anthropomorphized in the interests of clarity, the paraidgm ran as follows: A loud noise indicated that lever depressions would be rewarded with rather strong stimulation and this strong level could be earned eight times. Continued responses produced a weaker set of eight available stimulations but these were still worth working for. Progressive decrements in stimulation strength continued in this fashion and, eventually, the offered reward was too low to work for. The rat would quit responding altogether. About one-half min would elapse, the loud noise

would occur again, and the sequence of events would be repeated.

The Trial and the Dependent Measure. The control equipment automatically accumulated the number of responses that were made to earn, and the amount of time that was spent in earning, each set of eight stimulations. All test conditions were presented in three consecutive passes through the sequence and thus a trial lasted three min. The first min (or pass) served to familiarize the rat with the newly adjusted stimulation settings and the last two were reserved for the collection of data. These data were converted into rates of response and the rate-frequency data pairs were used to determine the frequency required to sustain a half-maximal rate of response.

All of the assessments of refractoriness in this investigation are based upon a single type of dependent measure, the calculation of which is here described. It is the frequency, interpolated between two observed rate-frequency points, which theoretically would support a half-maximal rate of response if it were presented to the rat. Its shortened name is "required frequency" and it is abbreviated as F followed by a string of subscripts indicating the exact condition tested. It

corresponds to "frequency threshold" (or FT_{XXX}) in other reports (Bielajew & Shizgal, 1980; Shizgal et al., 1980; Yeomans, 1975).

The method used to determine the required frequency is illustrated in Figure 4. In the figure, rates are plotted as a function of test frequency for a sequence beginning at 80 Hz. Note, first, that the abscissa is logarithmic and, second, that it is inverted to reflect the descending sequence of presented frequencies. The highest of the rates observed in the first three test frequencies is designated as the maximum rate. This rate is halved and the new value is designated 1/2 MAX. The required frequency is found by interpolation between the two rate-frequency points bounding the half-maximal rate. This interpolation is graphically represented by the horizontal 1/2 MAX line reflecting down to the abscissa. The abscissa is taken as the required frequency; in the graphic example it is 38 Hz.

Stimulation Conditions. Six different stimulation configurations or conditions were used (Figure 5). Two single-pulse conditions, designated single-pulse--lateral (SPL) and medial (SPM), consisted of pulse trains with equal temporal spacing between

Figure 4

The basic dependent measure. Rates of lever depression are plotted against frequency of brain stimulation. Required frequency is interpolated at half of the maximum rate.

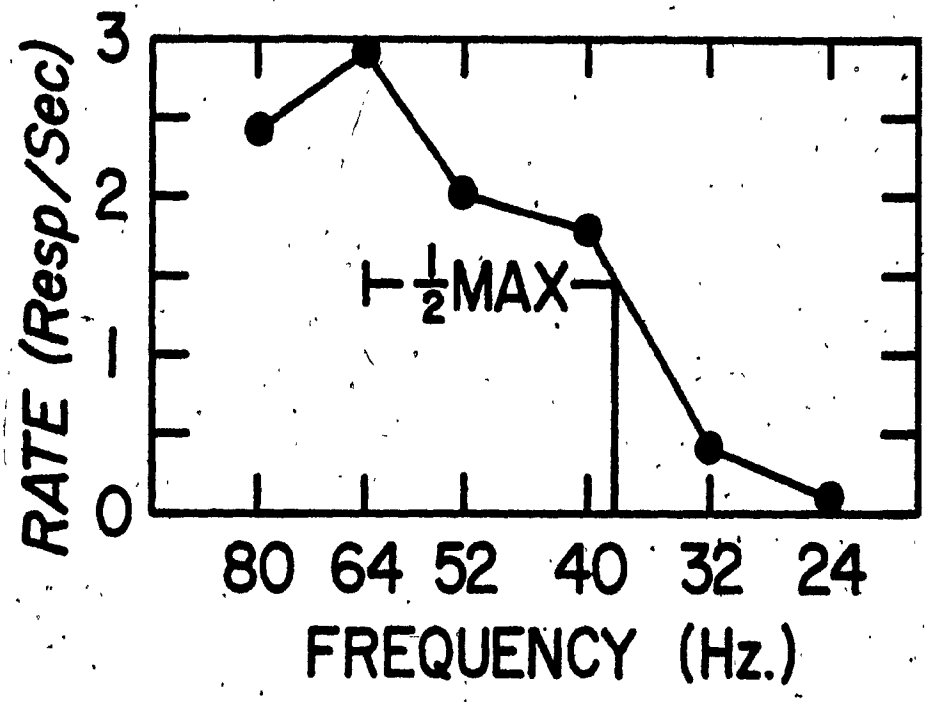
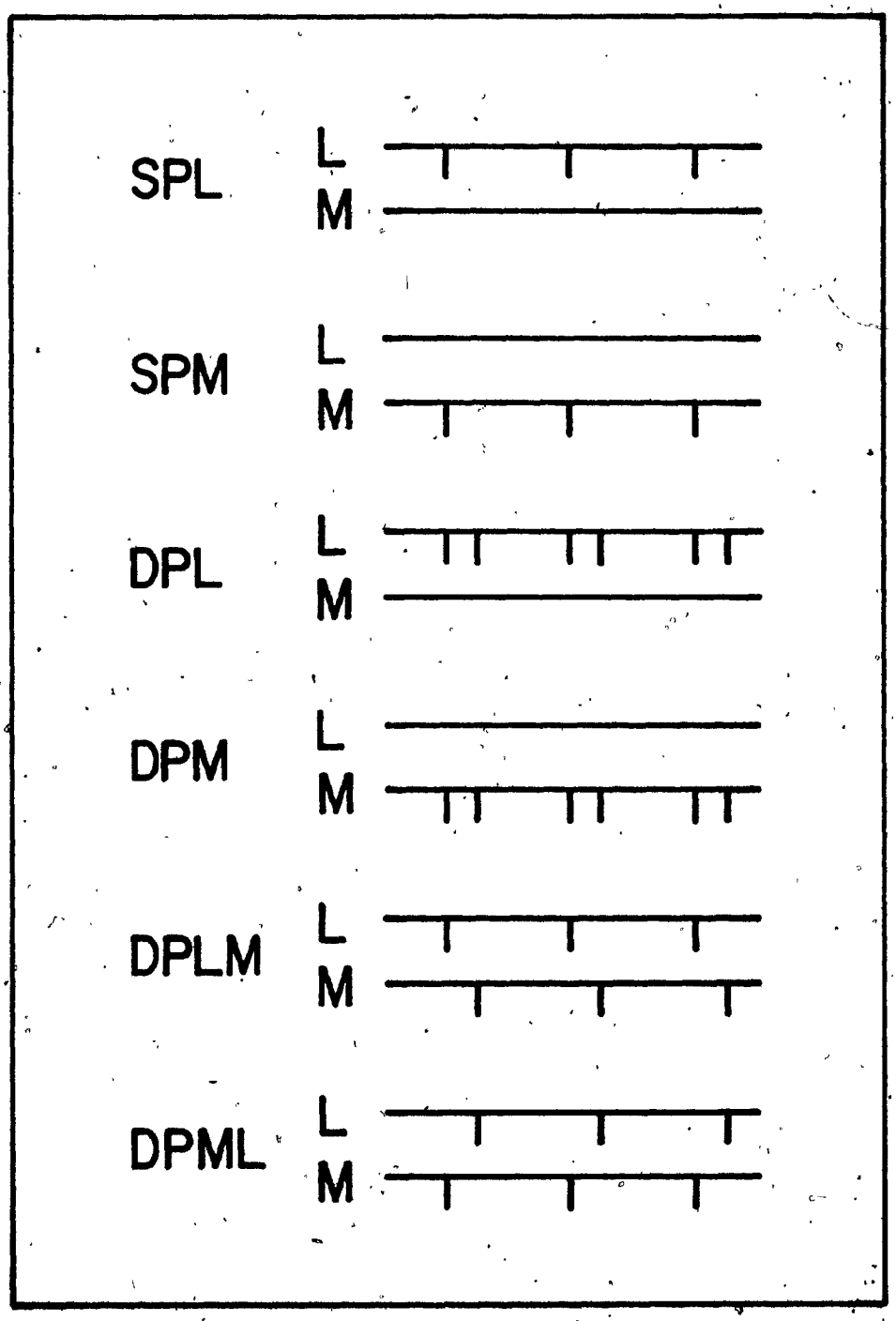

RATE (Resp/Sec)
3
2
1
0
½MAX
80 64 52 40 32 24
FREQUENCY (Hz.)

Figure 5

The pulse conditions. S, Single; D, double; P, pulse; L, lateral; M, medial. The clusters designate test conditions and the single letters refer to electrodes.


SPL
L
M
SPM
L
M
DPL
L
M
DPM
L
M
DPLM
L
M
DPML
L
M

each pulse. Lateral and medial refer, of course, to the position of the destined electrode. The single-pulse conditions were used to measure the required frequency of the individual electrodes and these frequencies, in turn, were used as standards against which the effectiveness of double-pulse stimulation was assessed. (The terms double-pulse and pulse-pair are used interchangeably in this text.)

In the four double-pulse configurations, trains of equally timed C-T pairs were delivered. Only five tested delays were interposed between the onsets of the C-pulse and the T-pulse and these were: 0.2, 0.5, 1.0, 2.0, and 5.0 msec. Two of the four double-pulse conditions, double-pulse-lateral (DPL) and medial (DPM), were used in the conventional assessment of refractoriness of cells at the individual electrode placements. In these two conditions both the C- and the T-pulse were applied to one electrode.

The last two double-pulse conditions were used to detect the presence of cells, refractory to stimulation, that lay between the two electrodes. Here, the C-pulse was applied through one electrode and the T-pulse was delivered through the other.

The order of presentation of pulses was fully counterbalanced; in half of the tests the C-pulse was passed through the lateral electrode (double--pulse-lateral-medial, DPLM) and, in the other half, the C-pulse was delivered through the medial electrode (DPML).

Training. After a post-operative recovery period of at least three days, the rats were screened for self-stimulation. The animals were connected, placed into the test cage, and allowed to explore it for three minutes. At first, the equipment was set up to deliver single pulses, 300 μA in intensity and with an initial frequency of 104 Hz. Any lever depressions during the three min exploration period were reinforced.

Conventional shaping procedures followed with the modification that shaping was restricted to times when the three highest frequencies were available. The current amplitude was adjusted early in these bouts of shaping towards the goal of getting the rat to self-stimulate for about the first 30 sec of each minute-long run. The second electrode was similarly tested.

Once the rat demonstrated proficiency in this paradigm (indicated by repeatedly quitting at about

the same frequency and by rapid returns to the lever following the signalled resets), the equipment was switched into an alternating mode. Now, each electrode was tested for one three-min trial and the machine alternated back and forth between electrodes. The training session continued in this manner until two hours had elapsed since the rat had first been introduced into the chamber. At this stage, rats that failed to self-stimulate for pulses delivered through either of the two electrodes were discarded.

Rats that responded for brain stimulation through both electrodes in the initial screen were, after a delay of at least one day, given a preliminary frequency-intensity workup. This involved documenting the required frequency of single pulses for each electrode over a wide range of test intensities. The intensities ranged from 100 to 1000 μA and they were presented in approximate 0.1 log unit steps. Specifically, they were: 100, 125, 160, 200, 250, 320, 400, 500, 630, 800, and 1000 μA. Appropriate adjustments in the initial frequency were made, again, with the aim of having the rat quit responding about half-way through the minute-long sequences. For example, the initial frequency for a test amplitude of 125 μA may have been 208 Hz;

an amplitude of 800 μA might have been accompanied by a starting frequency of 32 Hz.

The intensities were tested in an ascending order and consecutive trials alternated between electrodes. The frequency-intensity workup began with one electrode being tested at 100 μA in one trial, the second trial was a 100 μA test of the second electrode, the third was a 125 μA test of the first electrode, and so on. Alternating trials continued until all 11 intensities had been applied through both electrodes.

This preliminary frequency-intensity workup served three functions. First, it provided additional training to the rats in the test paradigm and it exposed the rats to a variety of parameter combinations. Second, it offered to the experimenter a guideline for the selection of initial frequencies in subsequent tests; often, with increases in intensity, debilitating motor effects would be recruited by one or the other electrode. Because there was no way to easily predict the influence of rate-reducing motor effects on subsequent tests involving pulse-pairs split between the two electrodes, rats demonstrating motor effects of brain stimulation were discarded.

Test Sessions; Primary Experiment. The primary

test sessions included the conditions DPLM and DPML in which the C-pulse was applied to one electrode, and the T-pulse to the other. Five consecutive trials, one for each of the C-T delays, formed a "block" and each session contained four blocks. Two blocks featured DPLM tests and in the other two the pulses' destinations were reversed (DPML). The four blocks were preceded, followed, and separated by two single-pulse trials, one each of SPL and SPM. Each session began after a 10 min warmup during which the rat was presented with a sample of the forthcoming conditions. The entire session thus comprised: a 10 min warmup period, 10 single-pulse trials (5 each of SPL and SPM), and 20 double-pulse trials (2 of each combination of C-T interval and destination of the C-pulse). With each trial lasting three min, a session requiring no repetitions of trial (because of either experimenter error or auditory disturbance of external origin) lasted one hour and forty min. No rat received more than two sessions in a single day.

Equal intensity C-pulses and T-pulses were used throughout. Although the overall design called for variation in stimulus intensity, each session featured a constant value for this variable. Tested intensities were varied from session to session but

the changes in amplitude followed no systematic pattern. Instead, the selection was guided by an exploratory intent. If, at some intensity, a rat had demonstrated a clear step in the E_{pp} vs. C-T function, indicative of overlap in the two stimulation fields, then the next session would be held at a lower intensity. Alternatively, if previously obtained functions were flat, higher intensities were then tried. All values of intensity fell within the range described for the frequency-intensity tests. Furthermore, the values used were consecutive in that sequence. Thus, if one rat demonstrated flat E_{pp} vs. C-T functions at 160 and 200 μA, and unequivocal steps at 400 and 500 μA, then this animal would be tested at all the intermediate intensities as well. The amplitudes tested would be: 160, 200, 250, 320, 400, and 500 μA.

It had been anticipated that the magnitude of the steps in the E_{pp} vs. C-T functions would be small. This anticipation was based upon the consideration that, relative to the total area encompassed by two equally sized, non-concentric, circles, the area intersected by them is small. Accordingly, two sessions were devoted to each tested intensity in the primary experiment. This gave four replications

of the primary conditions and since, for any chosen C-T interval, DPLM and DPML configurations should produce identical excitation, these two conditions were collapsed, giving a total of eight replications.

Test Sessions; Secondary Experiment. The secondary experimental sessions contained the tests in which C- and T-pulses were both applied to the same electrode. The experimental protocol here was identical to that of the primary sessions. The distinction was that now the DPL and DPM configurations were used. The four blocks were again preceded, separated, and followed by SPL and SPM trials and these sessions too followed a ten min warmup. The intensities at which these tests were conducted matched those used in the primary sessions and the order of presentation of the different amplitudes was similar but not identical. Only one session was held at each intensity and thus each condition was performed twice.

Test Sessions; Common Features. As much as was experimentally possible, both types of session contained fully randomized or counterbalanced orders of presentation. The order of blocks was counterbalanced; if, for example, a conventional refractory session began with a block of

double-pulse tests of the medial electrode, then the following session began with tests of the lateral electrode. Within a session the blocks alternated between electrodes or, in the case of C- and T-pulses with opposite destinations, blocks alternated between which electrode received the C-pulse. The order of presentation of C-T intervals within each block was pseudorandomly generated with the imposed provision that the four blocks of a session began with different C-T interval trials.

Within each session the current intensity was held constant and equal for both electrodes. Throughout the experiment the following variables remained unchanged: Monophasic cathodal stimulation was used; the pulse duration was always 0.1 msec; and the duration of the trains of stimulation was fixed at 0.5 sec.

Histology

At the end of testing, the rats were injected with a lethal dose of Nembutal and transcardially perfused, first with saline and then with formal saline. Each rat's head was fixed into a stereotaxic instrument at the same angle that was used for surgery and the crown was cemented to a screw held by the instrument's manipulator. The

dorsal part of the cranium, to which the crown was attached, was carefully separated from the rest of the skull and the entire electrode assembly, including the attached island of bone, was slowly raised away from the remainder of the head. The brain was then extracted and stored in formol saline for at least one day.

The tissue was frozen and serial sections were cut in the coronal plane. The forty-micra thick sections were mounted on gelatin-coated slides and, after the sections had dried, they were remoistened and examined through a projection microscope. Tracings were made of the median section containing damage due to the presence of the electrode tips.

Histological verification was not possible for one rat (#388). About a month after testing had been completed, this animal dislodged its crown.

RESULTS

Five rats completed all phases of the experiment. The others were rejected for failing to meet the imposed criteria for inclusion. These failures comprised: rats that would not self-administer stimulation delivered through one of the two electrodes (a frequent occurrence with the larger interelectrode distances), animals demonstrating motor effects of the stimulation, and rats not meeting an additional requirement that was based upon an inspection of the single-pulse data collected during the primary sessions. The criterion was that, at each of the tested intensities, the single-pulse required frequencies for the two electrodes had to lie within 0.2 log units of each other ($(F_{SPHi}/F_{SPLo}) < 1.58$). The rationale for this criterion is presented in Appendix II. For those not familiar with the idea of a counter model (Gallistel, 1976, 1978), Appendix I outlines the notions that are prerequisite to the content of Appendix II. Figures 6 to 10 show the single-pulse required frequencies for the five animals. The four rats for which verification was possible were found to have correctly oriented electrodes; examination of the histological material revealed that both of the

Figure 6

Frequency-intensity results, Rat 379

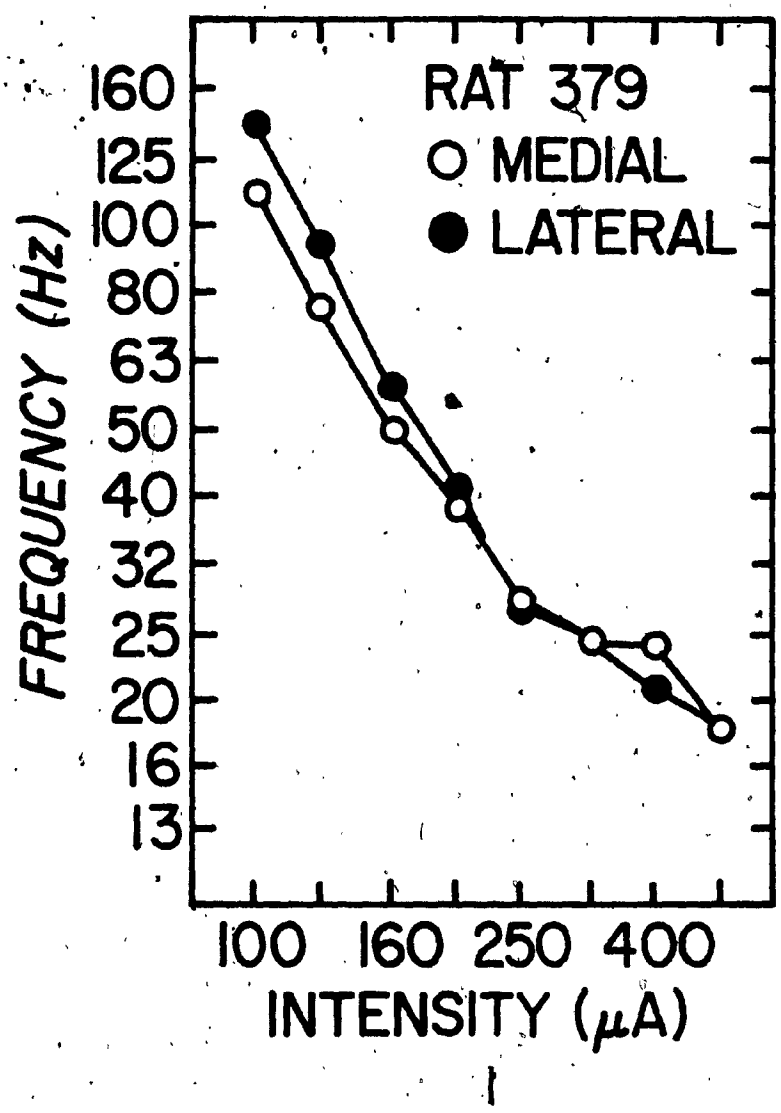

RAT 379
MEDIAL
LATERAL
FREQUENCY (Hz)
160
125
100
80
63
50
40
32
25
20
16
13
100
160
250
400
INTENSITY (μA)

Figure 7

Frequency-intensity results, Rat 386

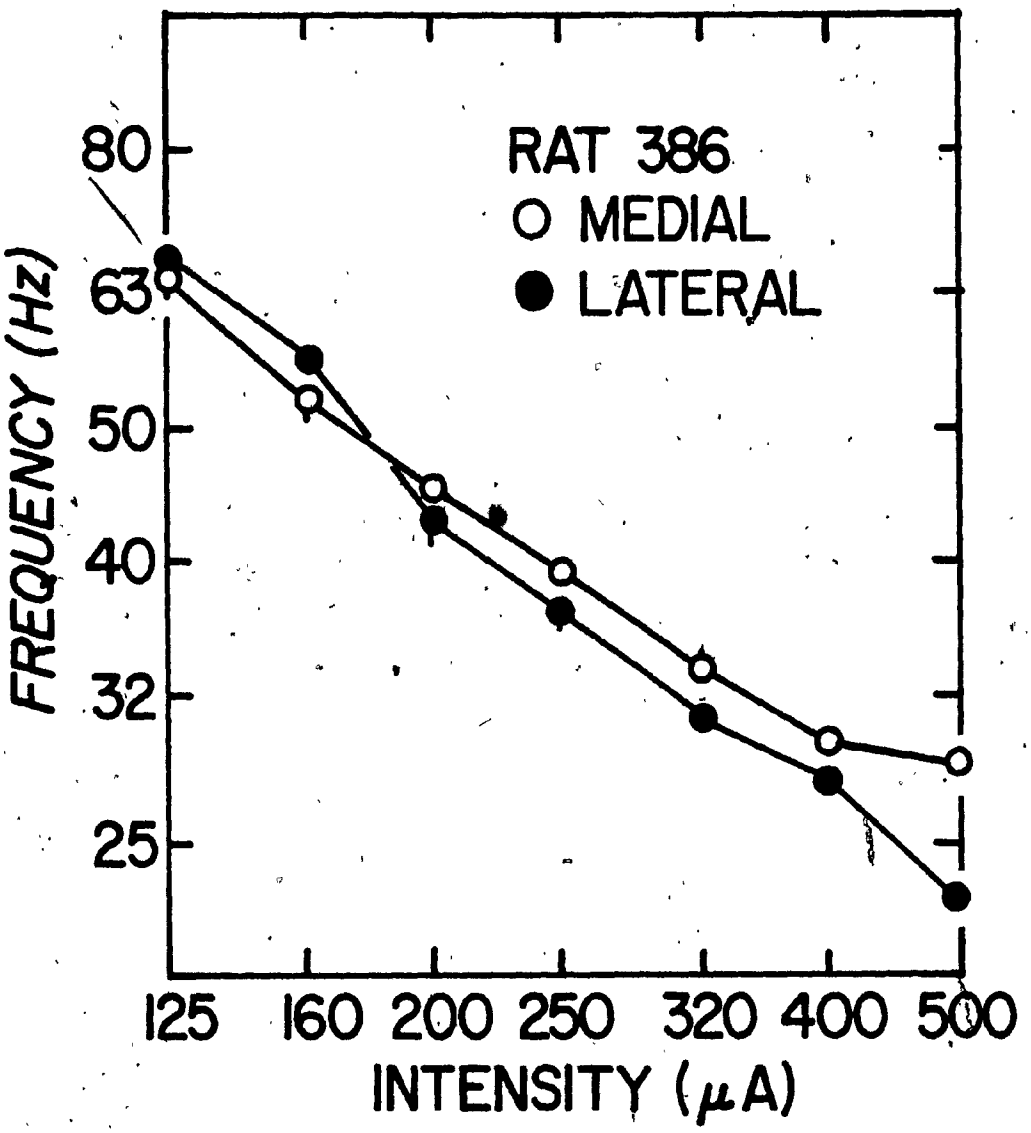

RAT 386
O MEDIAL
● LATERAL
FREQUENCY (Hz)
80
63
50
40
32
25
125
160
200
250
320
400
500
INTENSITY (μA)

Figure 8

Frequency-intensity results, Rat 388

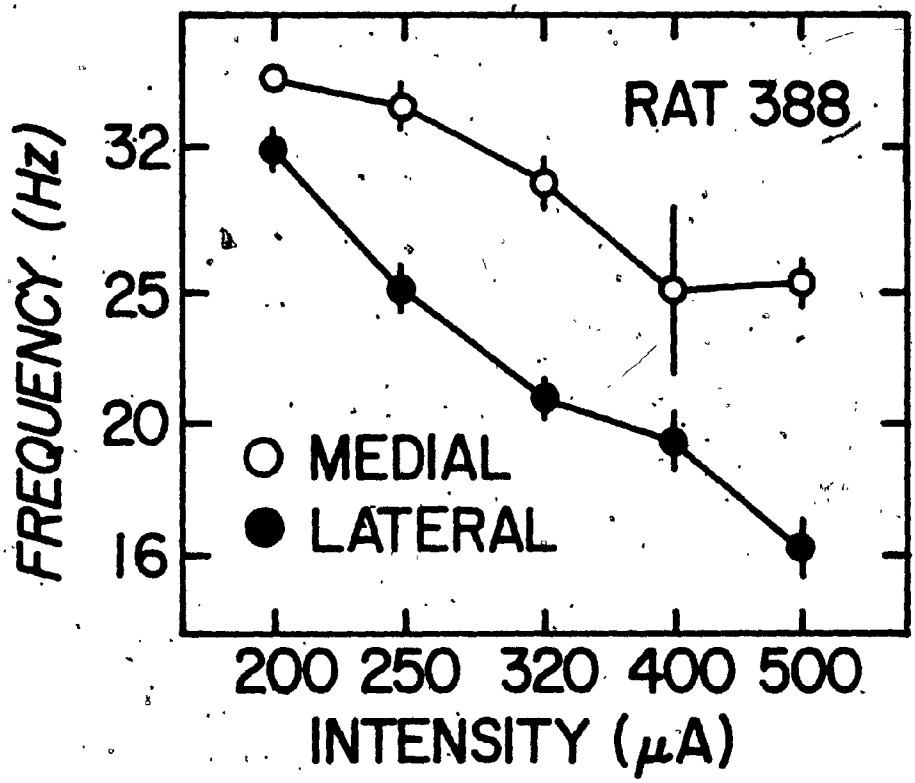

RAT 388
FREQUENCY (Hz)
32
25
20
16
MEDIAL
LATERAL
200 250 320 400 500
INTENSITY (μA)

Figure 9

Frequency-intensity results, Rat 398

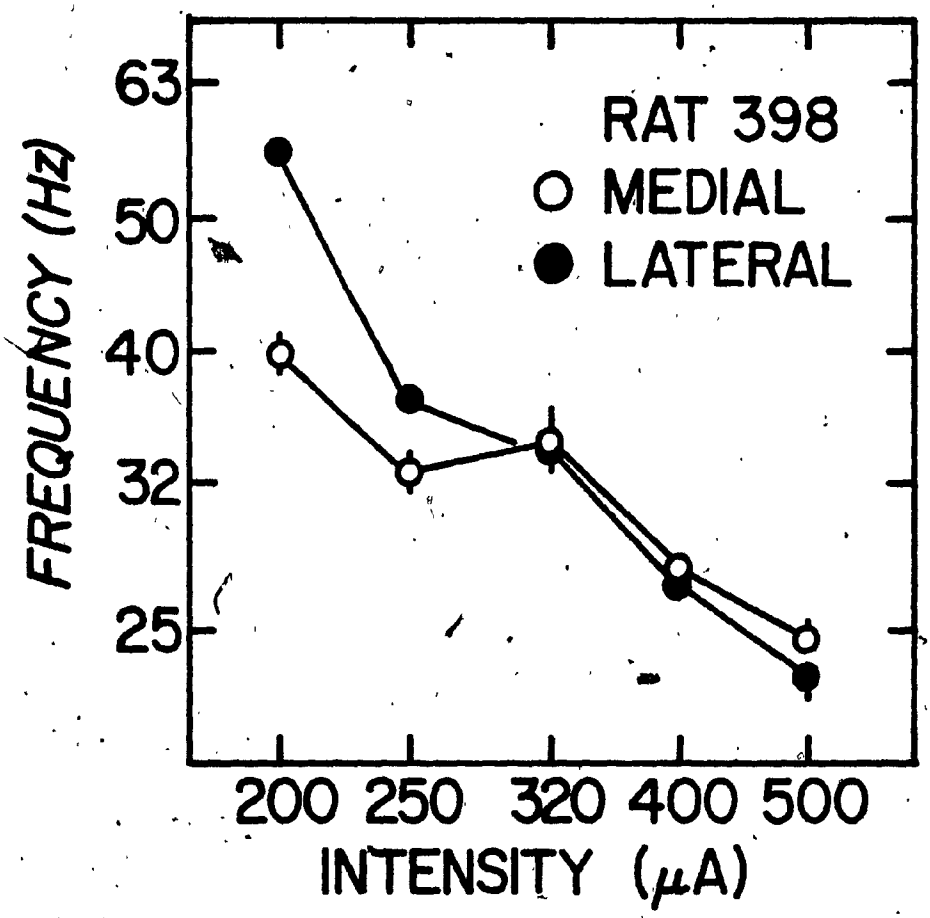
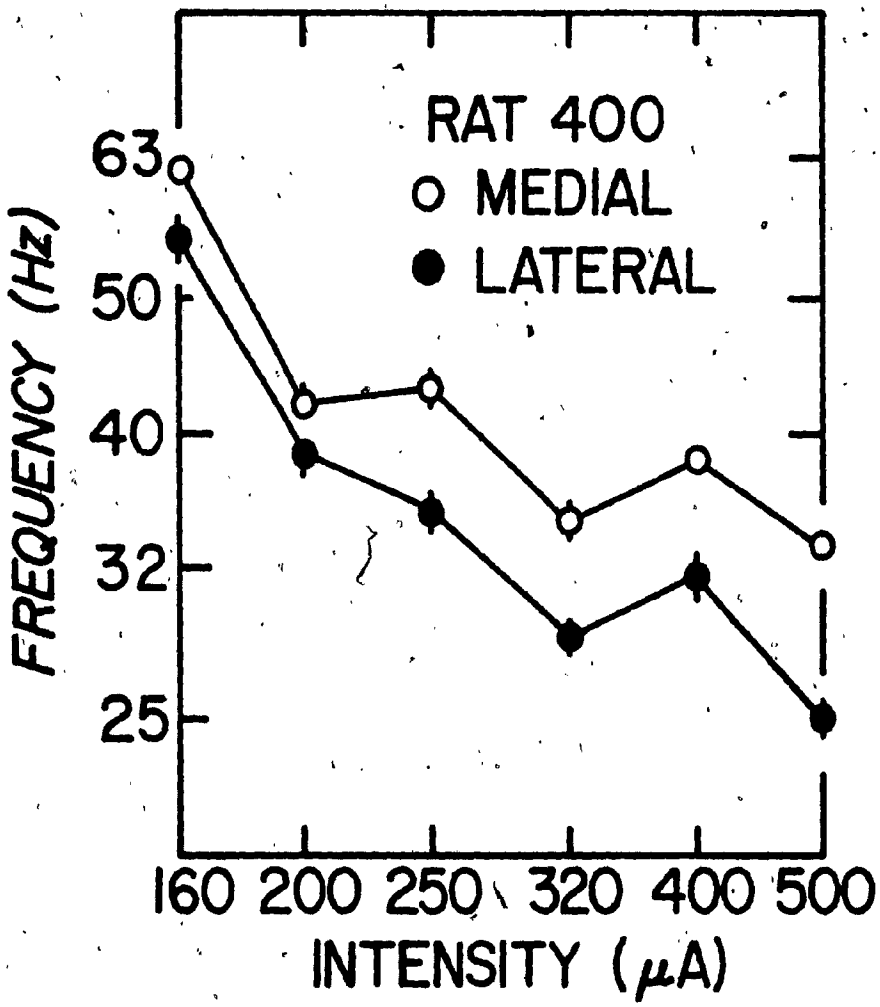

RAT 398
MEDIAL
LATERAL
FREQUENCY (Hz)
63
50
40
32
25
200 250 320 400 500
INTENSITY (μA)

Figure 10

Frequency-intensity results, Rat 400


RAT 400
MEDIAL
LATERAL
FREQUENCY (Hz)
63
50
40
32
25
160 200 250 320 400 500
INTENSITY (μA)

electrode tips were always located in the same coronal plane (Figure 11).

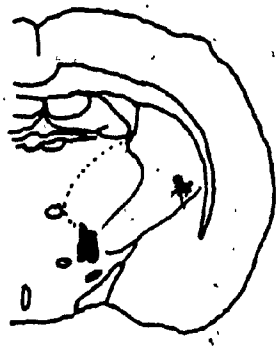
The results of the individual refractory determinations appear in Figures 12 to 16. Each figure is dedicated to one rat and it includes the refractory estimates at each of the intensities that were tested in the primary experiment. The interval between the C-pulse and the T-pulse is shown along the abscissa; for clarity of presentation, a logarithmic scale was used. The effectiveness of the T-pulse is plotted along the linear ordinate. The data obtained in tests of the lateral electrode are represented by filled circles and the open circles correspond to tests of the medial electrode. One point could not be determined. In the 100 μA test of the lateral electrode of Rat 379 (Figure 12), the required frequency for the 5.0 msec delay was estimated to be in the vicinity of 100 Hz. To make this assessment, pulse-pair frequencies of 160, 128 and 104 Hz would have had to be administered but the five msec delay would, at these frequencies, position the T-pulse beyond the halfway mark in the C-C interval. No attempt was made to measure this point.

Overall, T-pulse effectiveness was lowest at

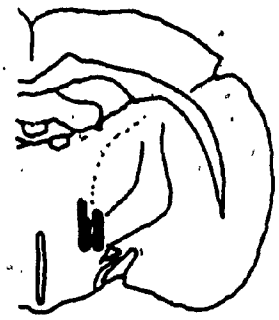
Figure 11

Histological reconstructions. Blackened area represents the damage produced by the electrodes.

RAT 379



RAT 386



RAT 398

RAT 400

Figure 12

Single electrode refractory determinations, Rat 379

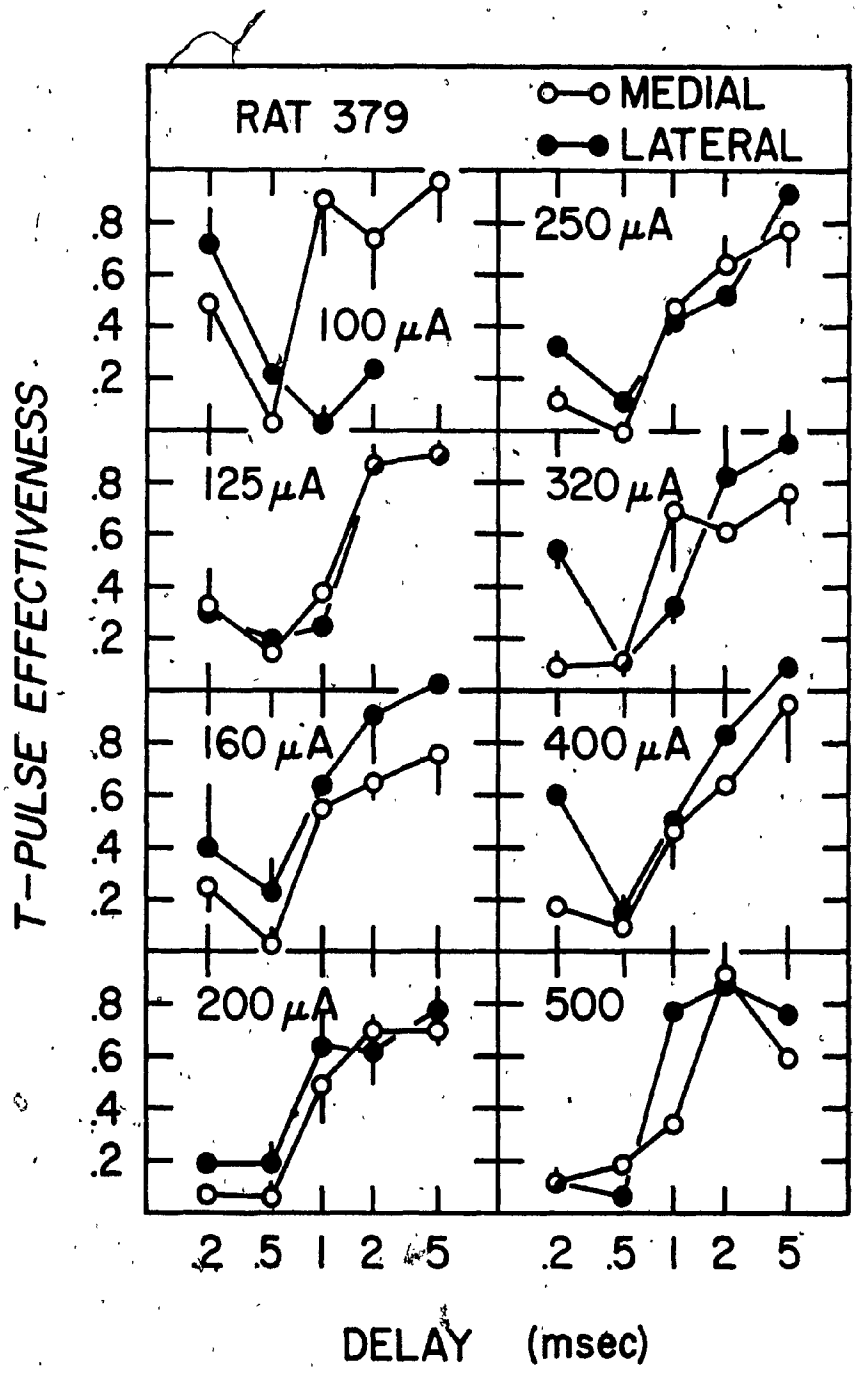

RAT 379
MEDIAL
LATERAL
100 μA
250 μA
125 μA
320 μA
160 μA
400 μA
200 μA
500
.8
.6
.4
.2
.2 .5 1 2 5
T-PULSE EFFECTIVENESS
DELAY (msec)

Figure 13

Single-electrode refractory determinations, Rat 386

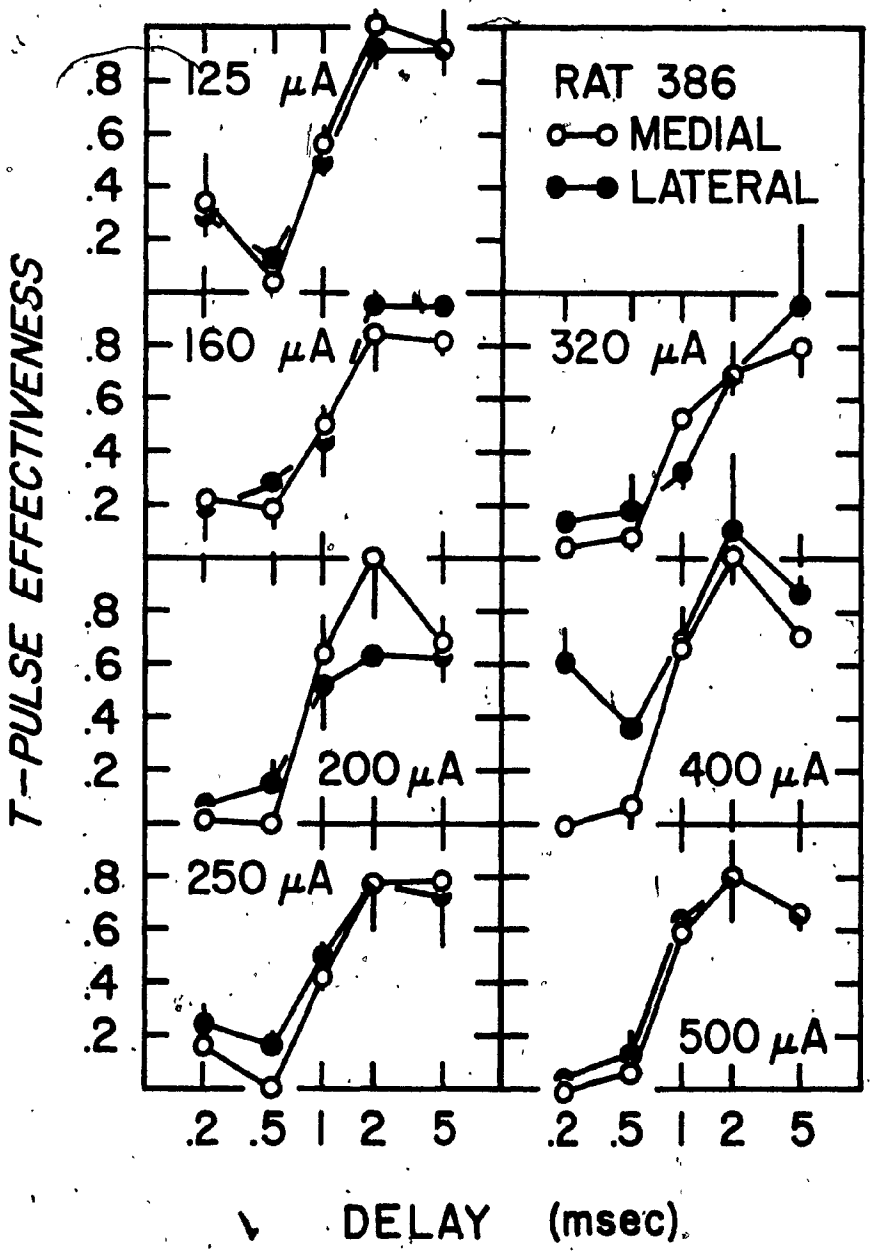

RAT 386
MEDIAL
LATERAL
125 μA
160 μA
320 μA
200 μA
400 μA
250 μA
500 μA
T-PULSE EFFECTIVENESS
.8
.6
.4
.2
.2 .5 1 2 5
DELAY (msec)

Figure 14

Single-electrode refractory determinations, Rat 388

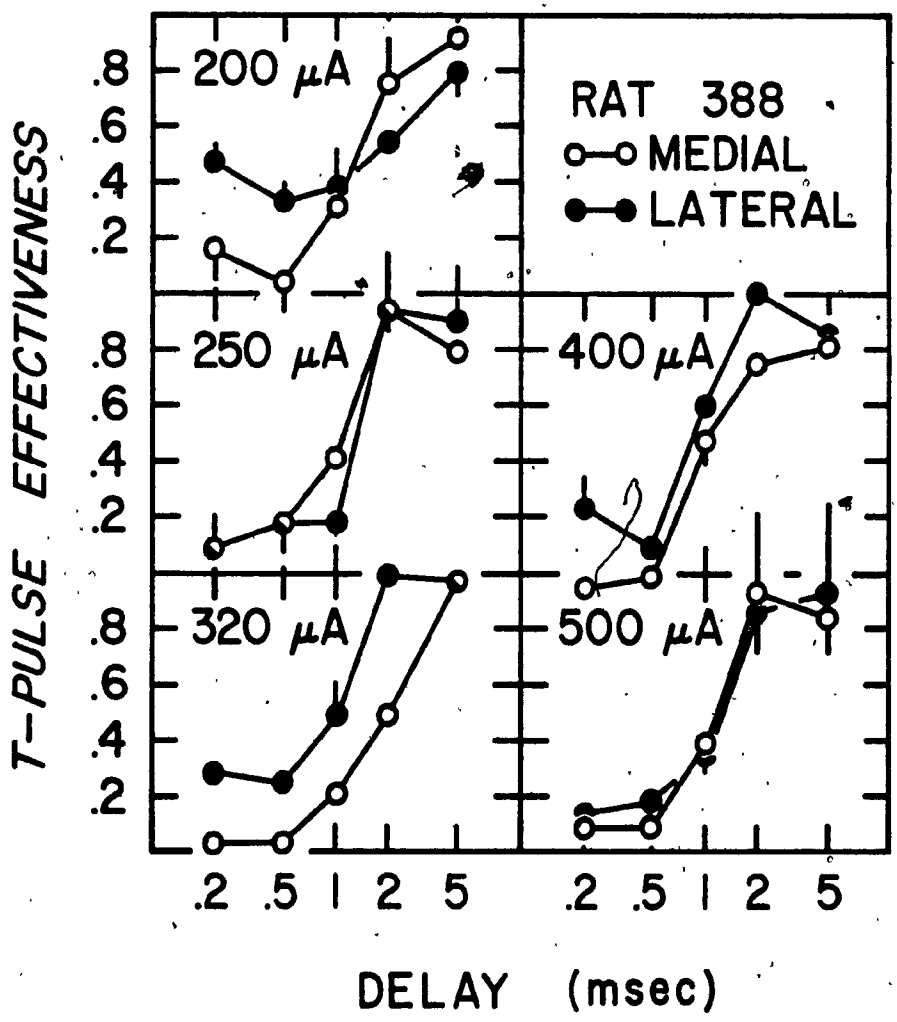

200 μA
250 μA
320 μA
400 μA
500 μA
RAT 388
MEDIAL
LATERAL
T-PULSE EFFECTIVENESS
.2
.4
.6
.8
.2 .5 1 2 5
DELAY (msec)

Figure 15

Single-electrode refractory determinations, Rat 398

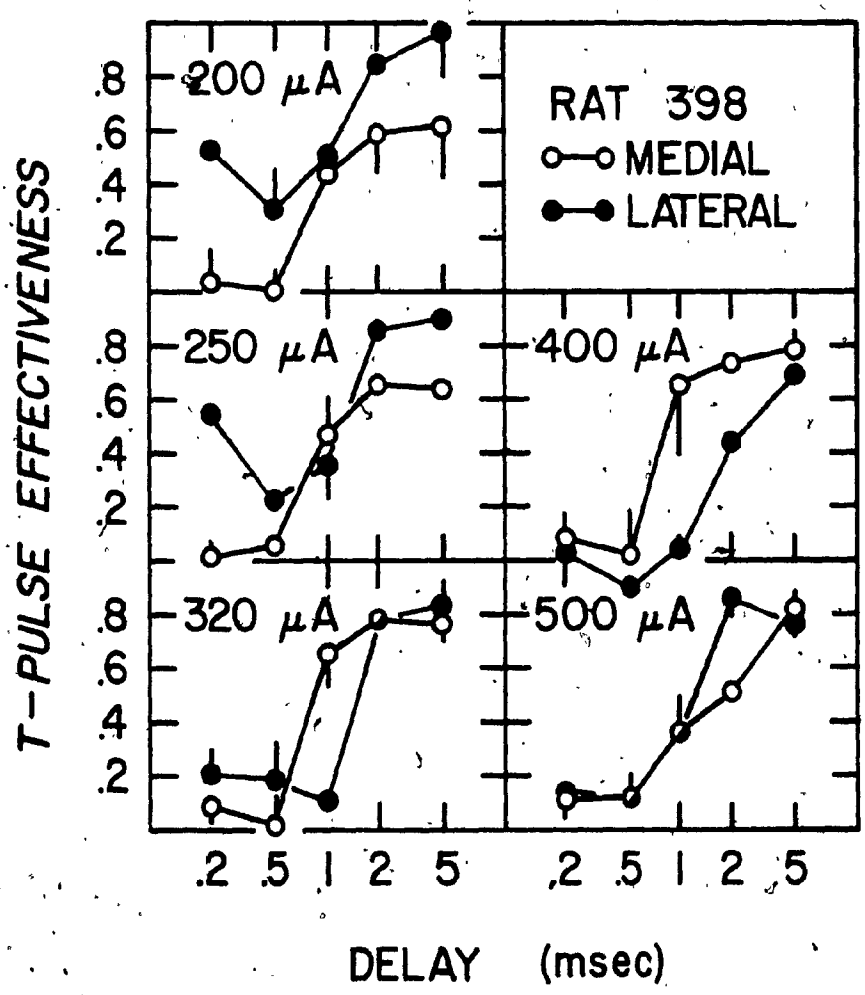
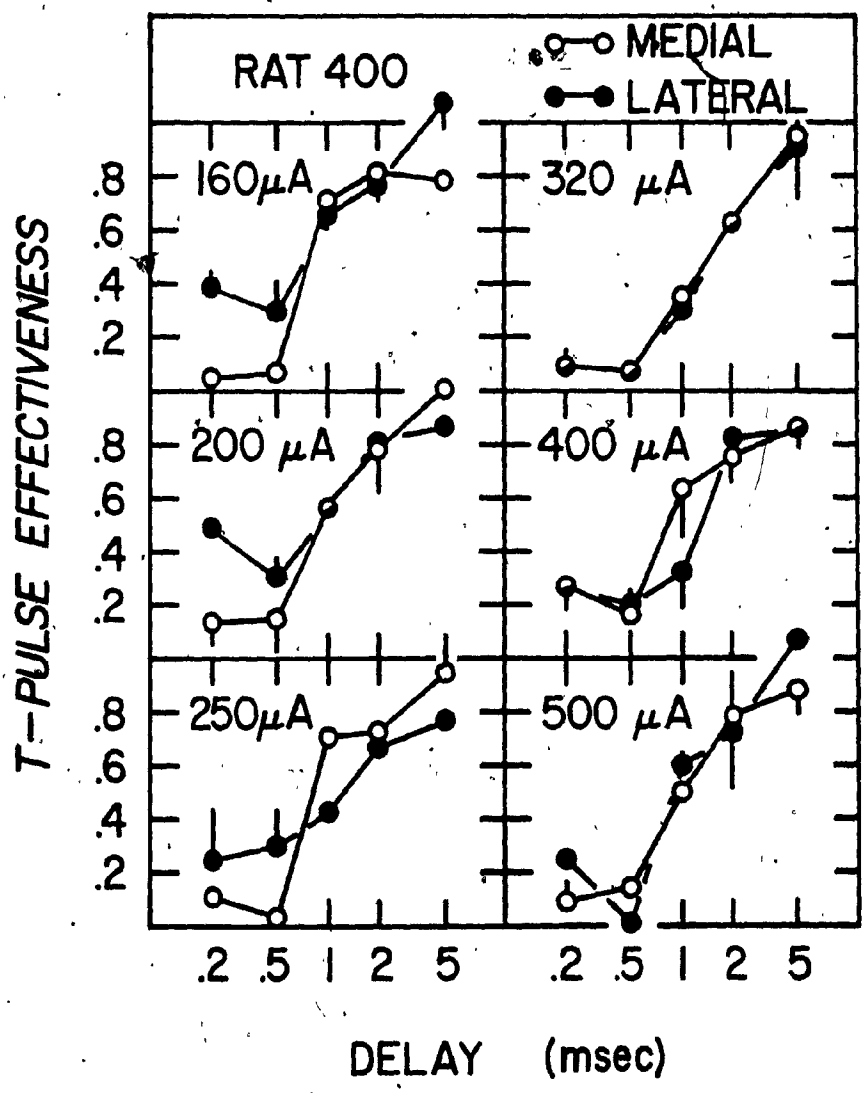

200 μA
250 μA
320 μA
400 μA
500 μA
RAT 398
MEDIAL
LATERAL
T-PULSE EFFECTIVENESS
.2
.4
.6
.8
.2 .5 1 2 5
DELAY (msec)

Figure 16

Single-electrode refractory determinations, Rat 400


RAT 400
MEDIAL
LATERAL
160μA
320 μA
200 μA
400 μA
250μA
500 μA
T-PULSE EFFECTIVENESS
.2
.4
.6
.8
.2 .5 1 2 5
DELAY (msec)

0.5 msec delays and the rising portion achieved a plateau at 2.0 msec. In some cases gains in effectiveness were witnessed beyond 2.0 msec (e.g. Rat 388, 320 μA test of medial electrode, Figure 14). The few exceptions to the general pattern of having the rising portion located between 0.5 and 2.0 msec did not appear to follow a trend; there was no systematic tendancy for their occurrence to be associated with a particular animal, electrode, or range of intensity. The location of the rising portion, given the coarse resolution of these data, is in perfect agreement with refractory estimates from other laboratories (Miliaressis & Rompré, 1980; Rompré & Miliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975).

In many cases, delays of 0.2 msec engendered effectiveness values that were higher than those seen at 0.5 msec delays (see, for example, Rat 398; lateral electrode test at 250 μA, Figure 15). This enhanced effectiveness at the very short delays is not the result of refractory failure; instead, it is explained by a temporal summation of partial depolarizations in reward cells that lie just beyond the stimulation field (Deutsch, 1964; Yeomans, 1975). This "local potential summation" has been

parametrically investigated and the reader is referred to Yeomans, Matthews, Hawkins, Bellman and Doppelt (1979) for more information.

The data derived from the primary experiment, in which C-pulses and T-pulses were applied through adjacent electrodes, appear in Figures 17 through 21. The format is identical to the individual refractory graphs. The ordinate is labelled differently, as Pulse-Pair Effectiveness, to reflect the distinction in the technique. The distance separating the electrode tips is indicated for each animal in the boxes containing the rats' identification numbers.

The interelectrode distances ranged from 0.30 mm (Rat 379) to 0.66 mm (Rat 388). If the logic behind this experiment is correct, then the steps in the E_{pp} vs. C-T delay functions should occur at low intensities with small interelectrode distances and they should emerge at comparatively higher intensities with the larger distances. A visual scan of these data suggests that this did indeed occur. The functions obtained with an interelectrode distance of 0.40 mm (Rat 386, Figure 18) appear to possess steps at and beyond a test amplitude of 200 µA whereas those witnessed with a 0.54 mm distance (Rat 398, Figure 20) are flat at 200 and 250 µA;

Figure 17

Between-electrode refractory determinations, Rat 379

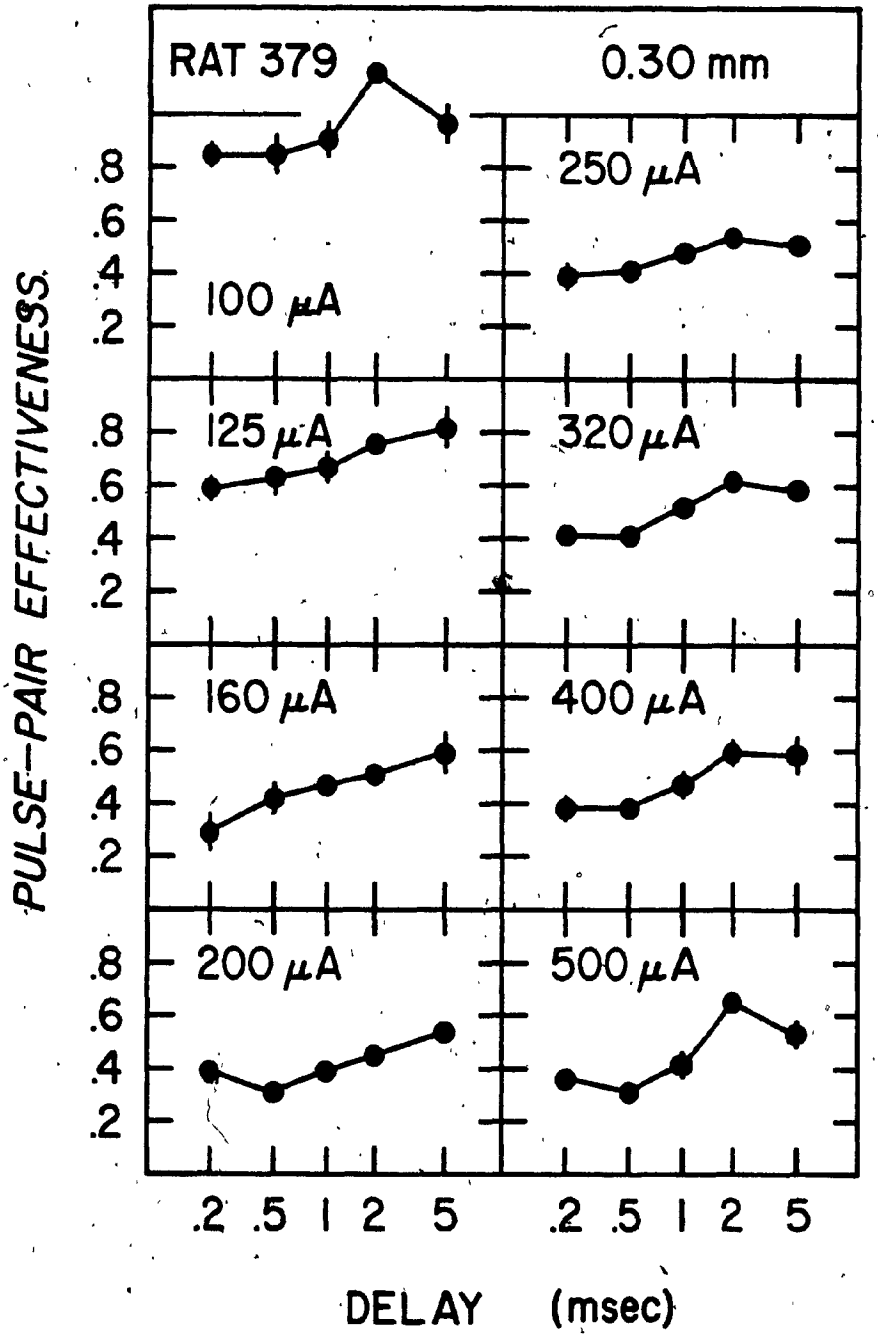

RAT 379
0.30 mm
100 μA
250 μA
125 μA
320 μA
160 μA
400 μA
200 μA
500 μA
PULSE-PAIR EFFECTIVENESS
.2
.4
.6
.8
.2 .5 1 2 5
DELAY (msec)

Figure 18

Between-electrode refractory determinations, Rat 386

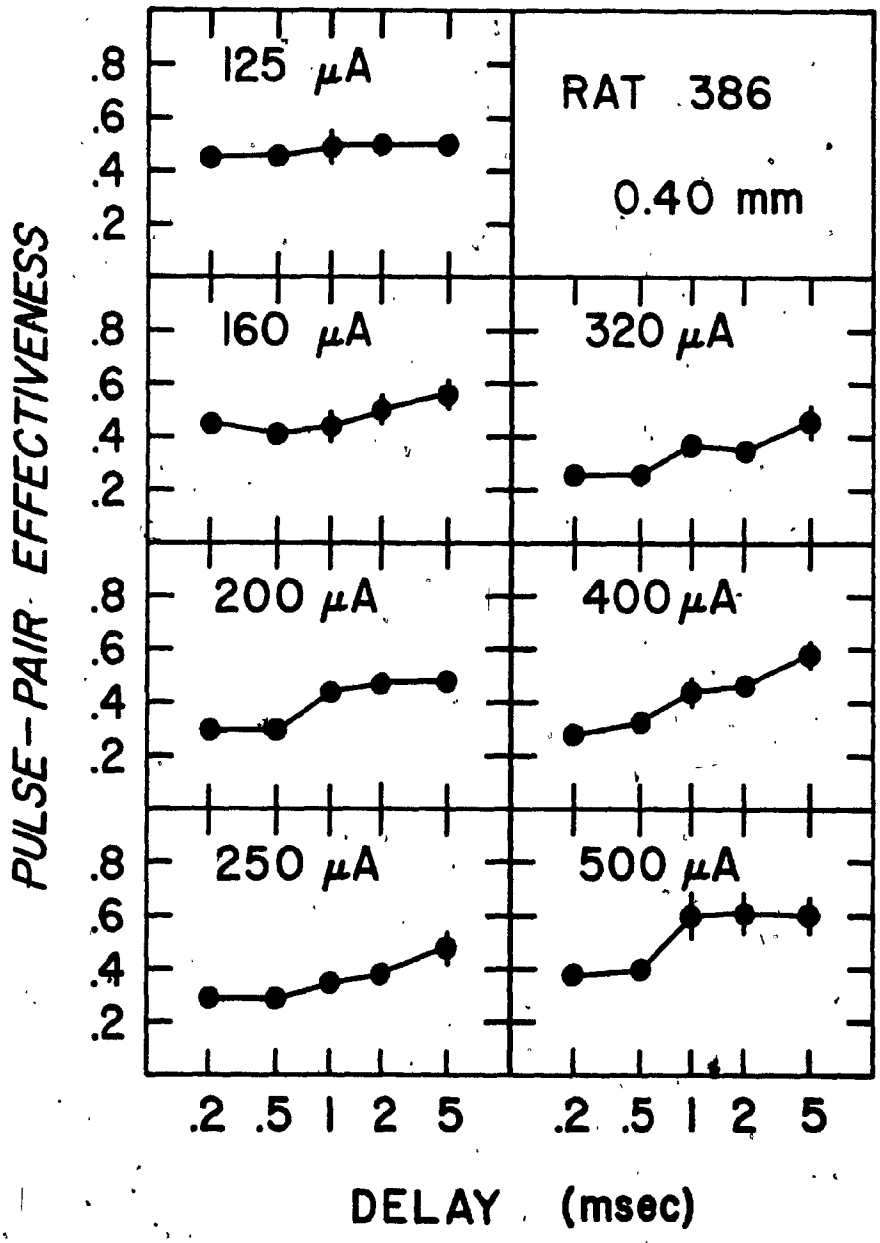

125 μA
RAT 386
0.40 mm
160 μA
320 μA
200 μA
400 μA
250 μA
500 μA
PULSE-PAIR EFFECTIVENESS
.8
.6
.4
.2
.2 .5 1 2 5
DELAY (msec)

Figure 19

Between-electrode refractory determinations, Rat 388

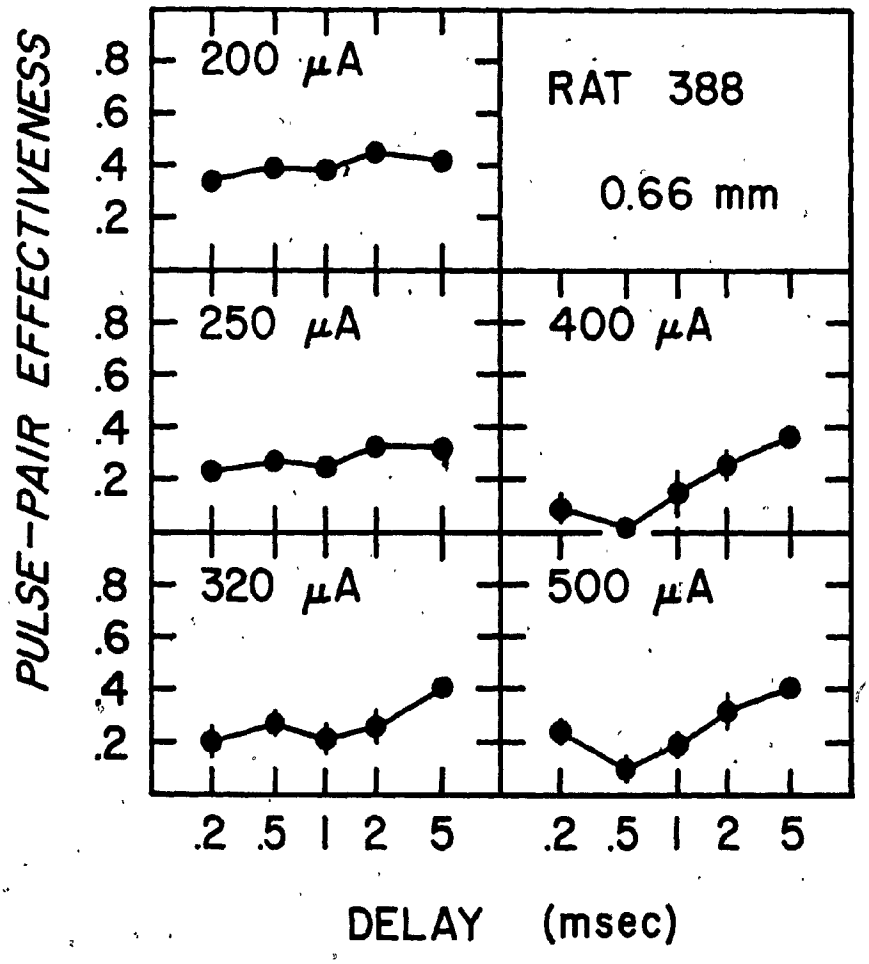

PULSE-PAIR EFFECTIVENESS
200 μA
RAT 388
0.66 mm
250 μA
400 μA
320 μA
500 μA
.8
.6
.4
.2
.2 .5 1 2 5
DELAY (msec)

Figure 20

Between-electrode refractory determinations, Rat 398

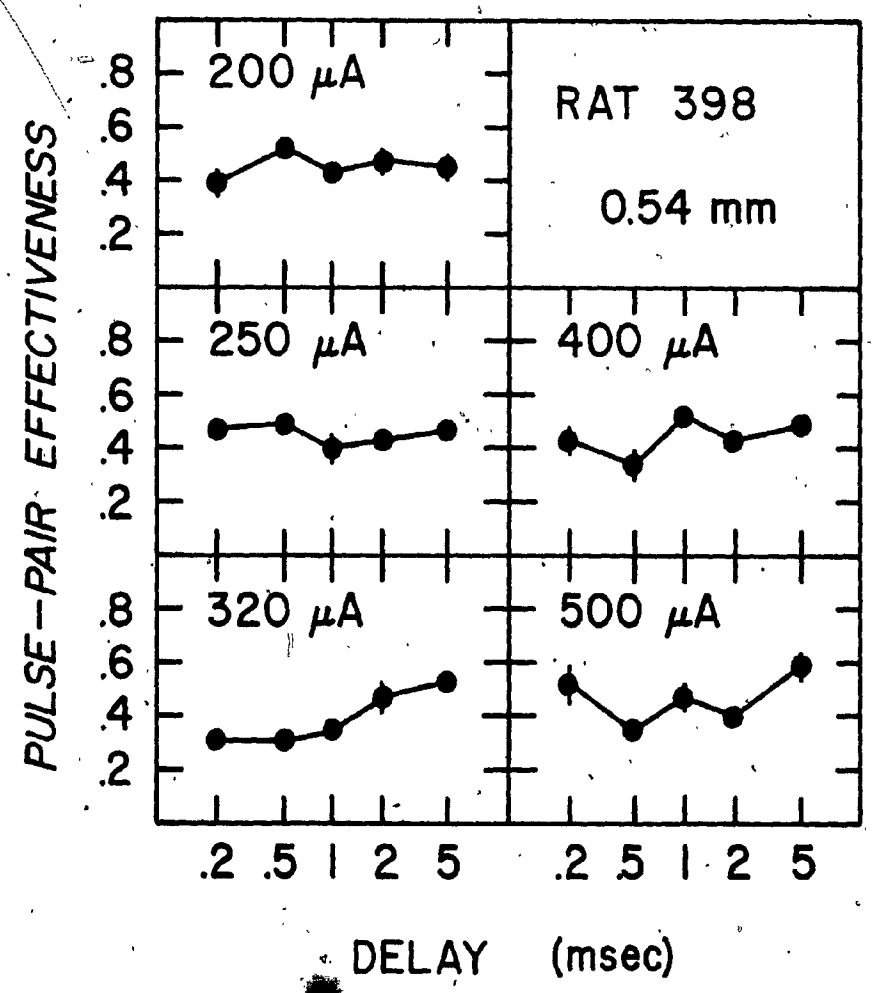
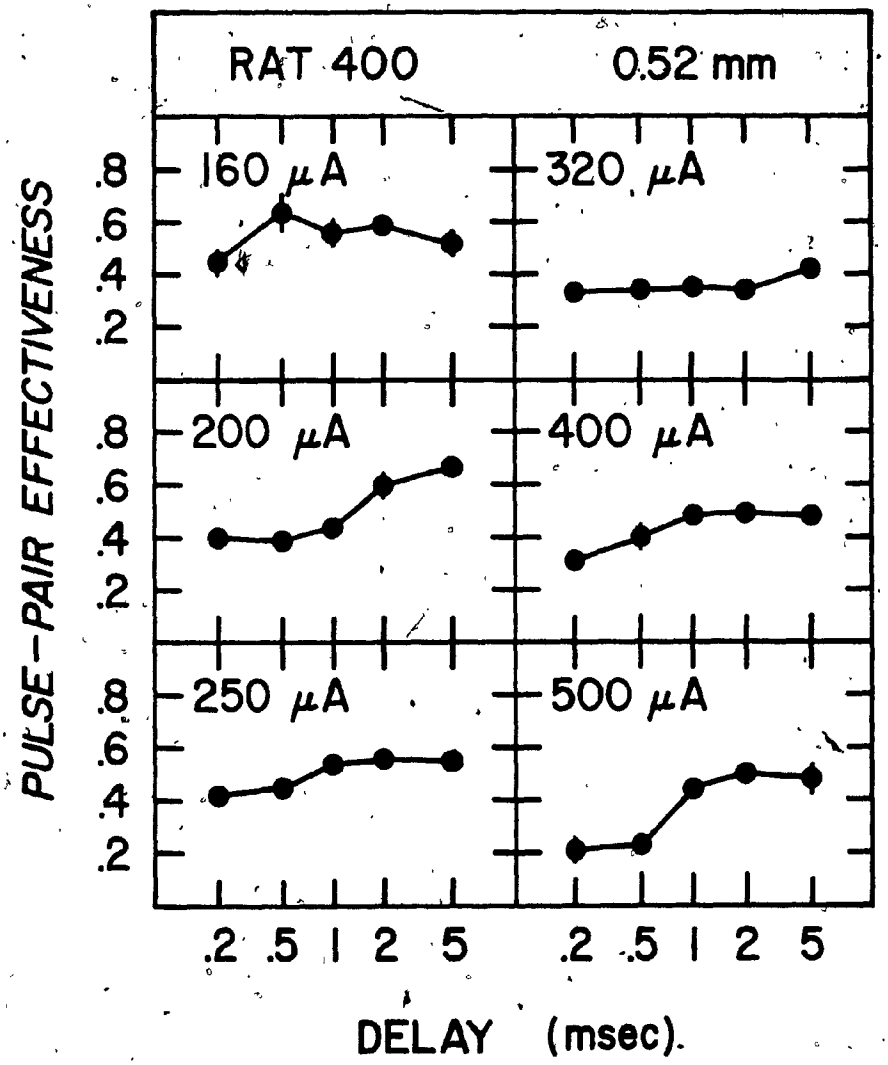

200 μA
RAT 398
0.54 mm
250 μA
400 μA
320 μA
500 μA
.8
.6
.4
.2
PULSE–PAIR EFFECTIVENESS
.2 .5 1 2 5
.2 .5 1 2 5
DELAY (msec)

Figure 21

Between-electrode refractory determinations, Rat 400


RAT 400
0.52 mm
160 μA
320 μA
200 μA
400 μA
250 μA
500 μA
PULSE-PAIR EFFECTIVENESS
.8
.6
.4
.2
.2 .5 1 2 5
DELAY (msec).

the step is not seen until a 320 μA test current was used.

The E_{PP} vs. C-T functions were individually probed at two points, 0.5 and 5.0 msec, with planned comparisons (Student's t-statistic; one-tailed alpha set at 0.05) in the attempt to determine the lowest intensity possessing a reliable step. The decision to use only two of the five C-T intervals for the comparisons was guided by the following rationale. The magnitude of the step was predicted to be small and thus, the detection of a reliable step necessitated the greatest sensitivity available. It was reasoned that if the probes were restricted to the two points that were likely to demonstrate the largest separation in E_{PP} values, then the power of these tests would effectively be enhanced.

The selection of the two points to be used was based upon a visual inspection of the results of the secondary, individual refractory, experiment. The 0.5 msec delay was chosen as the condition that would yield the lowest E_{PP} values at times when the stimulation fields intersected each other. Effectiveness levels were lowest overall at this delay in the individual assessments. Although a consideration of the post-stimulation excitability

cycle of a single neuron would lead to the selection of the shorter 0.2 msec delay, tests at this interval sometimes resulted in E values that were elevated because of summation in the subliminal fringe. It was suspected that a similar subliminal summation might also occur in the primary experiment, thereby engendering somewhat elevated E_{pp} measures at the 0.2 msec tests. Plateaus in T-pulse effectiveness were usually witnessed at 2.0 msec delays but, in some cases, additional increases in E values were seen with the longer 5.0 msec delay. Thus, if the stimulation fields overlapped at some tested intensity in the primary experiment, then the comparison that held the greatest potential for detecting the resulting refractoriness would be one between the E_{pp} values at 0.5 and 5.0 msec intervals.

The results obtained with Rat 379 were found to possess a step in the 160 μA test, for Rats 386 and 400 the step-like function first occurred with the 200 μA amplitude, and an intensity of 320 μA was required before evidence for between-electrode refractoriness was witnessed for Rats 388 and 398. That these intensities produced steps in the E_{pp} vs. C-T functions promotes the interpretation

that the stimulation fields had already intersected each other at these values of current. The task at hand was to estimate a current intensity that produced non-overlapping fields, the borders of which were just touching each other. The approach taken was the one that made the fewest assumptions about the homogeneity of distribution of the reward fibers surrounding the electrodes; the fields were deemed to be tangent at the next lower test intensity. The one assumption that is made by this decision is that the changes in stimulus spread, with 0.1 log unit increases in amplitude, are small enough to provide the resolution needed by the present technique.

These new values of current, conceived to result in fields that just meet, were termed "critical intensities" and they are listed in Table 1 under the column heading I_{Crit}. The critical intensities along with the corresponding interelectrode distances were used to estimate the constants of an equation that relates current and distance. The interelectrode distances were halved (recall that the fields are imagined to meet halfway between the electrode tips) and a linear regression of the critical intensities onto the squares of the

Table I

Summary of the key data: The interelectrode distance (IED) appears beside each animal's identification number (Rat). The value is halved (IED/2) to represent the radius of excitation achieved by the intensity (I_{Crit}) deemed to produce tangent stimulation fields. In turn, the radius is squared $(IED/2)^2$, towards the solution of individual current-distance constants (k).

Rat	IED	IED/2	I_{Crit}	$(IED/2)^2$	k
379	0.30 mm	0.15 mm	125 μA	0.0225 mm^2	1100 $\mu A/mm^2$
386	0.40	0.20	160	0.0400	1500
388	0.66	0.33	250	0.109	1400
398	0.54	0.27	250	0.0729	2100
400	0.52	0.26	160	0.0676	900

halved distances was performed. With intensity scaled in microamperes and distance in millimeters, the slope of the line was found to be 1500 μA/mm , the current intercept was 100 μA, and the correlation coefficient was 0.86 ($p<.05$). This line, the data points, and the 90% confidence corridor are graphically presented in Figure 22.

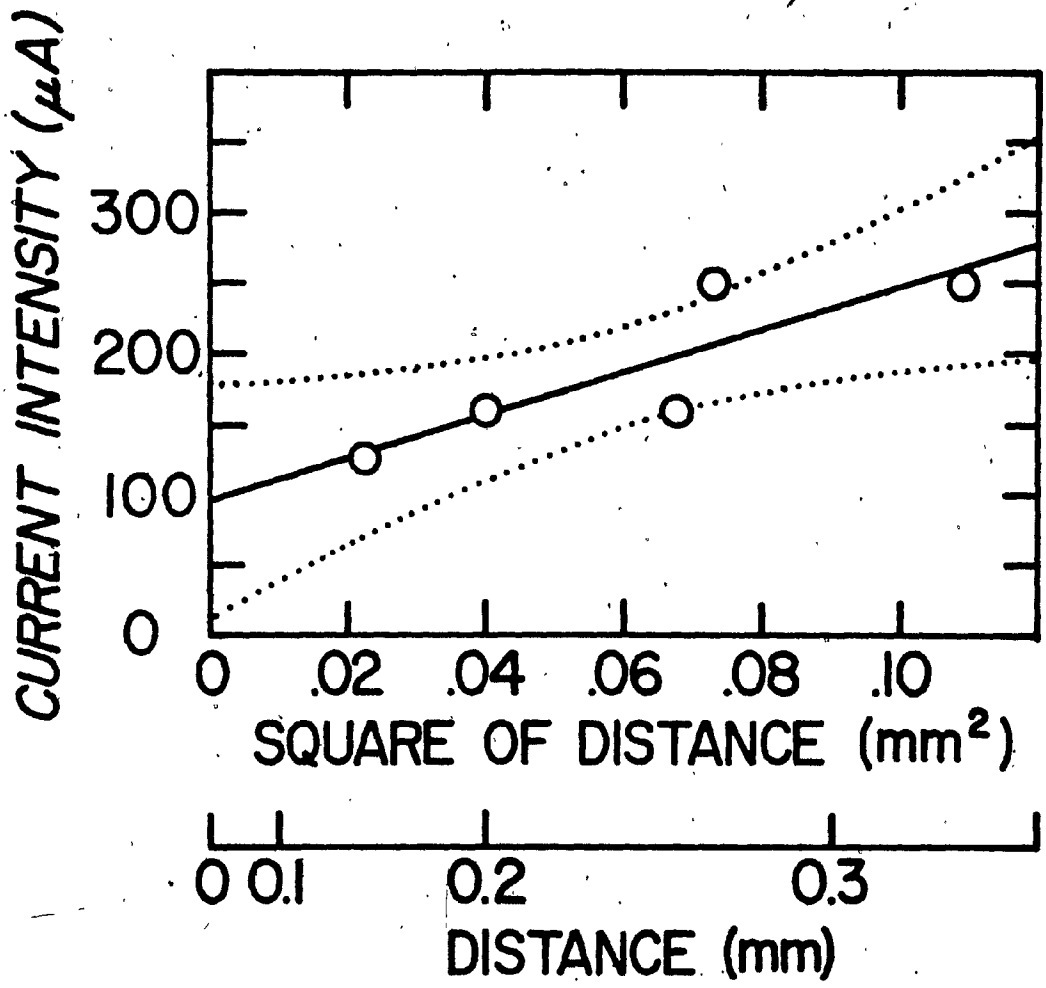
The data underwent one additional step. In order to judge individual fluctuation in the current-distance constant ($k = 1500$ μA/mm^2, overall), an estimate of k was made for each rat. The y-intercept of 100 μA (imagined to represent a threshold current for neurons right at the electrode tip) was used as I_o in the equation:

$$I_{Crit} - I_o = kr^2.$$

Each animal supplied its own set of I_{Crit} and r^2 values, and the relation was solved for individual current-distance constants. These constants, too, are listed in Table 1 and are under the heading k.

Figure 22

Current-distance estimate. Least-squares regression of current onto square of distance yields equation $I - 100\,\mu A = (1500\ \mu A/mm^2)r^2$. Dotted lines represent 90% confidence corridor.


CURRENT INTENSITY (μA)
300
200
100
0
0
.02
.04
.06
.08
.10
SQUARE OF DISTANCE (mm²)
0 0.1
0.2
0.3
DISTANCE (mm)

DISCUSSION

It appears that the technique employed in this thesis offers a viable approach to the estimation of current-distance relations for the behavioural consequences of brain stimulation. In the case of each preparation, the lower end of the tested current range produced flat effectiveness vs. delay functions and the higher levels resulted in step-like functions with the longer delays being more effective than the shorter ones. Furthermore, rats that had been prepared with larger distances separating their electrode tips required higher levels of current to demonstrate between-electrode refractoriness than did rats implanted with smaller tip separations. In the final analysis, the data were found to fit well with a parabolic expression for the relation between current and distance.

Specifically, the current-distance relation for the direct excitation of reward-related neurons in the lateral hypothalamic area was estimated to be:

$$I \doteq 100\,\mu A = (1500\ \mu A/mm^2)\,r^2$$

where I is the current amplitude of 0.1 msec

cathodal pulses in μA and r is the radius in mm of a circle, centered at the electrode tip, which encloses the stimulated axons. The form of this equation differs somewhat from the one developed in the introduction. The empirical results and the theoretical considerations found in neurophysiological studies converge in recommending that intensity is a scalar function of the square of the distance: $I = kr^2$, a form which holds that the current intercept is equal to zero. In contrast, the regression analysis performed on the present data yielded a substantial positive intercept (100 μA) which is represented by I_o in the form: $I - I_o = kr^2$.

Compared to the intensities required to span moderate distances (from Figure 22; 150 μA stimulates cells about 0.2 mm from the tip), the obtained intercept is large. Furthermore, the 90% confidence corridor constructed about the regression line fails to include zero as a candidate value for the current intercept. These data do not gracefully fit the scalar function; how might this apparent discrepancy between the neurophysiological recommendation and the obtained relation be resolved?

The following argument resolves the issue by making two main points: First, a positive

intercept is meaningful, not only in this determination of the relation for a behavioural effect of brain stimulation, but also in the case of attempts to estimate the relation for unit activation. Second, the value of the current intercept is bound to be high, compared to effective intensities, when large stimulating electrodes are used. Because of its relative magnitude, it cannot be considered to be negligible in behavioural work done with macroelectrodes.

The purpose of the current-distance relation is to map the intensity required to excite a neuron to the distance between it and the stimulus source. A strict interpretation of the scalar function forces the conclusion that no current is needed to stimulate an axon that is at no distance from the electrode tip. The arrangement is imaginary; spatial contiguity of the electrode and its target cannot be achieved. Nonetheless, the scalar form offers the unrealistic limit of zero μA as the distance shrinks towards zero. Surely some current must be passed through the electrode to bring the imagined cell to its threshold for the generation of an action potential. Provision for this initial depolarizing current is made by the inclusion of

the current intercept, I_o (Akaike, Fanardjian, Ito, Kumada & Nakajima, 1973). The meaning attached to this constant is that it represents a fixed quantity of the applied energy that is used to raise the contiguous cell to threshold.

Clearly, the intercept is meaningful irrespective of the nature of the investigation; it does not matter whether it is the response of a cell or that of an organism that is being measured. However, a consideration of the experimental situations, focusing upon the sizes of the stimulating electrodes, reveals why this current intercept may be negligible in the neurophysiological experiments and important in the behavioural ones.

Typically, stimulating microelectrodes measuring about ten micra across are used in unit recording work whereas behavioural studies are conducted with macroelectrodes often 250 micra in diameter. With area proportional to the square of the radius, the area over which current is dispersed differs by three orders of magnitude when micro- and macroelectrodes are compared. If the same amplitude pulse is delivered through these two electrodes, the cell closest to the microelectrode will experience a current density that is about

one thousand times greater than that felt by the neuron closest to the macroelectrode. This difference in current density achieved by the two types of electrode partly explains why current thresholds in neurophysiological experiments may be lower than one tenth of a microampere whereas tens of microamperes are required to witness behavioural effects of stimulation.

The intercept, I_o, takes current density at the tip into account; it reflects the amount of current needed to depolarize a cell imagined to be spatially contiguous with the electrode tip. The point stressed here is that a great deal of current must be passed through the macroelectrode before fully depolarizing levels are achieved. Perhaps in the case of stimulation through microelectrodes, the intercept is too small to be of consequence to the current-distance relation. With the large electrodes that are typically employed in behavioural work, this factor cannot be ignored.

The slope, k, of the regression line was found to be 1500 $\mu A/mm^2$. Using the obtained current intercept of 100 μA, individual values of k ranged from 900 to 2100. The spread of these current--distance constants compares favourably with the

range seen by Stoney et al. (1968) in assessing the excitability of individual pyramidal tract neurons (272 - 3460 $\mu A/mm^2$). Of course, very little between-preparation variation is predicted in the present work because these estimates were not derived from the activation of single axons. Instead, these data were based upon the excitation of a population of fibers. Presumably, individual extremes in excitability at the cellular level are overwhelmed in population assessments.

Departures of individual k values from the overall estimate might be explained by two potential sources of error associated with the technique used here. The first one stems from the unknown location of the behaviourally important neurons relative to the electrodes' positions. In presenting the underlying rationale, it was assumed that both electrodes would be situated within a region containing reward neurons or, at the very least, that the two electrodes would straddle such an area. In both cases, any diminished effectiveness of short C-T intervals in the primary experiment were thought to reflect a refractoriness of neurons that were properly situated between the two electrode tips. Another possible arrangement, however, of

electrodes and targets, is to have the electrode tips fall short of the mark. The supposition established here is that no element of the reward substrate is straddled by the two tips; instead, the targets are imagined to lie somewhere below both electrodes. (The alternate case, having the neurons straddled by the electrode shafts with the tips somewhere below, is equivalent.) In such situations, an assessment of the effective reach of the current, based upon the interelectrode distances, would seriously underestimate the true stimulus spread. If such an arrangement existed in one of the animals, its individual current-distance constant would be higher than the rest. This source of error is therefore biased.

It is noted that the experimental design incorporated a safeguard against all but the infrequent occurrence of this potential error. The rats had been tested at very low intensities as part of the initial frequency-intensity workup. These low current amplitudes, as it turns out, closely approached the obtained y-intercept. The intent was to verify that behaviourally relevant cells were to be found in close proximity to each of the electrodes. With the parsimonious idea

that if each electrode were near to reward neurons then the space between the tips would also include such elements, the safeguard offered adequate protection against this error. I leave it as a exercise to the reader to construct spatial distributions of reward elements for which the safeguard fails.

The second possible source of error results from the method that was used to determine the critical intensity for each animal. In brief, the lowest intensity with a reliable refractory effect was found and the next lower test amplitude was selected as the critical one. In fact, there are two separate facets to the error associated with this procedure. The first is minor and it emerges from the 0.1 log unit progression of the test amplitudes. Although discrepancies are small over the range employed here, intensities that follow equally sized excursions on a logarithmic scale do not give rise to equally sized differences in a quadratic progression. The non-linear relationship is illustrated by working backwards from the obtained current-distance equation. Increasing the intensity from 160 to 200 μA is calculated to enlarge the radius of the stimulation field by 0.058 mm. A

similar 0.1 log unit increase from 320 to 400 μA lengthens the radius by 0.064 mm. Once evidence for between-electrode refractoriness was observed, the next intensity down was chosen as the one producing tangent stimulation fields. The degree to which the radius would shrink depended upon the value of the test intensity. Compared to the magnitude of distances that were being assessed (0.15 to 0.33 mm), the errors of estimate introduced by this problem are thought to be insignificant.

The second facet concerns the inter-animal concordance on just how much overlap is required to measure between-electrode refractoriness. If the distribution of reward neurons is exceptionally dense between the electrodes and relatively sparse elsewhere, then a tiny intersection of the stimulation fields may suffice to yield a reliable effect. If, on the other hand, the situation were reversed and a comparatively sparse density were posited to exist between the tips, then a large degree of overlap would have to be established. In both cases, the amplitude imagined to produce tangent stimulation fields would be chosen by dropping down one step in test intensity. In

the former case, the result may fall short; the selected intensity may, in reality, produce stimulation fields that do not reach each other. In the latter case, the result may be too large; the chosen intensity may actually generate fields that continue to overlap. This source of error is thus conceived to be bidirectional. How powerful its influence would be on any particular estimate is difficult to assess. However, if the argument that the error is unbiased is correct, then the overall current-distance estimate would not be seriously affected.

All together, these sources of error do not introduce serious problems to the technique. Where systematic biases may exist, their occurrence is infrequent (absence of critical neurons between the electrode tips) or their influence is small (different reductions in r depending upon the value of I_{crit}). Where the source of error may be more significant (variation in the degree of overlap needed for the detection of between-electrode refractoriness), it is thought to be unsystematic. It is parenthetically noted that the magnitude of this unbiased source might be further reduced if additional measurements were focused in the range of intensities over which

the between-electrode refractoriness emerges. This would include smaller differences between tested intensities, test amplitudes that properly followed a quadratic progression, additional C-T intervals, and more replications per point. The refinement is costly in terms of the experiment's duration but the returns would justify this expense. For the present, the results of this experiment are thought to offer an excellent first approximation to the current--distance relation for rewarding brain stmulation.

The application of the obtained relation is here described with a pair of examples. Following the brief illustration, a more lengthy discourse explains why some constraints must be imposed upon its use. The equation may be arranged in ways to suit two purposes. In the first, it is written:

$$I = (1500\ \mu A/mm^2)r^2 + 100\ \mu A$$

and it may be used to adjust the intensity of the stimulation in order to generate a predetermined size of stimulation field. For example, if I wished to excite reward neurons of the lateral hypothalamus that lay within 0.3 mm of the electrode tip, 0.09 mm^2 would replace r^2 and the calculation would recommend a setting of 235 µA. Addition and subtraction of

one standard error of estimate (25 μA) to this recommendation yields the values 260 and 210 μA respectively.

The second arrangement of the terms:

$$r = ((I - 100\,\mu A)/(1500\,\mu A/mm^2))^{\frac{1}{2}}$$

allows one to determine, post hoc, the size of the stimulation field achieved by some already tested amplitude. If, for example, a test intensity of 300 μA had been used, substitution of 300 μA for I offers that the excited axons were within a 0.37 mm radius of the electrode tip. If a unit standard error of estimate (0.0145 mm^2) is applied against r^2, the corresponding high and low assessments of spread are 0.38 and 0.34 mm.

This estimate of the current-distance relation may not be used in all brain stimulation experiments. A handful of constraints must be imposed upon its application. The type of electrode used, the nature of the stimulation employed, the brain site targeted, and, of course, the behaviour under investigation are all critical variables. The restrictions associated with each of these factors is discussed here.

Ideally, the electrodes used should match the

ones used in the present experiment. The first of two concerns is with the electrode configuration: Monopolar stimulation was assessed here and the results of this work cannot be applied to the bipolar case. The second deals with the shape of the tip. The electrodes were 254 micra in diameter and they were insulated right down to their flattened tips. The key factor to be controlled is the total area of bared metal at the tip; changes in the exposed area affect the value of I_o, the current intercept. A procedure that must be avoided, if one intends to make use of the present relation, is the removal of insulation along the terminal length of the electrode. Even one quarter mm stripped of insulation at the tip would alter the bared area by a factor of five.

The relation was developed with the use of rectangular, monophasic cathodal pulses of 0.1 msec duration. Both the polarity and the duration of the pulses are critical. Except in situations where the electrode is positioned near cell bodies on the side distal to the axon hillocks, cathodal stimulation is more effective (has a greater reach) than is anodal (Porter, 1963; Ranck, 1975). Cathodal pulses are about twice as effective in

lateral hypothalamic self-stimulation as well, up to pulses of 5 msec duration (Matthews, 1977). This two-fold greater power of the cathodal pulse should not be taken to mean that the radius of excitation is double that of the anodal pulse; the more likely explanation is that the cathodal field encompasses twice the area of the anodal one (Gallistel, 1976).

Pulse duration and current intensity are two variables which, like other pairs of stimulation variables, trade-off against each other. Increases in pulse duration are compensated by decreases in intensity and vice versa. This trade-off is a general property of nerve cells and strength-duration relations have been specifically documented in brain stimulation reward (Matthews, 1977; White, 1976). Unlike other pairs of mutually compensatory stimulation variables, manipulations in current intensity and pulse duration affect the same mechanism. Consider two neurons, one immediately within the stimulation field and the other just outside of it. Both cells are depolarized by an applied pulse but the one inside achieves the threshold for firing within the time allotted by the duration of the pulse. If the pulse were

prolonged a little, then the one outside would, too, surpass its threshold for the generation of an action potential. Prolonging the pulse duration has the same result as increasing the current; the stimulation field is enlarged.

The present work featured 0.1 msec pulses. This value had been selected primarily for its popularity and partly for its efficiency (Barry, Walter & Gallistel, 1974). The recommendation made here is that the same duration be used by workers wishing to apply the current-distance relation in future studies. Data that have already been collected with other pulse durations may be assessed for effective distance, however, if the employed intensities are corrected for duration. The reader is referred to Matthews (1977) for the strength-duration function in brain stimulation reward.

Although the point has repeatedly been made that the relation is specific to cells involved in rewarding brain stimulation, it must be understood that self-stimulation obtained from a brain site other than the lateral hypothalamus does not immediately qualify for analysis by this current-distance relation. The behaviour may be the result of excitation of axons that differ

in size from the fibers of the lateral hypothalamus; the relations that would describe the spread for other self-stimulation sites might possess different values of k and I_o. The present relation may be used directly by researchers investigating lateral hypothalamic self-stimulation provided that the recommendations concerning electrodes, stimulation polarity, and pulse duration have been followed. In the case of other brain regions, some assurrance must first be obtained that the characteristics of the underlying elements match those of the behaviourally linked cells in the lateral hypothalamus. My belief is that it would suffice to demonstrate that the recovery from refractoriness profile is similar to the lateral hypothalamic one.

That the brain site is critical should not be hastily interpreted to mean that other stimulation-produced behaviours seen with lateral hypothalamic placements will follow the self-stimulation current-distance relation. The relation will be obeyed only to the extent that the subservient cells possess electrophysiological properties that match the characteristics of the neurons responsible for self-stimulation. Again, the rule of thumb ventured is that the

refractoriness of the substrate be documented.

Investigators who, for one reason or another, have been cautioned away from using this relation directly have, of course, the option of determining the current-distance relation that is appropriate to their own needs. With only minor modification of the procedure, the relation for any stimulation-produced change in behaviour may be determined.

The immediate impact of having such estimates touches attempts to locate the elements of brain circuitry involved in stimulation-induced behaviour. Mapping studies are afforded an extra measure of resolution; the boundaries of brain regions containing behaviourally related circuit elements may be delineated with a little more precision. This much is obvious and I do not wish to belabour the point. Instead, the discussion orients to the value of these estimates towards more delicate assessments. When moveable electrodes are used to probe brain regions in within-animal designs, the slope of the threshold changes (over distance moved) is determined by two important factors. One is that the stimulation field includes more and more elements as the electrode approaches its

target. The second is the distribution of the critical elements. The distribution might be perfectly homogeneous with abrupt demarcation or there might be irregularities of density. The simplest heterogeneous distribution, and the one that best illustrates the problem, has a dense core of critical fibers and a gradual decrease in density as one moves away from the center. Until now, the relative contributions of distance and distribution could not be distinguished. With accurate current-distance estimates married to moveable electrode mapping, the couple holds the promise of assessing relative densities of axons within the boundaries. I myself am looking forward to their tenth anniversary.

In itself, this thesis has not advanced our understanding of brain function. Specific contributions to brain-behaviour links have not been made, nor have new analytic methods been introduced. Instead, the contribution of this work has been to sharpen, if not to hone and to polish, the edge of an analytic tool that has served us faithfully for one-half century.

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APPENDIX I

This appendix describes a property of the reward system that is likened to a counter. The counter model stems from an observation made by Gallistel (1974) when he re-evaluated the results of a parametric assessment of rewarding brain stimulation (Edmonds, Stellar & Gallistel, 1974). He found that when values of pulse amplitude and pulse number (fixed train duration) produced criterion performance, the products of these settings equalled a constant. For example, if criterion performance were achieved with one set of values and the intensity were then doubled, the criterion performance could be accurately restored by halving the number of pulses (or pulse frequency).

The constant product was defended by the reward system over a very large range of individual combinations. This suggested to Gallistel (1977) that a neural network, somewhere downstream of the directly stimulated bundle of neurons, integrated excitation by simply counting the total number of arriving action potentials. As long as the delivery arrived over the same period of time, the integrator did not care how the impulses were packaged. Ten

axons might each conduct 50 action potentials for a total of 500 or twenty-five channels might each carry 20 firings, again, for a total of 500. The integrator treated these as equivalent combinations.

An important implication of this property concerns how rewarding effects are scaled. Each stimulation pulse is trusted to generate one action potential in each suprathreshold neuron, provided that the pulse duration is not longer than five msec (Matthews, 1977). The number of pulses that must be administered to produce criterion performance accurately reflects the relative number of fired axons. When many pulses are needed, the number of channels activated by each pulse is interpreted to be small. When only a few pulses suffice (through the same electrode), the number of fired neurons per pulse is understood to be greater. To the extent that an action potential in each behaviourally relevant axon provides the same contribution to the integrator, an expression of the effect of the stimulation in terms of number of pulses (or equivalents such as frequency and period) quantifies the number of excited channels.

APPENDIX II

This appendix outlines the rationale for the rejection of animals possessing large between-electrode differences in the single-pulse required frequency assessments. The criterion was based upon a consideration of how the measurement of a step in the E_{PP} vs. C-T function suffers when the two stimulation fields differ in their contribution towards self-stimulation behaviour. The need to impose it is explained with the development of an example.

Suppose that at some intensity the overlap in the two stimulation fields were such that 20% of the poorer field's reward contribution was derived from the excitation of the cells common to both stimulation fields. Suppose further that the single-pulse estimates of required frequency differed by a factor of two ($(F_{SPHi}/F_{SPLo}) = 2$). A pulse delivered to the richer field supplies twice the rewarding effect given by a pulse applied to the poorer one. For the sole purpose of developing this argument, let this two-fold difference be interpreted to mean that the number of reward cells in the poorer field (N) is half the number found in the richer (2N). The number of cells lying in the intersection of the two fields is represented by 0.2N.

A single pulse applied to the richer field produces 2N firings and one delivered to the poorer, N action potentials. A pair of pulses split between the electrodes, with an interposed delay that is sufficient to allow the common cells to recover from the first pulse, results in a total of 2N + N = 3N firings. When the delay is shorter, so that none of the common neurons has recovered, the corresponding count is 2N + (N - 0.2N) = 2.8N action potentials per pulse-pair. At the constant level of half-maximal behaviour, the products (number of firings per pulse-pair) x (required frequency of pairs) at all C-T intervals are equal (see Appendix I for background on this assertion). With F and F' standing for the required frequencies of pulse-pairs in the fully recovered and fully refractory situations respectively, the following relation restates the point:

$$3NF = 2.8NF'.$$

Dividing both sides by N and cross-multiplying to examine the relation between F and F' gives:

$$F/F' = 2.8/3 = 0.93.$$

This result indicates that the required frequency in the fully recovered condition may be expected to be about 93% of the value obtained when all possible cells are refractory to the second pulse.

This difference of 7% exists at the required frequency determination level; the application of the E_{PP} calculation actually yields the values 1.0 at full recovery and 0.8 for full refractory situations, in agreement with the first supposition that 20% of the poorer field's cells were common. If the same argument were applied to a situation with three times as many cells in the richer field, the result would be a 5% drop in required frequency once all cells had recovered. Again, the E_{PP} calculation promotes this to a 20% effect but this is not the level at which the dependent measures are obtained. The worry is that a 5% effect is beginning to enter the "error of measurement" region. That measurement errors along with real effects are promoted by the E_{PP} calculation adds no consolation.

The problem is anticipated to arise when a large discrepancy exists between the two stimulation fields in the relative densities of reward neurons. The best estimates of relative density are the single-pulse required frequencies; animals were

retained in the study if their electrodes possessed less than a 0.2 log unit difference in required frequency ($(F_{SPHi}/F_{SPLo}) < 1.58$) at each of the tested intensities. This criterion had to be met even when the intensities were low enough to result in flat E_{pp} vs. C-T functions.