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EVIDENCE FOR ANATOMICAL CONNECTIVITY BETWEEN LATERAL HYPOTHALAMIC AND LATERAL PREOPTIC AREA BRAIN STIMULATION SITES

Annette Boucher-Thrasher

Thesis presented to the School of Graduate Studies and Research, University of Ottawa, in partial fulfillment of the requirements for the degree of Master of Arts.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Introduction

The repeated performance of an operant task for rewarding brain stimulation describes the phenomenon of self-stimulation, initially discovered by Olds and Milner (1954). Following this first observation in the rat, self-stimulation has been demonstrated in a variety of other species including the gold fish (Boyd & Gardner, 1962), cat (Roberts, 1958), dog (Stark & Boyd, 1963), monkey (Porter, Conrad & Brady, 1959), dolphin (Lilly & Miller, 1962), and man (Bishop, Elder & Heath, 1963). The continued interest in this behavior stems from the complexity of the structures and pathways from which brain stimulation reward is obtained. Vigorous self-stimulation is obtained with electrode placements along the trajectory of the medial forebrain bundle (MFB) which has over 50 efferent and afferent connections to structures as anterior as the nucleus accumbens and as posterior as the ventral tegmental area (Nieuwenhuys, Geeraedts & Veening, 1982).

Research about brain stimulation reward has progressed despite the complexity of the MFB and the controversy about the nature of the phenomenon. The interest in self-stimulation by students of brain-behavior relationships is easily understood: Under certain conditions the pattern of responding for brain stimulation does not differ from that for conventional rewards such as food or water (Gibson, Reid, Sakai & Porter, 1965). Self-stimulation and naturally occurring appetitive behaviors are further linked by the results of several studies. For example, both behaviors display similar increases and

decreases in responding to such factors as deprivation and satiety (Hoebel, 1974; Hoebel & Thompson, 1969). In addition, approach to appetitve stimuli is facilitated and withdrawal from noxious stimuli is suppressed in response to rewarding stimulation (Stellar, Brooks, & Mills, 1979). Finally, activity recorded from neurons, which had previously responded to brain stimulation reward, increased with food presentation in food deprived animals (Rolls, Burton & Mora, 1976). Thus, self-stimulation seems to offer the possibility of being able to directly activate and study the neural circuitry underlying appetitively motivated behaviors.

Combined with the anatomical complexity is the fact that stimulation of the MFB produces a wide range of behavioral consequences. In addition to self-stimulation, electrical stimulation of the regions sustaining this behavior also induces feeding (Hoebel & Teitelbaum, 1962), drinking (Mendelson, 1967), sexual behavior (Herberg, 1963), exploration (Durivage, 1985; Milliaressis & Rompre, 1980; Rompre & Milliaressis) and escape (Bielajew & Shizgal, 1980; Skelton & Shizgal, 1980). In order to determine the neuronal characteristics of the substrate for brain stimulation reward one question to resolve is whether or not the same cells are responsible for the different behaviors. If the same fibers mediate the behaviors, changes in the stimulation parameters may account for the different behaviors observed (Valenstein, Cox & Kakolewski, 1969). It is also possible that different post synaptic elements account for the different behaviors. An alternative explanation for the variety of stimulation produced consequences is that the the

fibers controlling the different behaviors are intermingled. Psychophysical based measurement techniques have provided evidence that the reward substrate and the fibers mediating other stimulation induced behaviors are different although closely linked anatomically (Bielajew & Shizgal, 1980; Durivage, 1985; Gratton, 1985).

Any attempt to understand the mechanisms underlying the rewarding effect of brain stimulation would be strengthened by knowledge of the neuronal properties of the substrate mediating self-stimulation behavior. The studies in this thesis attempt to further characterize these substrates.

The Psychophysical Method

One approach to the study of brain stimulation reward is the psychophysical method. The neurophysiological properties of the underlying substrate are inferred from behavioral measurements with the use of trade-off functions. A trade-off function describes the linear relationship which results when one value of a stimulus parameter is counterbalanced by a specific value of another parameter to produce a constant behavioral output. In the present case, the response rate is maintained at a criterial level through compensatory variations between two parameters, frequency and intrapair interval. This constant level of responding is believed to reflect a constant level of neuronal excitation. For example, the frequency of pulse pairs and the intrapair interval are adjusted to maintain a criterial level of responding: fifty

neurons stimulated once have the same behavioral effect as five neurons stimulated ten time each. Intrapair intervals that are shorter than the neuronal refractory period are matched to a higher frequency of pulses than intrapair intervals that fall outside the neuronal refractory period (Yeomans, 1975). The assumption that a constant response reflects a constant level of excitation also applies to other trade-off functions which have been used to characterize the substrate for brain stimulation reward: Trade-offs between frequency and intensity and between intensity and duration will maintain a constant level of excitation (Yeomans, 1975). The intensity-duration trade-off results in a strength-duration function (Matthews, 1977; Scheck & Shizgal, 1985) and measures the temporal integrating properties of charge accumulation.

The argument that the input/output characteristics of intervening stages must be monotonic in order to infer the properties of the directly driven neuron or first stage properties from behavioral, or final stage, output is explained in greater detail in a review by Gallistel, Shizgal and Yeomans (1981).

The experiments presented here make use of the above rationale to examine the substrate mediating brain stimulation reward in the lateral preoptic area (LPO). This brain region was chosen because of its known anatomical connections with the lateral hypothalamus (LH) (Nieuwenhuys, et al., 1982). Although self-stimulation can be elicited at both sites it is not known whether the rewarding effect is a result of stimulation of a pathway common to both sites or not. The fibers which mediate the rewarding effect may not be the same ones which link the two sites. The

critical nature of the LPO's involvement in reward is emphasized by results which show that it displays high metabolic activity in response to rewarding LH stimulation as measured by (^{14}C)-2-deoxyglucose autoradiography (Gallistel, Karreman & Reivich, 1977; Gomita & Gallistel, 1982; Gallistel, Gomita, Yadin & Campbell, 1985; Gallistel, 1986). Based on this research an attempt has been made to assess the nature of the relationship between the LH and LPO.

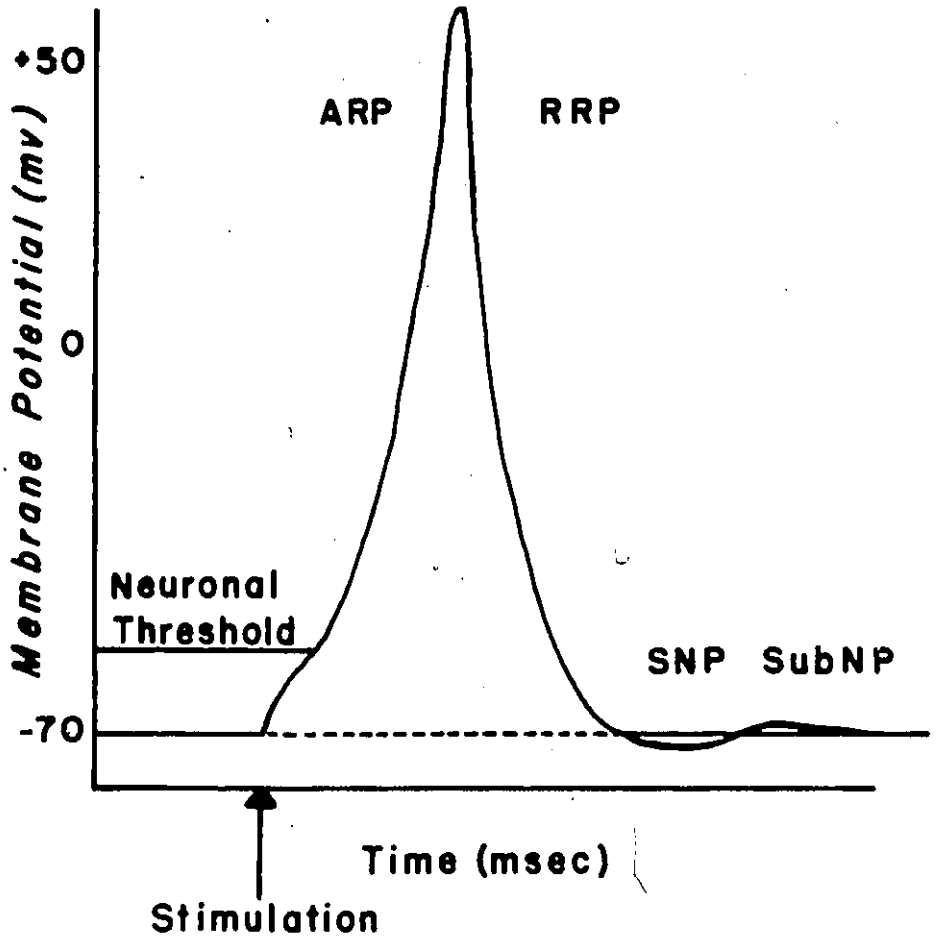
The first study presented here compares the refractory periods of self-stimulation neurons at the LPO placement to those of the LH placement. The possibility that the reward elements present at each site may be anatomically linked is assessed in the second experiment.

Excitability Cycles of Single neurons

Classical electrophysiological studies by Helmholtz (cited in Gallistel, 1973) in the peripheral nervous system made use of behavioral inference providing the rationale for the paired pulse technique used in this research. In 1854, Helmholtz estimated neuronal refractoriness from electrical stimulation of the nerve in a frog nerve-muscle preparation. Activation of the nerve was found to result in a muscle twitch, the amplitude of which was used as an index of the excitability of the nerve. Following an initial stimulation, a subsequent stimulation produced no increase in the amplitude of the muscle twitch until a minimum 1.6 msec interval had elapsed between the application of the two stimuli. The

Figure 1

Phases of the excitability cycle which include the absolute refractory period, relative refractory period, supernormal period and subnormal period in that order.


+50
0
-70
Membrane Potential (mv)
ARP
RRP
Neuronal
Threshold
SNP
SubNP
Time (msec)
Stimulation

amplitude of the muscle twitch associated with a pair of stimuli delivered within the 1.6 msec interval was indistinguishable from that produced by a single stimulus. The absolute refractory period was, thus, determined to be 1.6 msec.

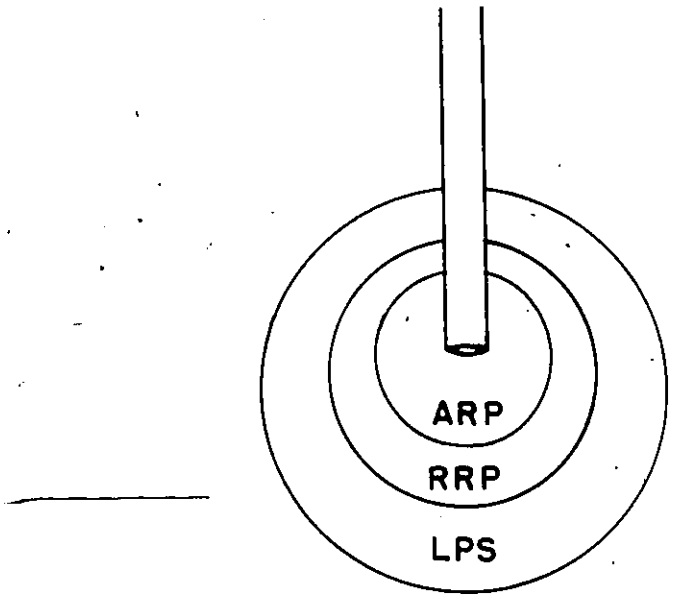
This estimate of the refractory period was later confirmed with electrophysiological recording of the nerve innervating the muscle (Adrian & Lucas, 1912; Boycott, 1899; Bramwell & Lucas, 1911; Gotch & Burch, 1899). Regardless of whether the refractory period determination was obtained from direct recording of the nerve or by using the associated muscle twitch as a measure, the results were the same.

Advances in technology have since allowed for more precise recording and, thus, for a more comprehensive understanding of the events involved in neuronal excitability. A complete description characterizing the excitability cycle of single neurons in the peripheral nervous system was provided by Erlinger and Gasser in 1937.

The principles underlying the excitability cycle have been generalized from the peripheral nervous system to the central nervous system (Swadlow & Waxman, 1978). The events which occur during neuronal activation differ depending on whether the stimulation received is above or below the firing threshold of the neuron. Subthreshold activation causes a slight change in the membrane potential in the area immediate to the stimulation; this small disturbance at the stimulation site is called a local potential and quickly decays. Subsequent subthreshold stimulation delivered before the membrane potential returns to its resting level can cause summation of local potentials resulting in the generation of an

Figure 2

The three regions around the electrode tip that may contribute to the recovery curve. The area immediately surrounding the electrode tip will produce a second action potential once the ARP is exceeded; the adjacent region is dependent on the RRP; the LPS region will only produce action potentials when the subdepolarizations produced by the C and T pulses summate.


ARP
RRP
LPS

action potential.

Suprathreshold neuronal activation produces an action potential which begins the excitability cycle. Figure 1 describes the phases of the excitability cycle. The absolute refractory period (ARP) coincides with the initial stage of the action potential. During this time a second action potential cannot be produced, regardless of how intense a stimulus is used. Following this ARP is a period of relative refractoriness (RRP). The membrane is somewhat excitable during this time but requires a higher than threshold stimulus to generate a second action potential. The supernormal period, during which the membrane is hyperexcitable, and the subnormal period , during which the it is slightly less excitable, complete the excitability cycle.

Excitability Cycles of Neuronal Populations

In order to facilitate the understanding of the excitability cycle of populations of neurons a homogenous population is assumed. Electrical stimulating pulses are delivered to a known fiber bundle via a stimulating electrode. Traditional nomenclature for double pulses designates the first pulse of the pair as the conditioning pulse (C pulse) and the second pulse of the pair as the test pulse (T pulse). The interval from C pulse onset to T pulse onset is then referred to as the C - T interval. A dramatic change in T pulse effectiveness, believed to reflect the excitability properties of the fibers, is observed when the C

- T' interval is lengthened. As with the single cell, the excitability curve, which is believed to reflect recovery from refractoriness of a population of neurons, is composed of phases. The relative contribution from fibers to these phases is dependent, however, on their relationship to the electrode tip.

The three regions which influence the shape of the excitability curve are described in Figure 2. The area immediate to the electrode tip receives the highest current density and is part of the suprathreshold area. Current density in this region is high enough that fibers here will produce action potentials in response to both C and T pulse stimulation provided the C - T interval exceeds the neuron's ARP. Adjacent to this region is a second suprathreshold area. The current density in this region is lower than in the first, however, making the propogation of an action potential by the T pulse contingent on the level of recovery from RRP. The fibers at the inner border of this region receive a higher current density than those on the outer border of this RRP region and thus, can be activated sooner.

Outside these suprathreshold regions is an area of subthreshold current density. Just as with the single cell, however, the local potentials produced by C and T pulses may summate and produce action potentials if the pulses arrive in close temporal proximity.

The location of the fibers in these regions is critical if a heterogenous population is being stimulated. If the relevant fibers are densest in the region nearest the electrode tip, the excitability curve will reflect this. Neurons will produce action potentials after T pulse

stimulation as soon as the C - T interval exceeds the ARP. If the highest fiber density is in the adjacent suprathreshold region, the critical C - T interval will have to be greater than the neuron's RRP in order for the T pulse to produce an action potential. Similarly, if the distribution of relevant fibers is dense in the subthreshold region a higher proportion of local potentials will summate and produce action potentials.

Refractory Period Experiments

Deutsch (1964) adapted the paired pulse technique in order to behaviorally determine the neuronal refractoriness of the substrate underlying brain stimulation reward (BSR). He based these estimates on the dramatic performance increases observed as the C - T interval was lengthened from .8 to 1.1 msec. He reasoned that if the C pulse and the T pulse were delivered to the animal at a C - T interval greater than the neuronal refractory period, then both pulses would reach the behaviorally relevant synapses and the animal would respond at a high rate. However, if both pulses were delivered at a C - T interval within the refractory period range, then the T pulse would not produce any further excitation of the substrate and the level of performance would be low or comparable to the condition in which T pulses were omitted. The rate of response would reflect activation by a single pulse at the short C - T interval and by both pulses at an interval outside the refractory period. Post-stimulation excitability characteristics were, thus, derived from the rate of response as a function of C - T interval.

This rate-based method has been used to determine the refractory period of reward neurons stimulated at the lateral hypothalamus (LH) (German & Holloway, 1973; Rolls, 1971; Smith & Coons, 1970; Ungerleider & Coons, 1970), preoptic area (POA) (German & Holloway, 1972) and ventral tegmental area (VTA) (Gallistel, Rolls & Greene, 1969) placements. Others have obtained refractory period estimates for stimulation induced eating (Hawkins & Chang, 1974; Rolls, 1973), drinking (Rolls, 1973) and exploration (Rolls & Kelley, 1972).

Such rate-based estimates of neuronal recovery rest on the assumption that there is a linear relationship between the response rate and the level of neuronal excitation. Yeomans (1975) noted that the floor and ceiling effects seen in refractory period studies violate this assumption. He showed that rate-based estimates of refractoriness varied with the stimulation parameters: Different stimulation frequencies resulted in different recovery estimates (1975). Indeed, at the highest and lowest frequencies used, rate did not vary at all as a function of C - T interval. Yeomans, therefore, based his estimates on a method of scaling which assumes that the relationship between the rate of response and the rate of neuronal excitation is a monotonic one. He also assumed that the magnitude of the observed reward is proportional to the total level of excitation produced at the different stimulation frequencies. That is, regardless of what stimulation frequency is used, the behavioral effect from a constant level of excitation is invariant; there is no behavioral difference between stimulation of many reward neurons once or stimulation of one reward neuron many times. By keeping the level of

neuronal excitation constant the behavioral output will also be kept constant.

Yeoman's scaling procedure required that the stimulation frequency necessary to produce responding at a constant rate be established for each C - T interval. When rate is plotted against frequency the resulting rate/frequency curve is shifted to the left as the C - T interval is increased. The required frequency for criterial performance is thus determined for each C - T interval. The required frequency for the double pulse condition at each C - T interval is then compared to the required frequency for the single pulse condition. The double pulse required frequency expresses how effective the T pulse is in proportion to the single pulses. In this way, an effectiveness function is calculated based on increases in T pulse effectiveness over C - T intervals.

Refractory period estimates arrived at using this procedure compare the number of pulse pairs needed to maintain a criterial level of performance to the number of single pulses needed to maintain the same level of perfomance. Because each of the single pulses is directly related to the number of action potentials produced, the T pulse has no behavioral effect when the double pulses are delivered at a C - T interval within the refractory period range. Under these conditions, twice as many double pulses as single pulses are required in order to maintain the same level of performance. Similarly, when the T pulse is delivered at a C - T interval outside of the refractory period range, the second pulse produces a behavioral effect equal to the first pulse, and only half as many double pulses as single pulses are needed to maintain

the same level of performance.

Unlike rate-based results, refractory period estimates obtained using Yeomans (1975) T pulse effectiveness scaling procedure show little between subject variation when the same site is stimulated both within and across laboratories. Approximately 80 % of the recovery from rewarding stimulation of the LH has consistently been shown to range from 0.4 to 1.2 msec (Bielajew, Jordan, Ferme-Enright & Shizgal, 1981; Bielajew, Lapointe, Kiss & Shizgal, 1982; Bielajew & Shizgal, 1982; 1986; Hawkins, Roll, Puerto & Yeomans, 1983; Gallistel, 1978; Milliaressis & Rompre, 1980; Rompre & Milliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975, 1979).

Refractory period curves obtained using Yeomans (1975) scaling method describe T pulse effectiveness with values ranging from 0 to 1. The typical function obtained is believed to characterize three major aspects of neuronal recovery from refractoriness: Local potential summation (LPS) or latent addition, the absolute refractory period and the relative refractory period.

The first of these, local potential summation, occurs at the shortest C - T intervals and is discernable because of an increase in effectiveness associated with these intervals. This effectiveness value is based on the fact that in addition to the circumscribed region of neurons depolarized by the C pulse and resulting in the generation of action potentials, a fringe region of neurons is also depolarized to a subthreshold level. At longer C - T intervals, the membrane potential will have returned to its resting state before the onset of the second

pulse.

The level of the LPS effect varies with the relation of the electrode tip to the substrate and with the intensity of the stimulation. The location of the electrode tip is critical as stimulation of an area bordering on densily packed fibers will result in a larger LPS effect than stimulation of an area composed of widely spaced fibers. Assuming a homogenous population with a uniform distribution, intensity may also affect the level of the LPS effect if the electrode is stimulating the center of the substrate. Under such conditions a lower intensity will result in a greater LPS effect than that produced by a higher intensity because fewer fibers are available for subthreshold stimulation when higher intensities are used as the boundaries of the substrate are exceeded.

The LPS effect only occurs at the short C - T intervals and begins to decay to 0 effectiveness as the C - T interval is lengthened. The decay of LPS sometimes interferes with the estimate of the absolute refractory period (ARP) phase. The ARP refers to the period during which the neurons stimulated to the above threshold excitation by the C pulse cannot be stimulated and produce a second action potential regardless of the intensity of the stimulation. Ideally, the ARP estimate is the longest C - T interval at which the effectiveness value is 0. In practice, however, the ARP is usually estimated as the longest C - T interval at which the effectiveness value is closest to 0 because the maximum decay of the LPS effect and the end of the ARP often overlap.

Following the ARP a relative refractory period (RRP) occurs during

which the neurons activated to above threshold levels by the C pulse can again be stimulated to produce an action potential by the T pulse-but their thresholds for activation are elevated. A refractory period curve with a gradual slope could be the result of activation of neurons with a long RRP. Alternatively, a slow rising refractory period curve might also result from stimulation of a heterogenous population of fibers with a range of ARP's and little or no RRP contribution.

In order to differentiate between ARP and RRP effects, Yeomans (1979) made use of unequal intensity C and T pulses. He felt that by using T pulses of higher intensity the higher threshold neurons in the RRP region would be activated. In this way, the delayed changes in effectiveness values attributable to a long RRP would be minimized and only ARP effects would be able to account for slow rising curves. Yeomans (1979) was able to show only a slight RRP contribution to the recovery from refractoriness at the sites tested; however, there has been some evidence to suggest that the magnitude of the RRP contribution is affected by the T-pulse/C-pulse ratio used (Bielajew, Lapointe, Kiss & Shizgal, 1982).

Yeomans (1975) scaling method has been used to determine the refractory period of self-stimulation neurons in the LH (Bielajew, et al., 1981; Bielajew, et al., 1982; Bielajew & Shizgal, 1982; 1986; Hawkins, et al., 1983; Gallistel, 1978; Milliaressis & Rompre, 1980; Rompre & Milliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975, 1979) the ventrolateral tegmentum (MacMillan, Simantikaris & Shizgal, 1985), VTA (Shizgal, et al., 1980), periaqueductal grey (Bielajew, et

al., 1981), LPO and nucleus accumbens (Fouriezos, Walker, Rick & Bielajew, in press), medial prefrontal cortex (Schenk & Shizgal, 1982; Silva, Vogel, Corbett, 1982) and mediodorsal thalamus (Bielajew & Fouriezos, 1985).

Similarly, the refractory periods of neurons involved in stimulation induced circling (Milliaressis, 1981; Rompre & Milliaressis, 1980; Yeomans & Linney, 1986), feeding (Hawkins, et al.,1983), exploration (Durivage, 1985), and escape (Skelton & Shizgal, 1980) have been determined.

Collision Experiments

A behaviorally derived estimate of the refractory period of the directly driven neurons is one property that characterizes the relevant substrate. Behavioral tests of the substrate mediating brain stimulation reward to date have also included evaluations of the strength-duration characteristics (Matthews, 1977), temporal summation properties (Edmonds, Stellar & Gallistel; 1984; Gallistel, 1974), current-distance relations (Fouriezos & Wise, 1984), anatomical linkage (Shizgal, et al., 1980), conduction velocity estimates (Bielajew & Shizgal, 1982) and direction of normal conduction (Bielajew & Shizgal, 1986).

These techniques have all provided important information about the substrate involved in brain-stimulation reward, however, one technique directly addresses the question of anatomical linkage. This paradigm, refered to as collision, is based on the conduction block that occurs

when antidromic and orthodromic impulses propagate along the same membrane.

Lucas (1913) first made use of this collision phenomenon to infer the conduction velocity of a known bundle of fibers. Pairs of stimulating pulses were delivered to a nerve-muscle preparation. Unlike the refractory period experiments, however, each of the pulses was delivered through a seperate electrode. The shortest C - T interval required to elicit a muscular contraction was measured. A muscular contraction was only observed when the C - T interval was sufficiently long to allow the action potential from one electrode to conduct past the second electrode and for the membrane in this area to recover from the refractory wake.

Neuronal collision can only occur when two action potentials propagate in opposite directions on the same axon. Collision is infered when there is a change in the net effect of activation from two stimulating electrodes if a delay is introduced between the pulses delivered to the first and second electrode.

When two electrodes are situated on the same axon and both are stimulated simultaneously the sequence of events at the level of the axon is as follows: The antidromic action potential produced by the electrode proximal to the cell body conducts towards the cell producing no post-synaptic effect and the orthodromic action potential generated by the electrode distal to the cell body propagates to the terminal causing transmitter substance to be released onto the adjacent cell. The orthodromic action potential from the proximal electrode and the antidromic action potential from the distal electrode collide resulting

in no further conduction. Although both stimulating electrodes generate action potentials, only one action potential reaches the synaptic termihal. When the delay between the two pulses is sufficiently long to exceed the sum of the conduction time and the refractory period of the neuron, the orthodromic action potentials generated at both the proximal and distal electrodes are able to propagate to the synaptic terminal.

The behavioral adaptation of the collision technique has aided in the characterization of the substrate for brain stimulation reward. For example, the studies of Shizgal, et al. (1980), Bielajew and Shizgal (1982) and Bielajew and Shizgal (1986) have provided evidence that the reward effect elicited by LH and VTA stimulation is mediated by small, myelinated fibers that conduct at speeds that range from 1 to 8 m/sec.

In addition to assessing anatomical linkage of reward fibers, the collision technique has been used to dissociate two stimulation induced behaviors. For example, Bielajew and Shizgal (1980) concluded that a common subset of fibers mediated the reward effect at the LH and VTA, while a unique substrate was responsible for stimulation induced escape at these sites. Similarly, Gratton (1985) showed dissociation of feeding and self-stimulation behaviors elicited at the same placement and Durivage (1985) found no common elements between the fibers mediating exploration and self-stimulation.

The experiments presented here make use of the pulse pair refractory and collision techniques to determine the relationship between the LH and the LPO.

Experiment 1

In this study, behavioral estimates of the refractory periods of neurons subserving brain stimulation reward at LH and LPO sites were calculated using Yeomans (1975) scaling formula.

Method

Subjects and Surgery

Five male Long Evans rats (Charles River Breeding Laboratories) weighing between 340 and 540 g at the time of surgery served as subjects. They were housed individually in clear plastic cages in a shared animal colony room that was kept on a 12 hour light/dark cycle with light onset at 700 hours. Food and water were freely available in the home cage.

The electrodes were fashioned from stainless steel wire, 250u in diameter, soldered to gold-plated amphenol plugs, and insulated with Formvar to the polished tip. The current return consisted of an electrode soldered to a tin-clad copper wire wrapped around four stainless steel skull screws. The entire assembly was secured to the skull with dental acrylic.

Prior to surgery the subjects were anesthetized with sodium pentobarbitol (65 mg/kg intraperitoneal) followed by a 0.2 cc subcutaneous injection of atropine sulphate to prevent fluid buildup in the lungs. Standard stereotaxic procedures were used to aim the

electrodes at the lateral preoptic area (LPO) and the lateral hypothalamus (LH). The following flat skull coordinates were used: LPO - 0.6 mm posterior to Bregma, 1.3 mm lateral to the sagittal suture and 8.5 mm below the skull surface; LH - 2.0 mm posterior to Bregma, 1.7 mm lateral to the sagittal suture and 8.2 mm below the skull surface.

Apparatus

Behavioral tests were conducted in a wooden test chamber, 46 cm x 28 cm x 50 cm high, with a clear Plexiglas front and a solid floor covered with sawdust. A Gerbrand rodent lever protruded from the wall approximately 3 cm above the floor of the chamber.

Stimulation consisted of 500 msec trains of rectangular, monophasic, cathodal pulses, 0.1 msec in duration, and were controlled by constant-current amplifiers (Mundl, 1980), and integrated circuit pulse generators. The current intensity was monitored on an oscilloscope by reading the voltage drop across a precision 1 K Ω resistor in series with the rat. To prevent the buildup of charge at the brain/electrode interface, the outputs of each channel were shorted through a 1 K Ω resistor in the absence of a pulse on either channel.

Procedure

Screening and Stabilization.

The subjects were screened for self-stimulation after a minimum

post-operative recovery period of three days. Conventional shaping procedures were used to train the animals to lever press for 500 msec trains of pulses on a continuous reinforcement schedule. Stimulation parameters were manipulated so as to avoid seizures and severe motoric side effects and still yield high rates of responding. Only animals that responded vigorously to the stimulation (> 1 response/sec) on each electrode were included in the experiment.

Once an animal responded consistently, usually after a single session, it was introduced to primes and timed trials. Five trains of pulses, 1 second apart, were delivered at the start of each 30 sec trial. The trial began either 12 sec after the last prime or following the first lever press, whichever came first.

Stable self-stimulation performance was based on a series of frequency threshold determinations. The threshold corresponded to the frequency required to maintain a half-maximal rate of responding and was calculated by interpolation. Starting with a current and frequency that supported vigorous responding, the number of pulses was lowered in 0.05_{10} logarithmic steps until responding fell below 10% of the maximum rate. Only in the case of one subject, A-8, were larger increments (0.1_{10} logarithmic steps) used.

Stability was achieved when the criterion, less than a 0.1_{10} logarithmic change in the frequency threshold across six consecutive determinations, was reached. Generally five sessions were required to establish stable performance.

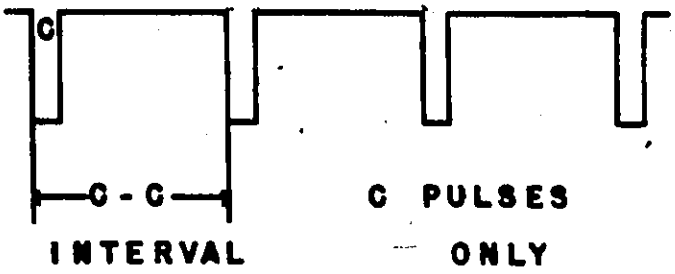
Figure 3

The difference between the single and double pulse test conditions is illustrated. The train duration is held constant for both conditions therefore the number of single pulses or pulse pairs is inversely related to the time between each C pulse; fewer pulses mean longer C - C intervals.

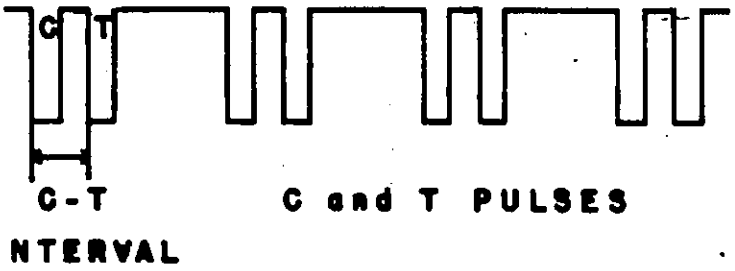
a) A train of single pulses (C pulses) is shown. The interval from the start of one pulse to the start of the next pulse is termed the C - C interval.

b) The double pulse condition is shown: The C pulse is followed by a T pulse. The interval form the start of the C pulse to the start of the T pulse is called the C - T interval. The C - T interval was always less than or equal to the T - C interval.

a) SINGLE PULSE CONDITION



b) DOUBLE PULSE CONDITION



Refractory Period Test.

Approximately 15 frequency threshold determinations made up a typical refractory period test. Four single pulse determinations, evenly spaced throughout each session, were used to assess the possible effects of fatigue or sensitization on the frequency threshold. The single pulse frequency threshold determinations had to maintain a less than a 0.1_{10} logarithmic shift across each session in order to be regarded as stable; no sessions were discarded due to unstable performance. Trains of pulse pairs were delivered in the remaining eleven determinations. The delay between the two pulses (C - T interval) was varied from 0.2 to 15.8 msec and was presented in a random order. Figure 3 shows the difference between the single and double pulse conditions.

Double pulse determinations of the frequency thresholds were based on individual C - T intervals. The frequency threshold for the double pulses was determined in the same way as for the single pulses: The frequency corresponding to half-maximal responding was interpolated from the rate-frequency curves. Four to six replications of the refractory period test were conducted for each electrode; the test presentation was alternated between electrodes.

Data Format.

The effect of adding the T pulse was assessed by comparing the frequency thresholds obtained from the single pulse tests to those obtained in the double pulse condition at a given C - T interval.

Yeoman's (1975) formula was used to scale the effectiveness of the T pulse stimulation. The formula:

$$E = FT_{(DP)}/FT_{(SP)} - 1$$

where E= the T pulse effectiveness

$FT_{(DP)}$ = the frequency threshold in the double pulse condition

$FT_{(SP)}$ = the frequency threshold in the single pulse condition

determines the contribution of the T pulse to the rewarding effect. If both C and T pulses are no more effective than the C pulses alone then the T pulses have no rewarding effect and an E of zero is obtained. If each T pulse is just as effective as each C pulse then an E of one is observed. In other words, an E of one indicates that the double pulse condition has twice the effectiveness or reward value of the single pulse condition.

Data Analysis.

The effectiveness of the T pulse was plotted as a function of the C - T interval. Refractory period curves typically have a decline in effectiveness at the shortest C - T interval (0.2 or 0.4 msec). The lowest effectiveness value is usually obtained at C - T intervals of 0.4 to 0.6 msec. At the longer C - T intervals, the E values gradually increase appoaching asymptotic values at 1.2 to 1.5 msec in the LH (Bielajew, et al., 1981; Bielajew, et al., 1982; Bielajew & Shizgal, 1982; 1986; Hawkins, et al., 1983; Gallistel, 1978; Milliaressis & Rompre, 1980; Rompre & Milliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975, 1979).

The asymptote test (Bielajew, et al .,1981) was used on the present data to statistically define the point at which the recovery from refractoriness approaches a plateau. The test involves comparing the mean E value and standard error obtained at the longest C - T interval to the mean E value and standard error obtained at the preceeding C - T interval. If these first two means and standard errors overlap, the E values associated with them are pooled and a new mean and standard error are established. This pooled mean and standard error are then compared to the mean E value and standard error of the next shortest C - T interval. If these means and standard errors again overlap, the E values associated with all three C - T intervals are pooled to provide a new mean and standard error. This process is repeated until the pooled mean E value and standard error no longer overlaps with the mean E value and standard error of the next shortest C - T interval. Note than when a shorter C - T interval had a higher, rather than the expected lower, effectiveness value associated with it the effectiveness value associated with the shorter C - T interval was included in the asymptote pool. The shortest C - T interval to be included in the asymptote pool describes the point at which recovery from refractoriness has reached a plateau.

The rising portion of the curve was thus defined as that part of the curve which begins at the C - T interval associated with the lowest effectiveness value and ends at the C - T interval established as the asymptote point. The part of the curve associated with the rising portion reflects the time course of the recovery from refractoriness. A problem exists, however, if one attempts to compare rates of recovery between

refractory period curves. The increased effectiveness associated with the local potential summation does not always decay to their lowest effectiveness values before the fastest neurons have begun to recover from refractoriness so that the resulting effectiveness value might reflect a cintribution from both local potential summation and recovery from refractoriness.. A further complication arises at the other end of the recovery curve because the maximum effectiveness, the point at which recovery is complete, does not always reach effectiveness values of 1 (Bielajew, et al., 1981; Bielajew, et al., 1982; Bielajew & Shizgal, 1982; 1986; Hawkins, et al., 1983; Gallistel, 1978; Milliaressis & Rompre, 1980; Rompre & Milliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975, 1979).

The problem of comparison is compounded if any differences exist in the slopes of the curves being compared. The hypothetical curves depicted in the left hand side of Figure 4 provide an example of the difficulty in assessing and comparing the rates of neuronal recovery of two behavioral curves. The solid line begins at 25 percent effectiveness at a C - T interval of 0.4 msec and rises to 78 percent effectiveness at 1.6 msec, while the curve represented by the broken line rises from 3 percent effectiveness at 0.6 msec to 97 percent effectiveness at a C - T interval of 6.3 msec. These hypothetical curves depict differences in the starting and asymptote points of the two curves despite parallel slope. Thus, it appears that the time course of the recovery of the solid line curve is the same as that of the broken line curve.

In order to avoid possible confounds caused by differences in

Figure 4

Hypothetical refractory period curves. The untransformed curves on the left hand side of the figure have similar slopes but different starting and asymptote points. The transformed curves on the right hand side of the figure are normalized so that the rising portion of both curves span the entire range of E values from 0 to 1.

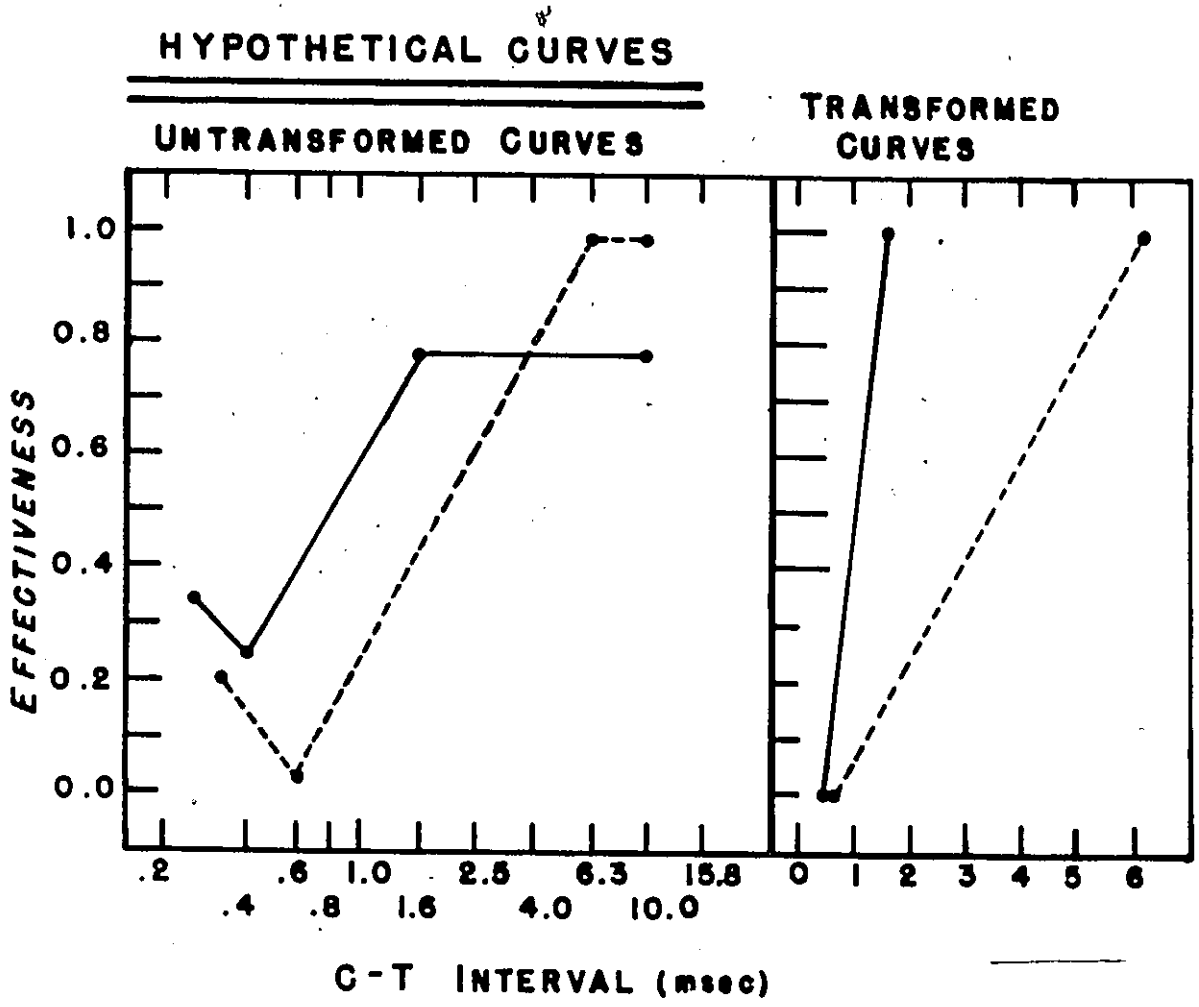
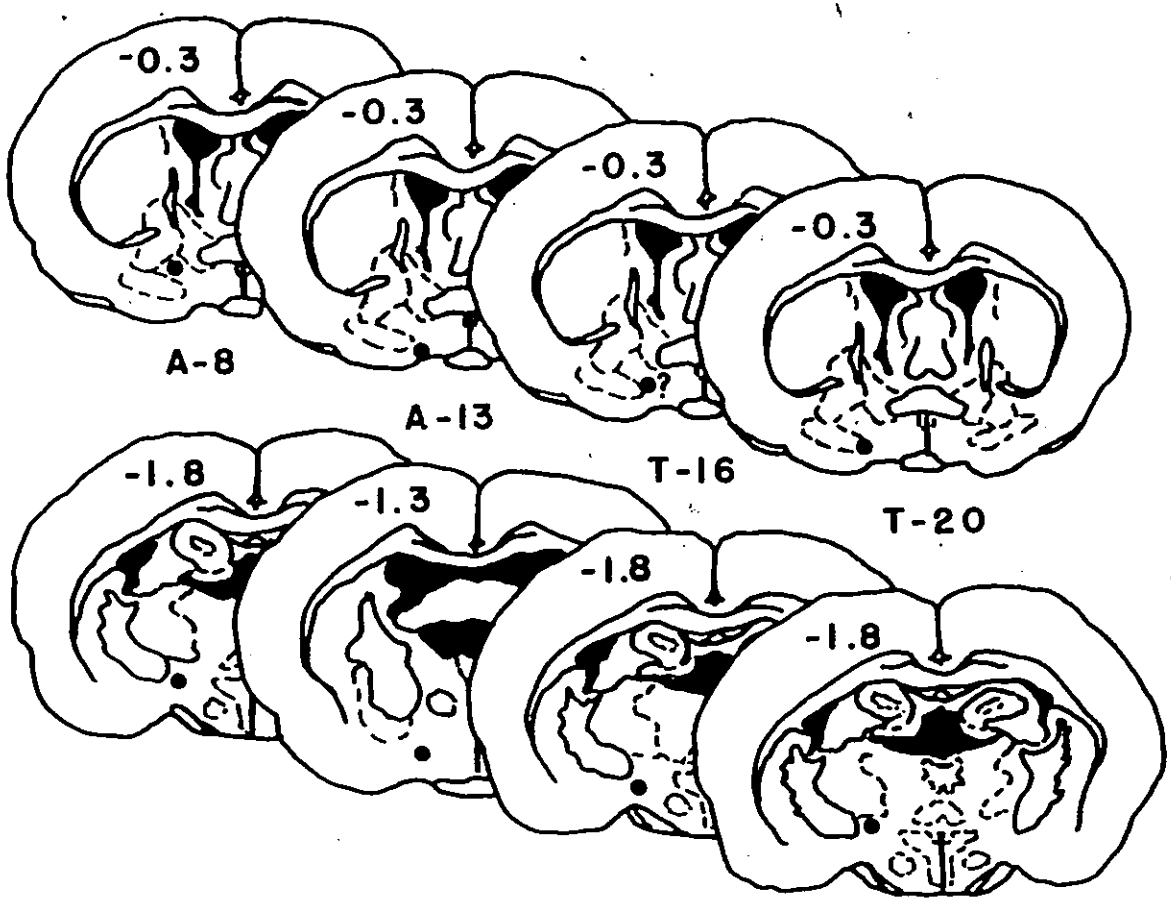

HYPOTHETICAL CURVES
UNTRANSFORMED CURVES
TRANSFORMED CURVES
EFFECTIVENESS
1.0
0.8
0.6
0.4
0.2
0.0
.2
.4
.6
.8
1.0
1.6
2.5
4.0
6.3
10.0
15.8
0
1
2
3
4
5
6
C-T INTERVAL (msec)

Figure 5

Drawings from the pertinent atlas plates in the Paxinos and Watson (1982) atlas. The location of the electrode tips is denoted by the filled circles. The question mark beside the electrode location of T - 16, anterior placement, indicates uncertainty as to the exact location of the placement. The distance from bregma is given in mm on each section drawing. The subject identification numbers are positioned between the anterior and posterior section for that subject.

ANTERIOR PLACEMENTS



POSTERIOR PLACEMENTS

slopes, minimum and asymptote C - T intervals, the rising portion of each refractory period curve in the present experiment was transformed. The transformation normalizes the rise such that it spans the entire range of effectiveness values from 0 to 1 (Bielajew, et al., 1981). The transformation procedure makes the assumption that even when the plateau lies below the theoretically predicted value of 1.0 it still reflects complete recovery from refractoriness. The failure of some curves to attain effectiveness values of 1.0 is not understood.

When this transformation is used on the hypothetical curves of Figure 4 the time course of the recovery of the solid line curve is shown to be clearly faster than that of the broken line curve (see Figure 4, right hand side). The formula used was:

$$E_{(transformed)} = E_{(C - T)} - E_{(min)} \; / \; E_{(asymptote)} - E_{(min)}$$

Although the transformation is successful in alleviating the interpretation problems caused by differences in the curves it is not without criticism. In normalizing the rising portion of the curve the effectiveness values span the entire range from 0 to 1 and by doing so may suppress the contribution of neurons with absolute refractory periods shorter than 0.4 to 0.6 msec. This problem would be more serious in a case where the lowest E value was associated with a longer C - T interval. At present it is not known if neurons with absolute refractory periods shorter than 0.4 msec exist in the CNS (Swadlow & Waxman, 1978).

Histology.

Following completion of the experiment the animals were sacrificed with a lethal dose of sodium pentobarbital. They were perfused intracardially with physiological saline followed by 10 % formalin.

The electrode assembly was removed by mounting the head in the stereotaxic and building up the crown with dental acrylic so that it was firmly secured to a large screw which could later be lifted vertically by the stereotaxic apparatus. After drilling throught the base of the skull the electrode assembly was free to be lifted, without incurring displacement of the two electrodes.

The brains were removed and stored for a minimum of 24 hours in 10% formalin and were then sliced to 40u sections on a cryostat. The sections were stained with cresyl violet. The electrode tips were located using the Paxinos and Watson (1982) atlas.

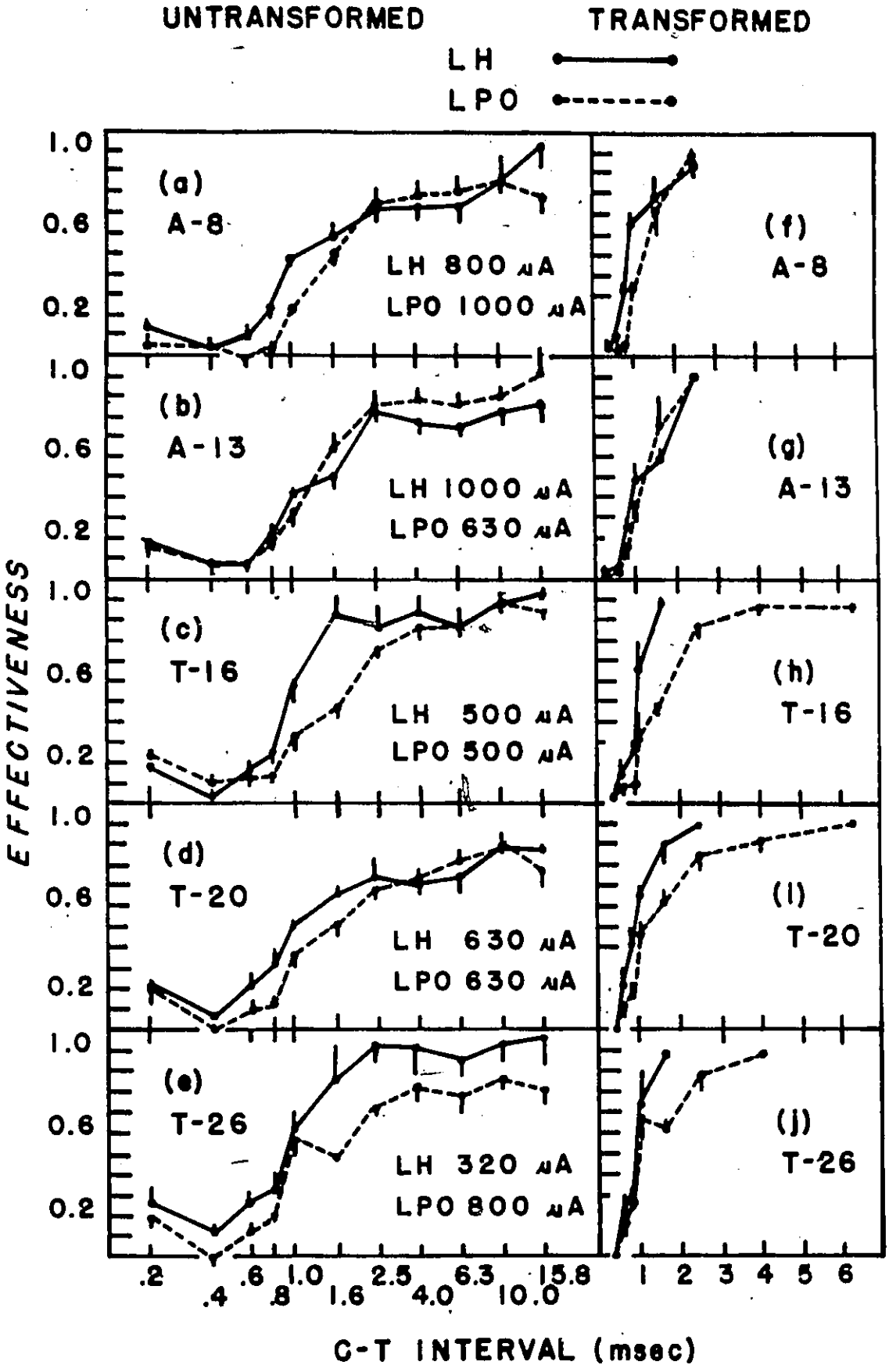
Results and Discussion

Histology

Figure 5 contains drawings from the Paxinos and Watson (1982) atlas that best corresponds to the sections bearing the electrode tip; the tips are represented as filled circles. The anterior electrode tips of subjects A - 8, A - 13, T - 16, and T - 20 were located in and about the LPO area. They were closely clustered in the anterior/posterior plane at the level of the nucleus of the horizontal limb of the diagonal band of Broca while showing some deviation in the dorsal/ventral plane. The

Figure 6

Results of the refractory period tests. The untransformed results are plotted on a logarithmic scale on the left hand side of the figure while the transformed results are plotted on a linear scale on the right hand side of the figure. The logarithmic scale is used for the untransformed results because of the wide range of C - T intervals tested. The LH results are depicted by the solid line; the broken line represents the LPO results. The identity of each subject is located in each box or panel. The currents used on each electrode are listed in the untransformed boxes.


UNTRANSFORMED
TRANSFORMED
LH
LPO
(a)
A-8
LH 800 μA
LPO 1000 μA
(f)
A-8
(b)
A-13
LH 1000 μA
LPO 630 μA
(g)
A-13
(c)
T-16
LH 500 μA
LPO 500 μA
(h)
T-16
(d)
T-20
LH 630 μA
LPO 630 μA
(i)
T-20
(e)
T-26
LH 320 μA
LPO 800 μA
(j)
T-26
EFFECTIVENESS
1.0
0.6
0.2
.2
.4
.6
.8
1.0
1.6
2.5
4.0
6.3
10.0
15.8
1
2
3
4
5
6
C-T INTERVAL (msec)

question mark near the anterior electrode tip of subject T - 16 indicates uncertainty about the location of this placement.

The posterior electrode tips were found in the region of the LH, adjacent to the internal capsule at the level of the paraventricular hypothalamic nucleus, for all subjects with the exception of A - 13. The electrode tip for this subject was located in the LH slightly more anterior and more ventral.

Histology was not available for subject T - 26.

Refractory Period Data

The results of the refractory period tests are shown in Figure &. The untransformed values for each animal are located on the left hand side of the figure while the transformed values are shown on the right hand side.

As expected, local potential summation effects are observed at the shortest C - T intervals (Yeomans, 1979); higher effectiveness values are associated with C - T intervals of 0.2 than of 0.4 msec. Minimum effectiveness values are observed at 0.4 msec in all cases except the LPO refractory period curve in the case of A - 8 which has its lowest value at a C - T interval of 0.6 msec. The effectiveness values then increase steadily approaching asymptotes at longer C - T intervals, 1.6 or 2.5 msec for the LH and 2.5 to 6.3 for the LPO with only slight increases observed at longer C - T intervals across animals. Table 1 lists the

minimum and asymptotic C - T intervals associated with recovery from refractoriness for each subject.

The rise in effectiveness reported for the LH refractoriness corresponds to the expected results for this placement (Bielajew, et al., 1981; Bielajew, et al., 1982; Bielajew & Shizgal, 1982; 1986; Hawkins, et al., 1983; Gallistel, 1978; Milliaressis & Rompre, 1980; Rompre & Milliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975, 1979). Typically, it has been reported that recovery from refractoriness in LH reward sites is complete by 1.2 to 2.5 msec with 80% recovery obtained by 1.2 msec. The asymptote point determined for the LPO placement, however, was well outside this range. The earliest plateau observed was at 2.5 msec in subjects A - 8 and A -13 while in the remaining subjects, the refractory period curves leveled off as late as 4.0 - 6.3 msec. The asymptote points for both the LH and LPO placements are given in Table 1. It would appear from these initial observations that recovery from refractoriness in the LH occurs earlier than in the LPO. As noted earlier, however, conclusions based on the untransformed scores can be misleading because of differences in minimum C - T interval, slope and asymptote point. For this reason, the statistical analysis of differences in the rate of recovery between the two sites was based on the rising portion of the refractory period curve.

The E values presented on the right side of Figure 6 (f, g, h, i, j) represent the transformed scores for the rising portion of the refractory period curves.

RECOVERY FROM REFRACTORY

	LH	LPO
SUBJECT	MIN - MAX C-T INTERVAL (msec)	MIN - MAX C-T INTERVAL (msec)
A-8	0.4 - 2.5	0.6 - 2.5
A-13	0.4 - 2.5	0.4 - 2.5
T-16	0.4 - 1.6	0.4 - 6.3
T-20	0.4 - 2.5	0.4 - 6.3
T-26	0.4 - 1.6	0.4 - 4.0

REGRESSION TEST RESULTS

SUBJECT	df	F RATIO
A-8	2/62	5.463*
A-13	2/44	1.251
T-16	2/48	27.803*
T-20	2/52	18.041*
T-26	2/44	16.136*

*Significant ($p < 0.01$)

RESULTS of T-TESTS on SLOPES

SUBJECT	LH SLOPE	LPO SLOPE	df	T SCORE
A-8	43.259	53.602	62	1.607
T-16	86.226	17.416	48	4.254*
T-20	45.922	16.504	52	6.091*
T-26	81.049	25.490	44	5.264*

*Significant ($p < 0.01$, 1-tail)

RESULTS OF T-TESTS ON INTERCEPTS

SUBJECT	LH INTERCEPT	LPO INTERCEPT	df	T-SCORE
A-8	-1.831	-27.682	62	3.607 *
T-16	-34.679	9.681	48	5.954 *
T-20	-0.061	15.817	52	1.694 *
T-26	-28.523	10.915	44	4.591 *

*Significant ($p < 0.05$, one-tail)

Statistical Analysis - Transformed Data.

The data from each refractory period session were subjected to the transformation procedure. Each point on the final transformed curve, therefore, represents the mean of four to six transformed scores. The transformation was done on the rising portion of each refractory period curve collected for all five subjects.

These data were analyzed using a linear regression analysis procedure (Neter & Wasserman, 1974) in order to assess whether or not the set of points obtained for each electrode placement was best described by one or two regression lines. A significant F ratio would be obtained if the points for each placement were better fitted by separate regression lines. A nonsignificant F ratio would result if the two sets of points were better described by a single regression line.

The results of the regression test appear in Table 2 and indicate that significant F ratios were obtained in 4 of the 5 subjects. Only in subject A - 13 were the results not significant.

The rising portion of the curve is believed to represent neuronal recovery from refractoriness. Differences between the slopes and intercepts of the two curves reflect differences in the time course of the recovery and in the point at which the recovery begins. A significant difference in slope indicates that the rate of recovery from refractoriness is faster at one placement than at the other. A significant difference in intercept is interpreted as differences in the point at which the curves begin to rise.

The significant F ratios suggest that there is a difference in

either the rate of neuronal recovery from refractoriness between the LH and LPO or in the time at which the recovery begins or a combination of the two. Because both factors may contribute to the F ratio, it was necessary to conduct t-tests on the differences between slopes and the differences between intercepts.

T tests for slope differences were conducted with the level of significance set at 0.01 for a one-tailed test. Table 3 lists the results of this analysis. Significant t scores were obtained for subjects T - 16, T - 20 and T - 26 suggesting that there are differences in the rate of recovery from refractoriness for the LH and LPO placements with the LH recovering earlier from refractoriness than the LPO in each case.

In addition to testing for differences in the rate of recovery, t-tests on the differences between intercepts were conducted to establish whether or not there were differences in the point at which recovery from refractoriness began. T-tests for intercept differences were performed on animals with significant F ratios to determine the contribution of intercept differences to the overall result.

The results of this test are presented in Table 4. A significant t score for intercept and a non-significant t score for slope are observed when the rates of recovery of the reward neurons at both placements are the same but the start of recovery occurs earlier at one placement. This was the case with subject A - 8. A significant t-test for intercept may also be obtained when the slope is significant which indicates that in addition to a difference in the rate at which the neurons recover there is also a difference in the point at which this recovery begins. This was

the case for the remaining three subjects: T -16, T - 20, and T - 26.

Because the F ratio was not significant for subject A - 13 it was concluded that neither the slopes nor the intercepts were significantly different. A single regression line based on the pooled results of both placements is able to account for a greater amount of the varience than a separate regression line for each placement.

It is interesting to note that A - 13's histological results also differed from all of the other subjects tested. The LH placement for this subject was slightly more anterior than the other four subjects and this placement difference, discussed below, could possibly account for the absence of refractory period difference.

There are at least two explanations that may account for the slow recovery from refractoriness observed at the LPO placements. It is possible that smaller fibers contribute more to the reward related neurons at the LPO site. LPO stimulation may excite a more heterogenous population of fibers than does LH stimulation or the smaller fibers may contribute more to the rewarding effect at the LPO placement than they do at the LH placement (Nieuwenhuys, et al., 1982; Swanson, 1986; Veening, Swanson, Cowan & Nieuwenhuys, 1982). Both placements have some contribution to the recovery from refractoriness at the short C - T intervals, however, this recovery continues at the longer C - T intervals with the LPO placements, several msec after the LH placements have reached an asymptote. If the LPO placement included multiple subpopulations of reward fibers with a wide range of refractory periods a continued rise in effectiveness at the long C - T intervals would be

expected. There is some evidence to support this notion as the refractory period estimates are much longer for anterior placements (Bielajew & Fouriezos, 1985; Fouriezos, et al., in press; Schenk & Shizgal, 1982). For example, sites in the anterior basal forebrain have refractory periods of 0.6 - 5.0 msec (Fouriezos, et al., in press), refractory periods in the mediodorsal thalamus have been reported to be 1.0 -10.0 msec (Bielajew & Fouriezos, 1985) and in the medial prefrontal cortex the refractory period estimates range from 0.8 - 6.0 msec (Schenk & Shizgal, 1982).

Alternatively, it is possible that the slower recovery observed at the two LPO sites results from stimulation of a homogenous population of fibers with a greater RRP contribution than at the LH. A long relative refractory period would decrease the slope of the recovery function. However, refractory period tests at similar placements using an unequal pulse procedure, in which the T pulses were 1.5 or 1.6 times the C pulse current, failed to influence the rate of recovery of these neurons (Fouriezos, et al., in press). These results suggest that the slow recovery from refractoriness cannot be easily attributed by a long relative refractory period contribution.

Experiment 2

Given the degree to which the LH and LPO refractory period curves overlap, it is possible that the two placements share a common set of reward fibers. Experiment 2 examines this possibility with a procedure

(Shizgal, et al., 1980) designed to test whether or not the two sites are directly linked by a subset of the same reward-related fibers.

Method

Subjects

The subjects and currents were the same as those used in Experiment 1.

Procedure

Collision Pretest.

A collision pretest was done on each of the animals following the stabilization procedure described in the first experiment. The collision procedure was analogous to the refractory period test except that instead of delivering C and T pulses to the same electrode, the C pulse was applied to one electrode and the T pulse to another such that in the anterior/posterior (AP) condition the LPO electrode received the C pulse and the LH electrode received the T pulse; in the posterior/anterior (PA) condition the C pulse was applied to the LH electrode and the T pulse was applied to the LPO electrode.

In the pretest sessions, currents were selected to yield frequency thresholds that were roughly matched across electrodes at three sets of current values: low, medium and high. Starting with the low current values, C - T intervals of 0.5, 5.0 and 10.0 msec were tested. A greater than 10 % increase in effectiveness between 0.5 and 5.0 or between 5.0

and 10.0 msec on six consecutive frequency threshold determinations constituted a successful pretest. Short, medium and long C - T intervals were used for the pretest because some animals showed increases in effectiveness between 0.5 and 5.0 and again between 5.0 and 10.0 msec while other animals showed increases in effectiveness only between one pair of C - T intervals. If the lowest pair of currents did not yield positive results, the animal was stabilizedd at the middle range of currents and the collision pretest rerun. If again no change in effectiveness values was observed between low, medium and high C - T intervals, the entire procedure was repeated at the highest currents. Three AP and three PA frequency threshold determinations were conducted per pretest. The currents used in the refractory period tests of Experiment 1 were determined by successful completion of the collision pretest.

Of the 24 animals that were implanted with LPO and LH electrodes, nine were easily shaped to self-stimulate on both electrodes. The collision pretest was positive for five of these nine.

Collision Tests.

Full collision tests were conducted on the five subjects that demonstrated collision effects in the pretest phase. Each collision test consisted of three LH and three LPO single pulse frequency threshold determinations, evenly spaced throughout the session. The number of double pulse determinations was based on the results of the collision pretest. Subjects A - 13 and T - 16 were tested with twelve C - T intervals which ranged from 0.2 to 15.8 msec. A C - T interval of 20.0

msec was included in the collision test of subject T - 26. The C - T interval was varied from 2.0 to 20.0 msec for subject A - 8 and from 0.63 to 5.0 msec for subject T - 20. In addition to being tested at currents that produced collision results, data were also collected for subject T - 20 at currents that failed to yield collision effects using the same range of C - T intervals.

The C - T intervals were randomly presented during each session. With the exception of the low current tests on T - 20, all of the collision sessions were interdigitated with the refractory period tests of Experiment 1. There were 4 to 6 replications of each AP and PA test.

Data Format.

Just as in the first experiment, the contribution of the second pulse to the rewarding effect of the double pulse was assessed. Although the currents were selected such that the LH and LPO electrode single pulse frequency thresholds would be comparable, it was not possible to have them perfectly equal. Thus, Yeomans (1979) formula :

$$E = FT_{(SPL)} / FT_{(C-T)} - 1 / FT_{(SPL)} / FT_{(SPH)}$$

where

E = T pulse effectiveness

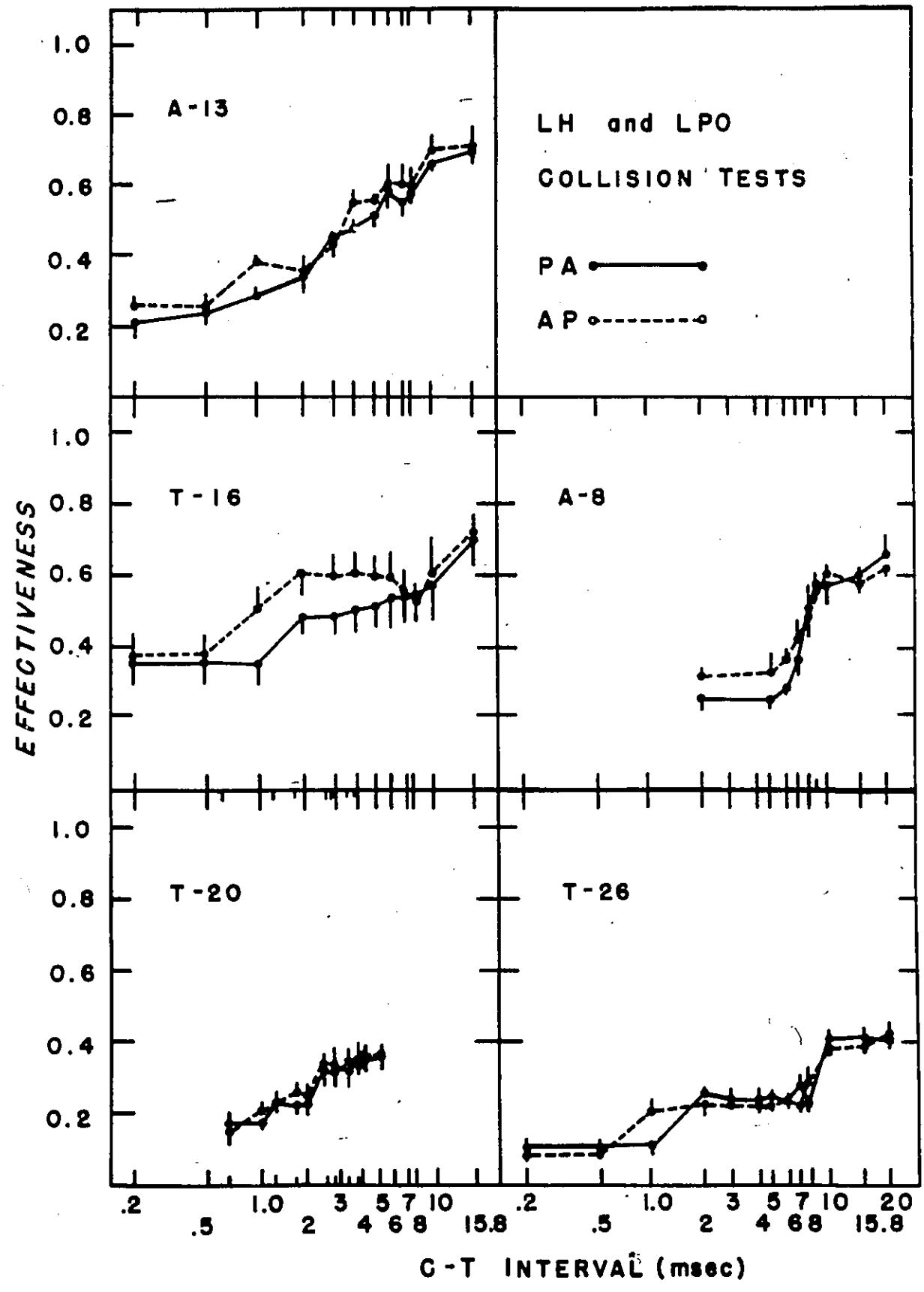
$FT_{(SPL)}$ = the single pulse frequency threshold which has the lower numerical value

$FT_{(C-T)}$ = the frequency threshold of the C - T interval under consideration

$FT_{(SPH)}$ = the single pulse frequency threshold which has a higher numerical value

Figure 7

LH and LPO collision test results are depicted: The PA tests are represented by the solid lines and the AP tests by the broken lines. In the PA tests the first pulse was delivered to the posterior electrode (LH) and the second pulse to the anterior electrode (LPO); in AP tests the C pulse was delivered to the LPO placement and the T pulse to the LH placement. The identification numbers and currents are given with the test results for each subject. Small dashes on the abscissa denote the C - T intervals tested with subjects T - 20 and A - 8 only.


A-13
LH and LPO
COLLISION TESTS
PA
AP
T-16
A-8
T-20
T-26
EFFECTIVENESS
1.0
0.8
0.6
0.4
0.2
.2
.5
1.0
2
3
4
5
6
7
8
10
15.8
20
C-T INTERVAL (msec)

COLLISION RESULTS: DIFFERENCE IN E VALUE

	AP CONDITION			PA CONDITION		
SUBJECT	Minimum E Value	Maximum E Value	Difference	Minimum E Value	Maximum E Value	Difference
A-8	.32	.62	.30	.26	.66	.40
A-13	.25	.71	.46	.21	.70	.49
T-16	.38	.73	.35	.36	.72	.36
T-20	.16	.36	.20	.18	.36	.18
T-26	.09	.42	.33	.11	.41	.30

Asymptote Test Results

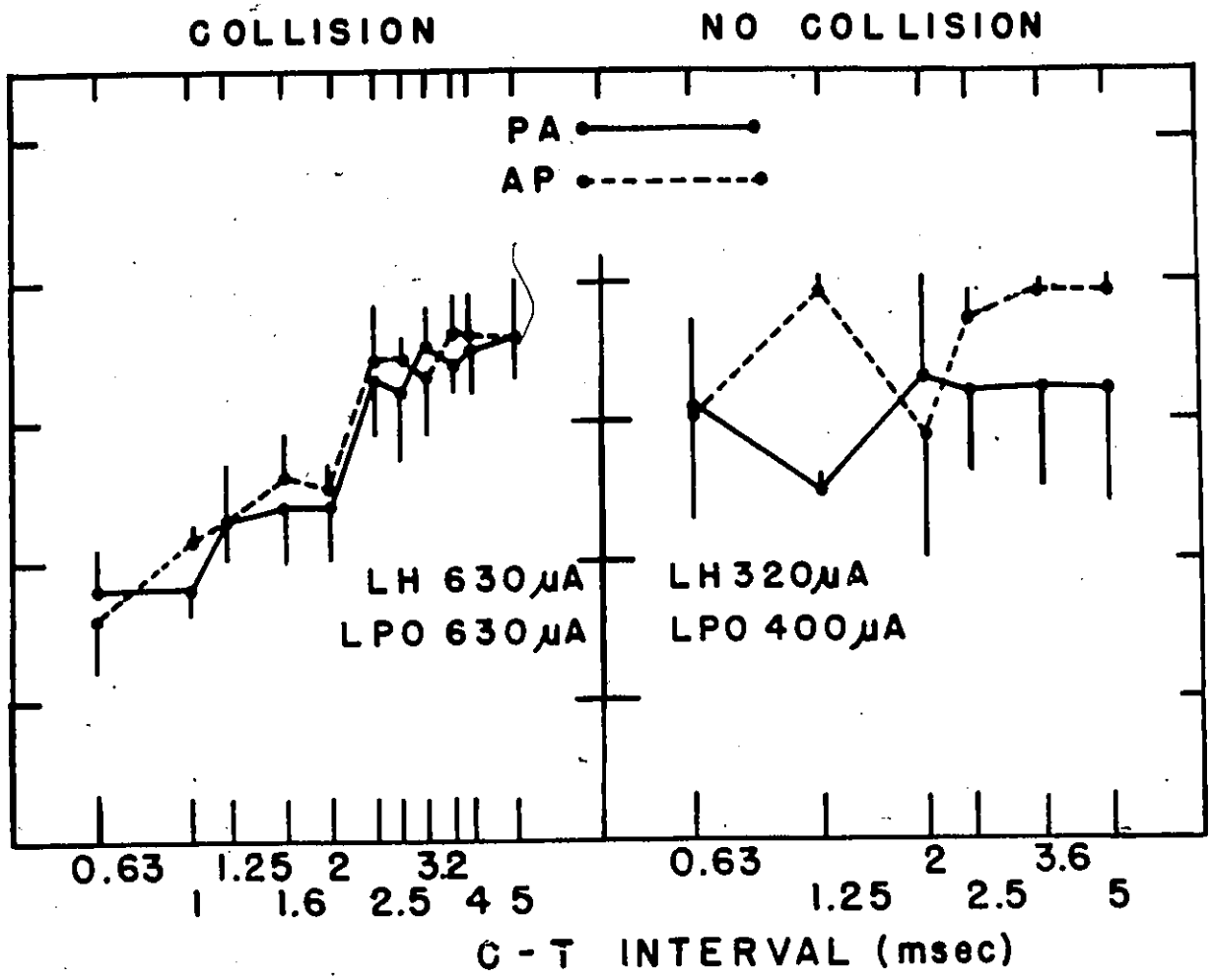
Subject	AP Condition		PA Condition	
	Minimum Point	Asymptote Point	Minimum Point	Asymptote Point
A-8	6.0	9.0	6.0	9.0
A-13	0.5	10.0	1.0	10.0
T-16	0.5	10.0	1.0	10.0
T-20	1.25	2.5	1.25	2.5
T-26	0.5	10.0	1.0	10.0

Figure 8

Collision and no-collision results for subject T - 20. Collision tests results are shown on the left hand side of the figure while the low current results are shown on the right hand side of the figure. The currents are given with the test results. PA condition results are denoted by a solid line; a broken line represents the AP condition results.

At high currents the effectiveness values increase across C - T intervals while at low currents the effectiveness values do not. These results are interpreted as evidence for collision at high currents but not at low currents.

T-20


COLLISION
NO COLLISION
PA
AP
LH 630μA
LPO 630μA
LH 320μA
LPO 400μA
0.63
1
1.25
1.6
2
2.5
3.2
4
5
0.63
1.25
2
2.5
3.6
5
C-T INTERVAL (msec)

corrects for the inequality in the frequency threshold. If the frequency thresholds for both electrodes were identical the lower term would reduce to one and calculation of double pulse effectiveness for the collision data would not differ from that used with the refractory period data.

Results and Discussion

Collision Data

The results of the LH and LPO collision tests are depicted in Figure 7. All subjects show increased effectiveness values across C - T intervals for both the AP and PA tests. The range of this increase, however, varies considerably. Table 5 lists the difference in effectiveness between the lowest and the highest effectiveness values across all C - T intervals tested. Subject A - 13 showed the greatest difference with the effectiveness value being increased by approximately 0.5 while subject T - 20 had the least difference with only 0.18 between the lowest and highest effectiveness values.

The asymptote test described in experiment one was performed on the collision data in order to statistically define an upper plateau and a lower shelf. The results of this test appear in Table 6. As in experiment 1, the asymptote point describes the shortest C - T interval to be included in the asymptote pool while the minimum point is defined as the longest C - T interval to be included in the lower pool before the rise in effectiveness values occurs.

In dealing with the LH - LPO collision curves it is disturbing that the collision plateau is very low for subjects T - 20 and T - 26. This is in contrast to collision curves obtained from LH - VTA stimulation which usually have effectiveness values that approach 1.0 once the rise is complete (Bielajew & Shizgal, 1982; Durivage, 1985; Gratton, 1985). It should be noted that T - 20 was only tested at C - T intervals up to 5.0 msec whereas all of the remaining subjects were tested with C - T intervals of up to at least 10.0 msec. Increases in effectiveness were observed in these animals at C - T intervals greater than 5.0 msec, thus, it is possible that the same would have occurred had T - 20 been tested at these delays. While the low collision plateau is easily explained in the case of T - 20, where the longest C - T interval tested was 5.0 msec, it is far more enigmatic in the case of T - 26. It was observed that the LPO refractory period results from this subject also displayed a low summation level. It is possible that since the effect was also seen in the single electrode data, it may be unrelated to the two-electrode stimulation. Support for this possibility comes from the observation of a similar phenomenon at LH - PAG sites where an effect of single electrode stimulation was observed in the double electrode data (Bielajew, et al., 1981) and from the low single electrode plateau of subject LL8's circling data (Yeomans & Linney, 1986).

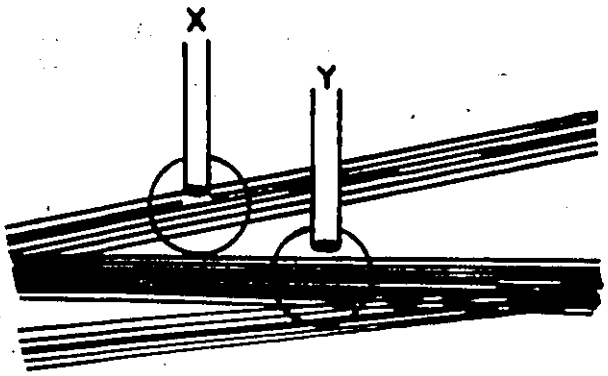
Although the magnitude of the collision effect between upper and lower plateaus in subject T - 20 was quite small (0.18 difference), reducing the intensities failed to produce any such difference. The collision and no-collision results are shown in Figure 8. The collision test results are shown on the left hand side of the figure while the low

Figure 9

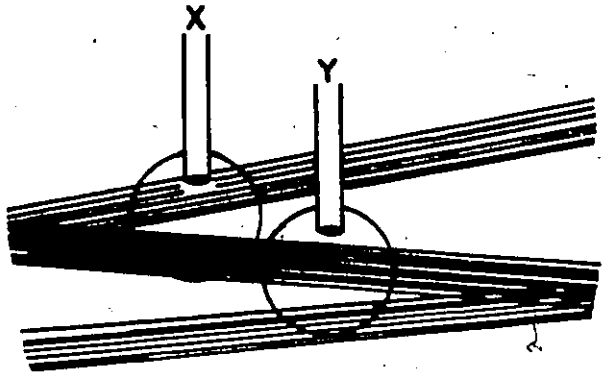
A possible fiber arrangement which would account for the present collision results is suggested. At low currents, the stimulation fields of both electrodes have no common elements. A subset of fibers is, however, common to both placements at high currents.

Misalignment of Electrode Fields

LOW INTENSITY



HIGH INTENSITY



current results are shown on the right hand side. The absence of collision is denoted as a flat line with little or no change in effectiveness values.

In all subjects, the shortest C - T intervals never yielded effectiveness values of 0; instead, the second pulse always added to the rewarding effect. The initial effectiveness value observed at a C - T interval of 0.2 msec was always between 10 and 40 %. It is unlikely that this initial effectiveness value could be due to local potential summation effects as there was no subsequent decline in the effectiveness value at 0.5 or 1.0 msec. Although one could argue that local potential summation effects are most typically seen at a C - T interval of 0.4 msec and the lack of decline in effectiveness at 0.5 msec merely reflects the recovery of local potential summation, the interelectrode distance of approximately 2 mm further argues against local potential summation effects as the cause of these initial effectiveness values (Fouriezos & Wise, 1984; Yeomans, Prior & Bateman, 1986). Thus, it appears that the collision effects obtained in this study reflect the activation of only a subset of the fibers common to the LH and LPO possibly due to misalignment of the two stimulation fields.

Figure 9 suggests a possible arrangement of fibers that could account for the results obtained in the present collision study (Shizgal, et al.,1980). At low currents, none of the fibers stimulated by one electrode are contained in the stimulation field of the second electrode so that no changes in effectiveness values will be seen as the C - T interval is increased. At high currents, however, a subset of fibers stimulated at one site will also be stimulated at the second site resulting in increases in effectiveness values at the longer C - T

intervals. This misalignment of electrode fields also serves to explain why the effectiveness values are never 0; even at the short C - T intervals, where orthodromic and antidromic action potentials collide, the second pulse always stimulates fibers that remain unaffected by the first pulse firings.

Conduction Velocity

The effectiveness values in the collision experiment rose slowly over a wide range of C - T intervals suggesting either the involvement of several subpopulations of reward-related fibers with a wide distribution of conduction velocities or a more homogenous population with a prominent relative refractory period contribution. The former explanation is more consistent with the unequal pulse refractory period results of Fouriezos et al., (in press) for the LPO site: The rate of recovery from refractoriness was unaffected by a T pulse current of 1.5 or 1.6 times the C pulse current.

Recbvery from refractoriness at the LH has been demonstrated to be faster than the LPO, therefore, if RRP contributes to the rate of recovery, it does so more at the LPO than at the LH. A long RRP contribution at the LPO site would result in a consistently delayed rise in the collision curve obtained from the PA condition only. The propagation of two sets of action potentials i a collision test requires that the C - T interval exceed the sum of the conduction time and the refractory period of the placement receiving the T pulse. When the T pulse was delivered to the LH rather than the LPO there was no change in

the shape of the collision curve regardless of the order presentation of the pulses. The reasonable symmetry in the AP and PA condition results suggests that the recovery of different populations of fibers rather than RRP effects at the LPO site contribute more to the shape of the collision profiles.

The conduction velocity is a function of the size of the axon and the presence or absence of myelination (Swadlow & Waxman, 1978). The rate at which the axon conducts and the point at which collision occurs depends on the calibre of fibers that are stimulated. When a heterogenous population of fibers is activated in a collision test an increase in effectiveness values at a given C - T interval may reflect the contribution of only a small portion of the relevant fibers. In addition, the fibers stimulated at one site may not necessarily be stimulated at the second site. The effectiveness values in a collision curve do not represent the contribution of all of the axons in the effective stimulation field unlike the refractory period condition in which all axons contained within the effective stimulation field undergo recovery from refractoriness and contribute to the change in effectiveness values.

In determining the conduction velocity of an axon the usual procedure involves an estimate of the collision interval and the conduction time. The interelectrode distance for each subject is then divided by the conduction time (Bielajew & Shizgal, 1982; 1986; Durivage, 1985; Gratton, 1985; Shizgal, et al., 1980; Yeomans & Linney, 1985). In dealing with a homogenous population of fibers the determination of conduction velocity is relatively straightforward. For example, Shizgal, et al., (1980) and Bielajew and Shizgal (1982) defined the collision

interval as the C - T interval at which an abrupt rise in effectiveness occurs. The conduction time was then arrived at by taking the difference between the collision interval and the corresponding refractory period interval. When the collision curve has a sharp, step-like increase the range of possible conduction velocities is smaller because the collision and refractory period curves are not as divergent. Estimating the conduction velocity in this manner minimizes the estimates of conduction velocity (Bielajew & Shizgal, 1982) because the criteria used for estimating the collision interval uses the shortest conduction time estimate. In attempting to compare their conduction velocity estimates to those of catecholaminergic neurons which have been electrophysiologically determined, Bielajew and Shizgal (1982) selected the method which would reduce their estimates and provide the most conservative difference between the behaviorally and electrophysiologically derived values.

When the abrupt step-like increase in effectiveness values typically associated with LH and VTA collision effects (Bielajew, et al., 1981; Durivage, 1985) is not produced a more comprehensive approach for estimating conduction velocities must be adopted. When the two placements being stimulated are linked anatomically an increase in effectiveness values at a given C - T interval represents the time necessary for an action potential to exceed the sum of the interelectrode conduction time and the refractory period. Changes in the effectiveness values occur over a wide range of C - T intervals if the fibers being stimulated have a range of conduction times or refractory periods. For slow rising collision curves Yeomans & Linney (1985) suggest transforming the collision data in two ways and then comparing

these transformed curves to three different places on the refractory period curve. Conduction times are determined at effectiveness values of 20, 50 and 80 % collision after each transformation.

The first transformation suggested is identical to that used in the refractory period experiment where the range of effectiveness values is normalized such that these values span the entire available range from 0 to one. Just as in the refractory period experiment the contribution to the recovery from the fastest fibers at the shortest C - T intervals is lost with this procedure. Also, by normalizing the collision curve such that the range of effectiveness values is close to that of the refractory period curve an assumption is made that the fibers stimulated at both placements during collision all contribute equally to the refractory period profile. Consequently conduction velocity estimates that are based on normalized collision curves are higher than would actually be the case because only the slowly conducting fibers are being considered.

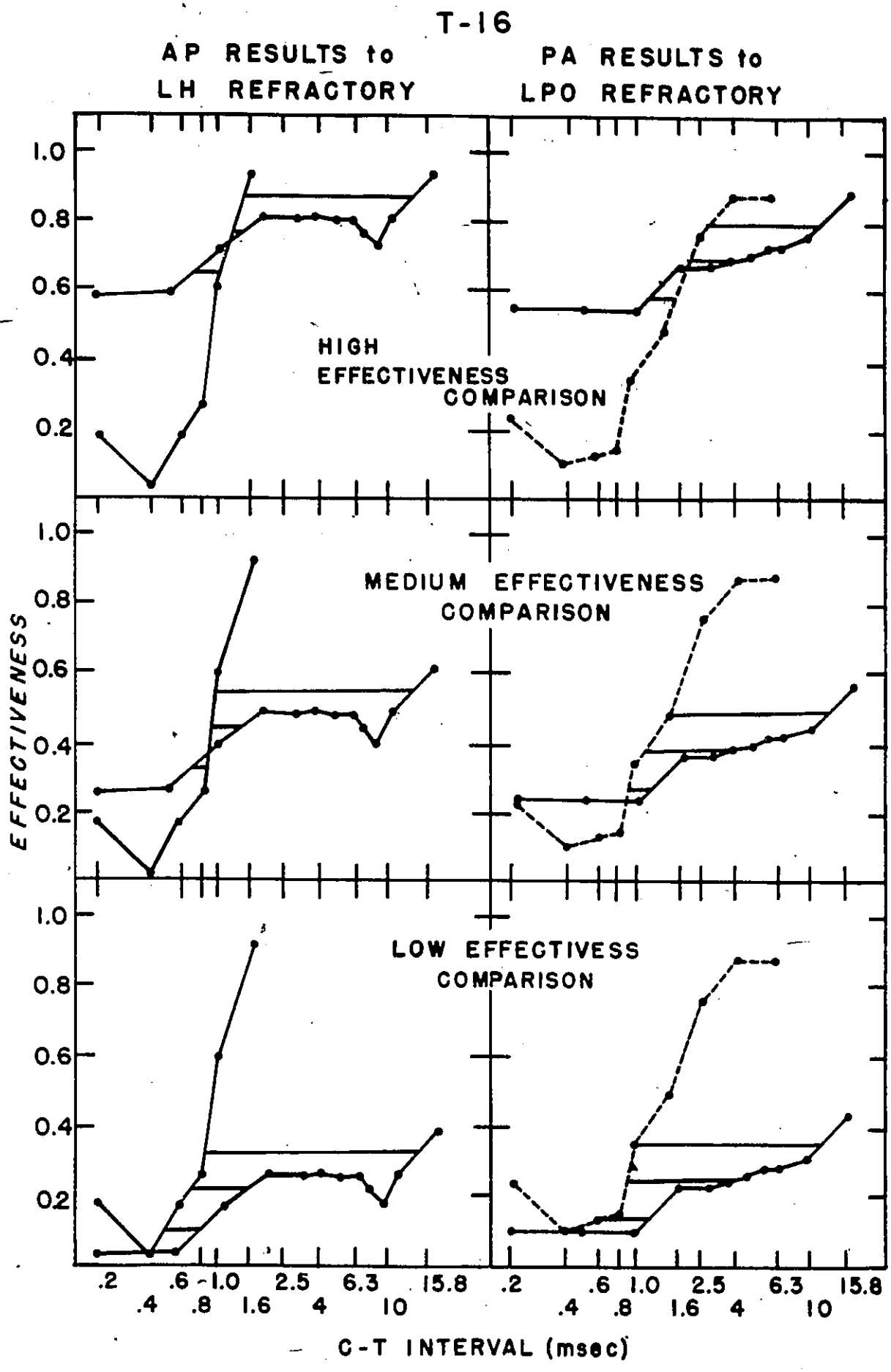
The second transformation takes into account the contribution from the faster fibers and involves zeroeing the collision curves. This consists of bringing the lowest effectiveness value to zero by subtracting a constant so that the resulting curve can be compared to the initial rise in the refractory period curve. Yeomans and Linney (1985) performed the transformation at each C - T interval by applying the following formula:

$$E_{zeroed} = E_{C-T} - E_{minimum}$$

Although this transformation does take into account the contribution of the fastest recovering neurons there is a further problem inherent in this approach. The fibers mediating the observed collision effect are

Figure 10

Conduction time estimates at 20, 50 and 80 % collision for low, medium and high effectiveness regions of the refractory period curve for a representative subject, T - 16.


T-16
AP RESULTS to LH REFRACTORY
PA RESULTS to LPO REFRACTORY
HIGH EFFECTIVENESS COMPARISON
MEDIUM EFFECTIVENESS COMPARISON
LOW EFFECTIVESS COMPARISON
EFFECTIVENESS
1.0
0.8
0.6
0.4
0.2
.2
.4
.6
.8
1.0
1.6
2.5
4
6.3
10
15.8
C-T INTERVAL (msec)

more likely to conduct slowly given the range of C - T intervals over which the effectiveness values increase. Estimates of conduction velocity based solely on these transformed curves would thus be too high.

The actual estimate of the conduction velocities lies somewhere in between that of the normalized and zeroed results. The discrepancies are, in part, due to the fact that while all of the neurons stimulated at a particular site contribute to the refractory period curve only a subset of these neurons are contributing to the changes in effectiveness in the collision curve. In order to arrive at the closest available estimate of conduction velocity, Fouriezos (personal communication) suggests using the conduction properties of the fibers to determine the best area of the refractory period curve from which to estimate conduction time. Because antidromically and orthodromically generated action potentials conduct at the same rate, there should be little or no difference between AP and PA estimates of conduction velocity. Conduction velocities were thus estimated for both the AP and PA conditions at low, medium and high effectiveness value areas of the refractory period curve. Conduction times were determined at 20, 50 and 80 % collision for each of the three areas.

Figure 10 depicts how conduction times were estimated at 20, 50 and 80 % collision for low, medium and high effectiveness values of the relevant refractory period curve for a representative subject, T - 16. The conduction time estimates were established for the AP condition compared against the refractory period results of the posterior placement. Similarly, the conduction time estimates for the PA condition were established with the refractory period results of the anterior

placement. Recall that in order to avoid collision, C pulses must propagate by the T pulse site and the latter region must recover from refractoriness. In the AP condition the T pulse site is the LH while in the PA condition the T pulse site is the LPO. The low effectiveness comparisons were done by shifting the collision curves such that the lowest effectiveness values for both the collision curves and the refractory curves were equivalent while maintaining the same range of C - T intervals. The medium effectiveness conduction times were established by shifting the collision curve up so that the 50 % collision point was equal to the 50 % total effectiveness point of the refractory period curve. It should be noted that the maximum effectiveness of the refractory period curves used for these calculations was also the asymptote point established in Experiment 1. The high effectiveness value conduction times were done by raising the collision curve. The collision curve's maximum effectiveness point was brought up equal to the asymptote point of the refractory period curve estalished in Experiment 1.

The conduction velocity estimates for both the AP to posterior (P) placement comparisons and the PA to anterior (A) placement comparisons are reported in Table 7 for all subjects at the three areas of the refractory period curve. The conduction velocities are obtained by dividing the interelectrode distance by the conduction time estimates. The conduction time estimates for all subjects are given in Table 8. The negative conduction velocities represent cases in which moving the collision curve resulted in it having a higher effectiveness value at a shorter C - T interval than the refractory period curve at that point. Because there is no difference in the conduction velocity regardless of

whether the conduction direction is antidromic or orthodromic, the conduction velocity estimates with the least AP - PA difference are the most meaningful.

The AP - PA differences are listed in Table 9. Based on these differences, the best conduction velocity estimates are obtained with the low refractory period effectiveness comparisons for subjects A - 8, A - 13, and T - 16 while comparisons with the medium refractory period effectiveness values are more appropriate for subjects T - 20 and T - 26. The best conduction velocity estimates arrived at for each of the subjects are located along with the individual interelectrode distances in Table 10. Conduction velocities fall within a range of 0.24 m/sec to 11 m/sec. With the exception of A - 8, the conduction velocity estimates arrived at using 20 % collision are fast, ranging from 3 m/sec to 11 m/sec. At 80 % collision the conduction velocity estimates for all subjects are much slower, ranging from 0.24 to 1.5 m/sec.

This is consistent with expected conduction velocities; at short C - T intervals, the fastest conducting fibers will be implicated whereas slower conducting fibers will contribute more at longer C - T intervals. The greatest difference in AP and PA conduction velocity estimates occurs at the 80 % effectiveness region. One possible explanation for this is that the effective stimulation field at the LPO site includes a greater number of somatic elements. The end of the recovery profile as well as the 80 % collision effectiveness would reflect activation of both cell bodies and axons.

Previous behaviorally derived estimates of conduction velocity have been reported for fibers mediating self-stimulation between the LH and

the VTA (Bielajew & Shizgal, 1982; Durivage, 1985, Gratton, 1985; Shizgal, et al.,1980) and for circling fibers between pontine and midbrain sites (Milliaressis & Phillippe, 1983; Yeomans & Linney, 1985). The conduction velocity estimates obtained for fibers linking the LH to the VTA ranged from 1.0 to 8,0 m/sec (Bielajew & Shizgal, 1982). A single conduction velocity estimate was calculated for each subject in this study as both the refractory period and collision curves showed sharp increases in effectivess values. Although the refractory period curves rose abruptly, a range of conduction velocity estimates at different regions of the refractory period curve would be more informative when different refractory periods are obtained. When the collision curve rises abruptly, however, it may be that a single population of fibers is contributing to the effect making it unnecessary to analyze the contributions of different regions of the refractory period curves to the collision profile.

Conduction velocity estimates obtained for axons mediating circling range from 2.5 to 19 m/sec (Yeomans & Linney, 1985). Although these estimates are obtained using two different transformations procedures at 20, 50 and 80 % collision, neither transformation is entirely satisfactory. When the transformation involves normalizing the data, the conduction velocity estimates are too high and when the data are transformed by zeroeing, the estimates of conduction velocity are too low.

The present conduction velocity estimates, ranging from 0.24 to 11 m/sec, take into account the differences in the LH and LPO refractory periods and the possible contribution of only a subset of fibers from

Conduction Velocity Estimates (m/sec)

		AP results to LH refractory			PA results to LPO refractory		
	% COLLISION	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
SUBJECT	20	0.38	0.40	0.28	0.33	0.34	0.30
A-8	50	0.29	0.30	0.33	0.29	0.30	0.32
	80	0.24	0.25	0.41	0.24	0.25	0.28
	20	11.0	-22.0	-5.5	4.4	5.5	22.0
A-13	50	0.88	1.0	1.38	1.16	1.22	1.57
	80	0.31	0.33	0.36	0.30	0.31	0.34
	20	10.5	-21.0	-7.0	3.0	4.2	-5.25
T-16	50	3.5	5.25	21.0	0.41	0.43	0.55
	80	0.23	0.23	0.24	0.23	0.25	0.26
	20	4.0	9.0	-4.5	2.57	9.0	-1.38
T-20	50	1.64	2.25	18.0	1.13	1.64	-0.9
	80	1.0	1.2	4.5	1.06	1.5	-0.72
	20	10.0	4.0	-6.67	2.5	4.44	-3.33
T-26	50	0.36	0.37	0.39	1.48	1.82	-6.67
	80	0.24	0.24	0.26	0.24	0.24	0.31

Conduction Time Estimates

	AP results to LH refractory				PA results to LPO refractory		
SUBJECT	% COLLISION	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
A-8	20	5.1	4.8	6.7	5.75	5.6	6.3
	50	6.55	6.4	5.8	6.65	6.3	6.0
	80	7.9	7.5	4.6	7.9	7.5	6.9
A-13	20	0.2	-0.01	-0.4	0.5	0.4	0.1
	50	2.5	2.2	1.6	1.9	1.8	1.4
	80	7.2	6.6	6.2	7.3	7.2	6.4
T-16	20	0.2	-0.1	-0.3	0.7	0.5	-0.4
	50	0.6	0.4	0.1	5.1	4.9	3.8
	80	9.2	9.0	8.6	9.0	8.5	8.0
T-20	20	0.45	0.2	-0.4	0.7	0.2	-1.3
	50	1.1	0.8	0.1	1.6	1.1	-2.0
	80	1.8	1.5	0.4	1.7	1.2	-2.5
T-26	20	0.2	0.5	-0.3	0.8	0.45	-0.6
	50	5.5	5.4	5.1	1.35	1.1	-0.3
	80	8.4	8.3	7.8	8.4	8.2	6.4

Differences Between AP to LH and PA to LPO Conduction Velocity Estimates

SUBJECT	%COLLISION	LOW	MEDIUM	HIGH
A-8	20	0.05	0.06	0.02
	50	0	0	0.01
	80	0	0	0.14
A-13	20	5.5	27.5	27.5
	50	0.28	0.22	0.19
	80	0	0.03	0.01
T-16	20	7.5	25.2	1.75
	50	3.09	4.82	20.45
	80	0	0.01	0.02
T-20	20	1.43	0	3.12
	50	0.51	0.61	18.9
	80	0.06	0.3	5.22
T-26	20	7.5	0.44	3.33
	50	1.12	1.45	7.06
	80	0	0	0.06

CONDUCTION VELOCITIES CHOSEN (m/sec)

SUBJECT	Interelectrode Distance (mm)	Effectiveness Level		AP results to LH refractory	PA results to LPO refractory
A-8	1.9	Low	20	0.38	0.33
			50	0.29	0.29
			80	0.24	0.24
A-13	2.2	Low	20	11.0	4.4
			50	0.88	1.16
			80	0.31	0.31
T-16	2.1	Low	20	10.5	3.0
			50	3.5	0.41
			80	0.23	0.23
T-20	1.8	Medium	20	9.0	9.0
			50	2.25	1.64
			80	1.2	1.5
T-26	2.0	Medium	20	4.0	4.44
			50	0.37	1.82
			80	0.24	0.24

each refractory period curve to the collision curve by locating that part of the curve that is most likely to contribute to the collision results. The range of conduction velocities reported here supports the notion that fibers with a wide range of conduction velocities mediate the rewarding effect of brain-stimulation reward.

General Discussion

The work reported here investigates the possibility that a common set of reward fibers mediates self-stimulation elicited from the LH and the LPO. Anatomical linkage of self-stimulation fibers has been shown between the LH and the VTA (Bielajew & Shizgal, 1982; 1986; Shizgal, et al., 1980) but not between any other pair of sites. Failure to obtain collision effects from rewarding sites has been reported from LH and PAG (Bielajew, et al., 1981), LH and MPFC (Schenk & Shizgal, 1982) and rostral medial cortex and cingulate cortex (Silva, et al., 1982). This constitutes the first demonstration of collision in far anterior placements.

Results from both the refractory period and collision experiments are consistent with the view that self-stimulation behavior is subserved by several subpopulations of fibers. The refractory period estimates obtained from the LPO differed from those of the LH: Recovery from refractoriness began later and continued longer with the anterior site. The LPO refractory period ranged from 0.4 to 6.3 msec which overlaps with results obtained from other anterior placements (Bielajew & Fouriezos, 1985; Fouriezos, et al., in press; Schenk & Shizgal, 1982). The LH

results were consistent with those previously obtained from this placement (Bielajew, et al., 1981; Bielajew, et al., 1982; Bielajew & Shizgal, 1982; 1986; Hawkins, et al., 1983; Gallistel, 1978; Milliaressis & Rompre, 1980; Rompre & Milliaressis, 1980; Skelton & Shizgal, 1980; Yeomans, 1975, 1979) with recovery in this study ranging from 0.4 - 2.5 msec.

The most parsimonious explanation for the collision data presented in this thesis is that rewarding LH and LPO stimulation is subserved by multiple populations of fibers with conduction velocities ranging from 0.228 to 11 m/sec. Furthermore, this range suggests that a wide distribution of fiber diameters underlies this effect.

The collision curves obtained here differ in two ways from collision data obtained from stimulation of the LH and VTA (Bielajew & Shizgal, 1981; Durivage, 1985; Gratton, 1985). The LH - LPO collision curves do not have the sharp increases in effectiveness typically associated with LH - VTA collision (Bielajew & Shizgal, 1982; Durivage, 1985; Gratton, 1985). Increases in effectiveness range from 0.5 to 10.0 msec in the former case whereas the effectiveness values rose rapidly within 0.6 msec in the latter. This difference suggests that LH - LPO stimulation activates different fibers. The conduction velocity estimates range from 1.0 to 8.0 m/sec in LH - VTA collision and implicate a population of small, myelinated, fast-conducting fibers. The preent conduction velocity estimates range from 0.24 to 11 m/sec; both faster and slower conducting fibers are included in this range. The second difference relates to the asymptote level of the collision plateau. The asymptote points of LH - LPO collision curves are low when compared to

those of LH - VTA collision data. As explained earlier, the reason for the low summation level at these sites is not known but may be due to the low plateau observed in the single electrode data.

In summary, the rewarding neurons of the LH and LPO appear to be anatomically linked. The fibers mediating this effect probably differ from those linking LH - VTA collision sites that produce collision effects. Unlike previous collision results, the present findings are consistent with the view that several subpopulations of both myelinated and unmyelinated fibers participate in brain stimulation reward at these sites. Given the overlap in conduction velocity estimates in fibers that course between the LH - VTA and LH - LPO it is possible that a common subset of fibers project from the LPO to the VTA or from the VTA to the LPO. Using the behavioral pulse pair procedures it may be possible to establish the relationship which exists between the two pairs of collision sites that mediate brain-stimulation reward.

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