THE SUBSTRATE FOR PREFRONTAL CORTICAL SELF-STIMULATION : A PSYCHOPHYSICAL INVESTIGATION

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ABSTRACT

THE SUBSTRATE FOR PREFRONTAL CORTICAL SELF-STIMULATION: A PSYCHOPHYSICAL INVESTIGATION

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Several investigators have suggested that the substrates mediating the rewarding effects of stimulation of the lateral hypothalamus (LH) and prefrontal cortex (PFC) are comprised of different neurons. Psychophysical methods were used in the present study to estimate post- stimulation excitability characteristics and strength- duration characteristics of these substrates. Tests of the anatomical linkage between the PFC and LH reward substrates were conducted as was a study of spatio-temporal integration in the PFC substrate.

In the tests of post- stimulation excitability pairs of pulses were delivered through PFC or LH electrodes, and the number of pulse pairs required to maintain a criterial level of responding was determined at various within pair intervals. Recovery from refractoriness began later and spanned a longer interval at the PFC site than at the LH site.

The rewarding effects of pairs of pulses applied one to the PFC and one to the LH electrode summated poorly

regardless of the intra-pair interval. If the rewarding effects of stimulating the two sites resulted from the direct activation of the same cells, two results are expected. The level of summation should be high at long intra-pair intervals and should decrease abruptly as the within-pair interval is reduced due to the collision of antidromic and orthodromic volleys. That summation levels were both low and invariant across pulse pair interval suggests that the rewarding effect of stimulating the two sites was due to the activation of different cells that do not effectively pool their outputs.

In an attempt to further characterize the PFC reward substrate, anodal and cathodal strength duration functions were obtained. The chronaxies of the cathodal strength-duration curves for the two sites overlapped. These values are long (PFC median = 2.839 msec; LH median = 1.646 msec), in comparison to the available electrophysiologiocal values for single axons. Both reward substrates must include some neurons with very long chronaxies or cells that fire repetitively during long duration pulses. At several LH and PFC placements, the anodal chronaxie exceeded the cathodal value, suggesting that anode break excitation increased the effectiveness of long duration anodal pulses.

The largest difference between the PFC and LH strength-duration curves was that the rheobasic intensity for PFC self-stimulation exceeded the rheobase for LH self-stimulation. This finding, coupled with the refractory

period data, suggest that the substrate for PFC self-stimulation is comprised of smaller, less excitable neurons than the LH substrate.

The anodal and cathodal strength- duration curves were roughly parallel at pulse durations less than 10 msec. When averaged across pulse duration, the ratio of anodal/cathodal currents was lower (mean=1.54) than the values previously reported for LH sites.

In the final experiment, the stimulation frequency and intensity were traded off in order to characterize spatio-temporal integration in the PFC reward substrate. With high frequency stimulation, the minimum current that would support PFC self-stimulation was considerably higher than the minimum current reported for LH self-stimulation. The frequency/ intensity trade-off was fairly linear at least over a range of several hundred microamperes, suggesting that there was an extensive range of reward relevant axons in approximately constant density around the electrode tips. This is in contrast to a report in the literature in which current intensity increases were hypothesized not to enhance the rewarding effects of PFC stimulation.

Taken together, these data provide quantitative characteristics of the neural substrate for PFC self-stimulation that can be used to guide the electrophysiological identification of these cells and provide a basis for differentiating the PFC and LH reward substrates.

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INTRODUCTION

By determining the chemical and physiological characteristics of the neural substrate for brain stimulation reward (BSR), students of this phenomenon hope to shed light on central mechanisms of motivation and reinforcement. Several lines of evidence have suggested that the rewarding effects of stimulation are due to the activation of neural elements that mediate naturally occurring appetitive behavior. For example, BSR facilitates approach to appetitive stimuli while suppressing withdrawal from noxious ones (Stellar, Brooks and Mills, 1979). Electrical stimulation of brain sites that support BSR can induce feeding (Hoebel & Teitelbaum, 1962), sexual behavior (Herberg, 1963) and drinking (Mogenson & Stevenson, 1966). Factors that influence the vigor of appetitive behavior, such as satiety or deprivation, also modify self-stimulation (Olds, 1958; Hoebel, 1969). In sum, responding for BSR fluctuates in the way one would expect if the stimulated tissue were the substrate for conventional, appetitive rewards. Support for this interpretation comes from the finding that neurons activated by rewarding stimulation of a number of brain sites respond to the presentation and consumption of food in food deprived animals (Rolls, Burton & Mora, 1976).

An alternative interpretation of the electrophysiological data is that the neurons recorded by Rolls et al. are not those that are responsible for the rewarding effects of stimulation but subserve some other

function. There may simply be anatomical overlap between the stimulated substrates for both conventional rewards and BSR. How can one determine whether a cell driven by rewarding stimulation is part of the BSR substrate?

Surely not all cells activated by a stimulating electrode are involved in BSR. Stimulation through an electrode that supports BSR also produces a multitude of effects that seem not to be directly related to reward. For example, stimulation of BSR sites can also elicit exploration (Rompre and Miliaressis, 1980), circling (Miliaressis, 1981), copulation (Herberg, 1963), changes in nociception (Rose, 1974) and a variety of autonomic responses including changes in heart rate and blood pressure (Malmo, 1961; Perez-Cruet, Black & Brady, 1963). The question of how to tease out reward- related neurons from other cells that are also fired by rewarding stimulation has been a fundamental issue for students of BSR. One way to resolve this issue is to derive neurophysiological and anatomical characteristics of neurons that subserve the rewarding effects of stimulation and to compare these values to the characteristics of the cells from which Rolls has recorded. In this manner, one could assess the likelihood that the cells producing the recorded potentials are also part of the substrate for BSR. The relationship between the BSR substrate and the substrate for conventional rewards could then be investigated.

The suggested resolution of this problem involves the

psychophysical approach to the study of stimulation- induced behaviors, an approach that has, to date, been applied principally to the study of BSR sites that lie along the trajectory of the medial forebrain bundle (MFB). The present thesis extends previous work by using psychophysical techniques to determine (1) neurophysiological characteristics of the substrate for prefrontal cortical (PFC) self-stimulation; (2) whether the same directly stimulated substrates subserve the rewarding effects of PFC and lateral hypothalamic (LH) stimulation; and (3) whether the spatio-temporal integration of rewarding effects of stimulation is similar in the substrates for LH and PFC self-stimulation.

The psychophysical approach

This approach provides means for estimating neurophysiological characteristics of the substrates for behaviors elicited by electrical brain stimulation. These characteristics place quantitative constraints on the properties that a cell must possess if it is to be considered as part of the reward substrate. These characteristics may serve as a basis for differentiating the neural substrates subserving BSR and other stimulation-produced behaviors.

Psychophysical experiments, of the type to which this thesis is devoted, are trade-off or equivalent stimuli experiments. They determine combinations of two parameters

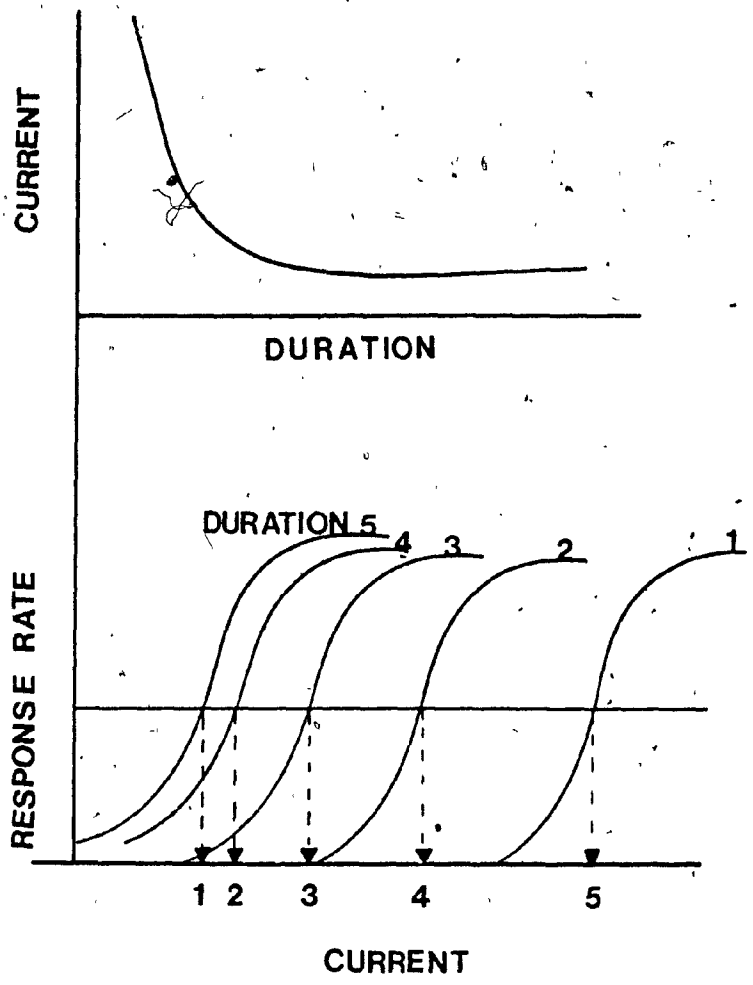

Figure 1 Derivation of the current-duration trade-off. The current required to maintain a criterial level of lever pressing is determined for different pulse durations (lower panel). These currents are then plotted against the duration (upper panel). Long duration, low current pulses will produce the same level of responding as short duration, high current pulses.

that produce the same behavioral output. An example is the trade-off between pulse intensity and duration in producing a criterial level of self-stimulation performance. Figure 1 illustrates the derivation of such a trade-off function. The lower panel contains a hypothetical set of response rate/current functions for different pulse durations. The current required to produce a criterial level of responding is determined from each curve by interpolation and is plotted against pulse duration (upper panel). As is implied by the term "trade-off", long duration low current pulses can produce the same level of performance as high current, short duration pulses.

The trade-off between the current strength and pulse duration yields the strength-duration curve, which has been used as a basis for inferring physiological characteristics of the neural substrate for BSR. To infer such characteristics from constant behavioral output data, one must be sure that a given level of behavior always reflects the same level of activity in the directly stimulated neurons. That is, the behaviorally derived constant output function relating required current to pulse duration must be the same as the constant output function that relates these parameters in the directly stimulated substrate. This will be the case as long as a monotonic relationship exists between inputs and outputs of each successive stage between the directly stimulated cells and the behavioral output. If every output of each stage arises as a consequence of one

and only one input then the input/output relationship of the entire set of concatenated stages is monotonic. By holding the final output (behavior) constant, one also holds constant the output of all preceding stages. For example, if the output of stage N is held constant then its input must also be held constant. Since the input to stage N is the output of stage N-1, the output of stage N-1 must be held constant as well. This constancy propogates backwards to the stage that combines the effects of the two parameters that are traded off. Thus, if the system behaves monotonically over the tested range, behavioral output is a valid measure of the neurophysiological events occuring at the stage where the input parameters are combined.

A monotonic relationship between the input parameter and the vigor of behavior has been observed for BSR over the commonly tested ranges of current intensity, frequency and pulse duration (Gallistel, 1978). Gallistel, Shizgal and Yeomans (1981) have shown that a monotonic relationship between initial input and final output requires that all intervening stages also be monotonic. If nonmonotonicity existed as some stage in the substrate, then there is no way that the nonmonotonicity could be corrected at some later stage (Gallistel et al., 1981). It therefore seems that the assumption of monotonicity is valid.

SELECTIVE LITERATURE REVIEW

Psychophysical studies of BSR have employed both one and two electrode stimulation techniques. The one electrode studies have provided estimates of refractory periods (Yeomans, 1975; Bielajew & Shizgal,1980; Rompre & Miliaresis,1980), strength-duration characteristics (Matthews, 1977), and temporal summation characteristics (Gallistel, 1974; Edmonds, Stellar & Gallistel, 1974) of the neural substrate for BSR. The two electrode studies have estimated conduction velocity (Shizgal, Bielajew, Corbett, Skelton & Yeomans,1980; Bielajew & Shizgal, 1982)anatomical features of the substrate such as direct axonal linkage (Shizgal et. al, 1980) and direction of conduction (Shizgal, Kiss and Bielajew, 1982). The plausability of the behaviorally derived neurophysiological characteristics have recently been tested electrophysiologically (Kiss, Lapointe and Shizgal, 1981; Shizgal et. al, 1982). The findings have shown that neurons with the proposed characteristics are indeed activated at stimulation parameters that support BSR.

The next section will review the results of psychophysical experiments concentrating first on methodological issues. This will be followed by a summary of the major findings.

REFRACTORY PERIOD ESTIMATES

Deutsch (1964) developed the first technique for inferring recovery from refractoriness from behavioral data obtained in the self-stimulation paradigm. His technique

relies on the observation that over a substantial range, response rates increase monotonically with the number of pulses per stimulation train. If the same number of pulse pairs as single pulses are delivered through a stimulating electrode, response rates are expected to increase provided that the second pulse in each pair is delivered at an intrapair interval that exceeds the refractory period of the BSR substrate. In this case, both pulses in a pair will be effective in stimulating the substrate. As a result, the number of effective pulses per train will be doubled. At intrapair intervals shorter than the refractory period, the second pulse will be ineffective in firing cells activated by the first pulse. Response rates should then drop as the interval between the first (conditioning or C-) pulse and the second (Test or T-) pulse in a pair is reduced to values within the refractory period range.

Scaling

Since Deutsch's first attempt to measure the refractory period of the substrate for BSR, a number of replications and extensions of this work have appeared in the literature. Using a similar procedure, Rolls (1973) estimated refractory periods for directly stimulated neurons subserving stimulation-induced feeding and drinking. These estimates were in the range of those reported by Deutsch for BSR (0.5 - 0.7 msec.).

The validity and reliability of both Deutsch's and Rolls's estimates have been challenged by Yeomans (1975). He

argued that because the function relating response rate to the number of pulses is non-linear, manifesting both ceiling and floor effects, the C-T interval at which recovery will appear to begin will be dependent on the stimulation frequency. He supported this argument with a dramatic demonstration: refractory period estimates obtained with response rate as the dependent measure did indeed vary as a function of the stimulation frequency. To circumvent this problem Yeomans derived a scaling procedure that determines the stimulation frequency required to produce a criterial level of performance as a function of C-T interval. In effect, the procedure asks the rat how many pairs of pulses it requires to maintain a criterial level of behavior and then compares the results for each C-T interval to the required number of single pulses. When the C-T interval exceeds the refractory period of the stimulated neurons, the T-pulse is effective in firing all the reward-related neurons and, as a result, the rat is willing to work for half as many pairs of pulses as single pulses. As the C-T interval is reduced, the rat requires more pulse pairs. The number of pulse pairs reaches a maximum at the C-T interval equal to the absolute refractory period of the substrate. When this frequency threshold procedure is used to estimate refractory periods, the across subject variance on this measure is substantially lower than that obtained with response rate measures. The technique has also yielded consistent data across studies and laboratories (Skelton and

Shizgal, 1980; Miliaressis, 1981; Yeomans, 1979; Bielajew et al., 1981; Bielajew and Shizgal, 1982; Hawkins, Roll, Puerto and Yeomans, 1982).

Interpretation of pulse-pair data

Figure 2 depicts the characteristic relationship of T-pulse effectiveness to C-T interval obtained with LH self-stimulation. The curve has been interpreted to represent 3 phenomena:(1) local potential summation;(2) the absolute refractory period; and (3) the relative refractory period.

Local potential summation (LPS)

When pairs of pulses are applied through a stimulating electrode, the C-pulse will fire neurons within a circumscribed region surrounding the electrode tip; neurons just beyond the stimulated region will undergo subthreshold depolarization. If the T-pulse is delivered before the membrane potential has returned to its resting value, the subthreshold depolarizations caused by both C and T- pulses may summate and trigger an action potential. The interval over which summation occurs is determined by the rate at which the local potentials decay to the resting level (this is taken up in detail in the section on strength duration functions) and by the magnitude of the subthreshold depolarizations.

The magnitude of the summation effect is dependent on the current intensity as well as on the spatial relationship

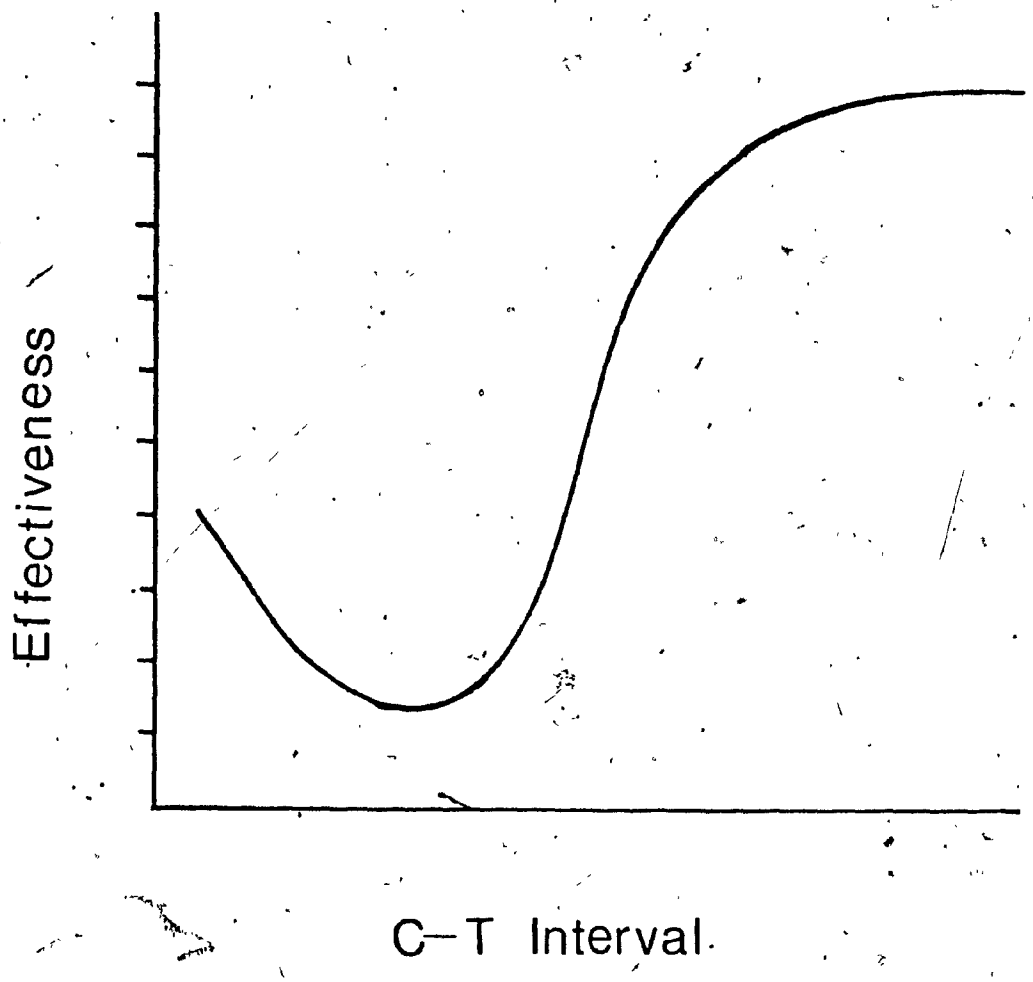

Figure 2 Sample refractory period curve. The effectiveness of the T-pulse is plotted against the C-T interval. Local potential summation, and refractory period effects are shown.

between the electrode and the substrate. For example, an electrode situated on the fringe of a bundle of reward-related neurons may be expected to produce a larger LPS effect than one located in the center of the bundle. Low currents are more likely to produce a large LPS effect than are high currents. In the former case, the population of neurons that receive subthreshold stimulation from the C-pulse is likely to be larger relative to the stimulated core than when high currents are used. Although the physiological basis of the LPS effect is quite different from the basis of the refractory period, LPS does influence the function relating T-pulse effectiveness to C-T interval.

Absolute refractory period (ARP)

During the absolute refractory period, neurons that have been stimulated by the C-pulse are unable to fire again regardless of stimulus intensity. If the T-pulse is delivered during this interval, it will be ineffective in activating those neurons fired by the C-pulse. In principle one may hope to estimate the ARP by determining the longest C-T interval at which T-pulse effectiveness is 0. However a complication arises when local potentials have not decayed to zero at the end of the ARP of the neurons stimulated by the C-pulse. The temporal overlap of these two phenomena will result in T-pulse effectiveness values that always exceed 0. One way to minimize this problem is to use large currents so that LPS effects are reduced. Alternatively, C

and T pulses of unequal amplitude have been shown both empirically and theoretically to yield smaller LPS effects than equal intensity pulses. (Yeomans, 1979; Miliaressis, 1981).

In Figure 2 there is a gradual rather than abrupt change in T-pulse effectiveness following the ARP. This gradual increase can be attributed to two factors. It is possible that a heterogeneous population of reward-relevant neurons with different ARP's contribute to BSR at such sites. Alternatively, the gradual increase in T-pulse effectiveness with C-T interval could be a consequence of the relative refractory period.

Relative refractory period (RRP)

After the ARP there is a period of relative refractoriness during which neurons can again be fired but have elevated thresholds. When current flows through a stimulating electrode, the neurons closest to the electrode tip receive the highest current density; the effective current decreases with distance from the electrode tip. Yeomans (1979) has made use of this relationship in trying to separate the contributions of ARP and RRP effects to the behaviorally derived refractory period curve. He reasoned that if the T-pulse intensity were larger than the C-pulse intensity, then a greater number of neurons would receive supra-threshold stimulation during the RRP and the T-pulse effectiveness vs. C-T interval function would have a steeper

slope than when equal pulse intensities were used. In his initial application of this technique, he found no evidence to support the hypothesis that the RRP effect was responsible for the gradual increase in effectiveness at C-T intervals beyond the ARP. More recently, Bielajew, Lapointe, Kiss and Shizgal (1982), testing higher T-pulse/C-pulse intensity ratios (1.73:1), and using more powerful statistical methods, demonstrated an RRP effect, although the across animal variance in the magnitude of this effect was large. It therefore seems that the RRP contributes somewhat to the behaviorally derived refractory period estimate for BSR at MFB sites, although the full extent of this contribution is unknown due to limitations on the magnitude of the T-pulse/C-pulse intensity ratios that can be tested.

Super- and Sub-normal periods

After the RRP there is often a period of hyperexcitability called the supernormal period which is followed by the subnormal period, a period of hypoexcitability. The super-normal effects are only manifested when the T-pulse is less intense than the C-pulse (Yeomans, 1979). The sub-normal effects are very small (Yeomans, 1979) and have a much longer time course than the excitability phenomena examined in this thesis (Raymond and Lettvin, 1978). Therefore, these effects are not depicted in Figure 2.

Applications of the frequency-scaling technique

Using Yeoman's scaling technique, refractory period estimates for stimulation-produced feeding (Hawkins et al., 1982), circling (Rompre & Miliaressis, 1980), escape (Skelton & Shizgal, 1980) and self-stimulation of various sites including the LH (Yeomans, 1975; Miliaressis, 1981; Shizgal et. al, 1980), ventral midbrain (Shizgal et. al, 1980; Bielajew & Shizgal, 1982), central gray (Bielajew Jordan, Ferme-Enright and Shizgal, 1981) and dorso-medial thalamus (Bielajew, Shizgal & Fouriezos, note 1) have been determined.

With medial forebrain bundle electrodes, the refractory period estimates for feeding, exploration, self-stimulation and stimulation-induced escape are quite similar. Recovery in the substrate(s) for these behaviors begins at 0.5-0.8 msec. and is roughly 80% complete by 1.2 msec. Subtle differences in LPS have been observed between self-stimulation and stimulation-escape (Skelton & Shizgal, 1980) and self-stimulation and stimulation-induced exploration (Rompre and Miliaressis, 1980) which may indicate a difference in the spatial distribution of the substrates for these behaviors.

The most marked differences in refractory period estimates have been observed across some stimulation sites for self-stimulation and between the substrates for BSR and stimulation-induced circling. Bielajew et. al (1982) have reported long estimates for the substrates for central gray (1981) and dorso-medial thalamic (note 1) self-stimulation

as compared to sites along the trajectory of the medial forebrain bundle. The simplest interpretation of these data is that different substrates mediate the rewarding consequences of stimulation at these sites. Miliaressis and Rompre (1980) have reported marked differences in ARP for LH self-stimulation and median raphe induced circling.

How can one be sure that the estimates obtained actually represent the refractory period of the directly stimulated substrate? First, it is unlikely that all of the changes in effectiveness with C-T interval can be accounted for by synaptic phenomena. T-pulse effectiveness values change with C-T interval increments as small as 0.1-0.2 msec. It is unlikely that such small changes in the delays between 2 inputs could effect the output at a synaptic terminal. Second, an observer recording the synaptic output of the directly driven cells might not be able to differentiate the effects of varying C-T intervals due to the entrainment phenomenon (Kocsis, Swadlow, Waxman and Brill, 1979). Since conduction velocity decreases during the RRP and increases during the super-normal period, the propogating action potentials tend to entrain to a fixed interval. If structures significantly beyond the stimulated site were responsible for the observed recovery curve, then during the RRP and super-normal period the curve would be flat. Since changes in E-value consistent with RRP (Bielajew et. al, 1982) and super-normal period (Yeomans, 1979) have been demonstrated, it is most likely that the behaviorally

derived curve reflects excitability characteristics at or near the stimulation site. Finally, since the refractory period of directly stimulated cell can be determined electrophysiologically, the plausability of the estimates can be checked. Electrophysiological data have confirmed that neurons with the proposed refractory periods are activated using stimulation parametrs that support self-stimulation at LH sites (Kiss et al., 1981; Shizgal et al., 1982). Taken together, these arguments support the notion that the behavioral data reflect the refractory period of the stimulated substrate at or near the electrode tip.

The results of behavioral experiments (reviewed by Gallistel et al., 1981) have provided criteria that neurons must possess in order for them to be considered as part of the reward substrate. Neurons that do not have excitability cycles consistent with those derived from the behavioral experiments must either serve some function not related to BSR, or their role in BSR must be at some stage beyond the directly stimulated stage.

Refractory period is related to fiber diameter (Hursh, 1939; Swadlow and Waxman, 1978). As such, it provides information concerning the anatomy of directly stimulated tissue and therefore provides criteria for distinguishing reward related neurons from other directly stimulated cells. The next section continues in the same vein, reviewing work on the anatomical linkage between two sites that subserve a stimulation produced behavior.

ANATOMICAL LINKAGE

Assume that the refractory periods of directly driven cells in two neural areas differ. It would then be tempting to conclude that different substrates mediate the effects of stimulation at these two sites. Nonetheless, it is possible that the behavioral effects of interest arise from stimulating different portions of the same neurons. The different estimates may reflect changes in size or other properties along the trajectory of the same axons. Conversly, when similar refractory period estimates are obtained for a given behavior using two different stimulating electrodes, one cannot conclude that that the same directly stimulated cells were resposible. Rather, the estimates may represent the activation of similar caliber cells with different trajectories. Hence, refractory period tests cannot unambiguously determine whether the same directly stimulated fibers are responsible for the behavioral effect of stimulating two different sites.

A behavioral version of the collision test (Shizgal et. al, 1980) has been developed to obtain such information so that the trajectory of fibers that subserve stimulation-produced behaviors can be traced. This technique also provides estimates of the conduction velocity in the directly driven fibers (Shizgal et. al, 1980; Bielajew & Shizgal, 1982), adding yet another property to the characterization of the substrate.

Collision occurs when an antidromic and orthodromic

action potential approach each other along an axon. The cancellation of the two equal but opposite longitudinal currents results in the failure of either action potential to propogate further (Tasaki,1949). Figure 3 illustrates this phenomenon.

Assume that two segments of the same fiber are stimulated. When a cathodal pulse of sufficient amplitude is applied at the electrode proximal to the cell body (electrode "A"), both an antidromic and an orthodromic action potential are elicited. Although the antidromic potential has no post-synaptic effect, the orthodromic potential will continue propogating to the terminals of cell X, causing transmitter substance to be released onto cell Y. A similar effect will be produced by passing a stimulating pulse through electrode B (the electode distal to the cell body). When pulses are concurrently delivered through the two electrodes, the antidromic action potential triggered by stimulation through electrode B will collide with the orthodromic potential triggered by stimulation through electrode A. Although two stimulating pulses were passed through the stimulating electrodes, only one action potential will reach the synaptic terminals. However, if prior to stimulating through electrode B, a delay is introduced to allow the orthodromic action potential elicited via stimulation through electrode A to clear electrode B and the tissue under B to recover from refractoriness left by the propogating action potential,

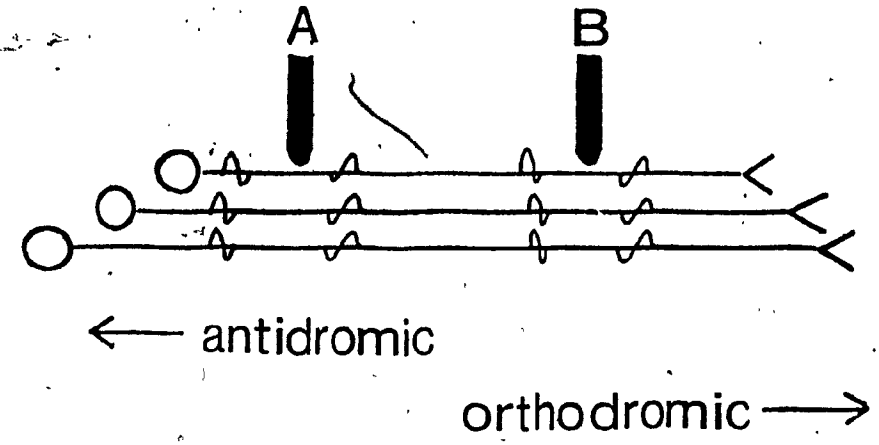

Figure 3 Illustration of the collision phenomenon (From Shizgal et. al, 1980). Two electrodes are situated in a fiber bundle. When stimulating pulses are concurrently delivered through the electrodes, each pulse will elicit an orthodromic and antidromic action potential. The orthodromic potential elicited via stimulation through electrode A will collide with the antidromic potential elicited via stimulation through electrode B; only one action potential will reach the synaptic terminals.

then two orthodromic action potentials (one from each electrode) will reach the synaptic terminals.

The collision experiment is procedurally similar to the refractory period experiment described in the previous section. In collision tests, the effectiveness of paired pulse stimulation is assessed by comparing the number of pulse pairs required to produce criterial performance to the required number of single pulses when either stimulating electrode is used alone. By varying the spacing between the pulses in a pair, the minimum delay necessary to elicit maximum paired pulse effectiveness (the collision interval) can be obtained. By subtracting the behaviorally derived refractory period estimate, one arrives at a measure of conduction time. Division into the interelectrode distance then translates the conduction time to conduction velocity. Shizgal et. al (1980) have suggested that in the case where a cell body intervenes between the two electrodes, the collision profile will vary as a function of which electrode receives the C-pulse. As a result, collision tests are routinely performed under two conditions; under each condition the C-pulse is presented to a different electrode.

Following the rationale presented above, Shizgal and co-workers (Shizgal et.al, 1980, Bielajew & Shizgal, 1982) tested the hypothesis that medial forebrain bundle (MFB) self-stimulation sites were directly linked by reward related fibers. They reasoned that if continuous MFB fibers formed part of the BSR pathway, then collision would occur

when stimulating pulses were alternately applied to sites along the trajectory of this bundle. The data from rats with unilateral LH/ventral tegmental (VTA) electrodes showed collision-like effects; paired pulse effectiveness at short C-T intervals was markedly lower than at longer intervals. Conduction velocity estimates ranged from 1.0 to 7.8 m/sec. Regardless of which electrode received the C-pulse, the collision curves were identical in shape; both were characterized by an abrupt change in paired pulse effectiveness with increased C-T interval. In contrast to the gradual increase in paired pulse effectiveness observed in refractory period experiments, the collision data suggested that the neurons which contribute to the collision effect are fairly homogeneous in excitability. Recent electrophysiological data have indicated that fibers with the proposed characteristics are indeed activated by stimulation of the LH and VTA (Kiss et al., 1981; Shizgal et al., 1982).

In order to test the inference of collision from the behavioral data, Shizgal et al. used this paired pulse technique with BSR sites that have no known direct axonal connections. With stimulating electrodes placed bilaterally in the MFB at the level of the LH, no collision-like effects were observed. Paired pulse effectiveness was invariant with C-T interval; the summation between the reward effects at these two sites was relatively low (20-40%). The failure to observe collision supported the inference of collision in

the LH/VTA study and further suggested that the technique could be used to determine whether reward signals from anatomically distinct fibers were integrated (summated) at some site distal to the first stage cells.

Reward Summation

When both the C and T-pulses are effective, the rewarding effects they engender may summate over time and space. At sites where collision is observed, paired pulse effectiveness will often reach 100% as the collision interval is exceeded; the required number of pulse pairs is exactly half the required number of single pulses. The maximum paired pulse effectiveness reflects the efficacy of summation. In the case where collision is observed, the summation level is expected to be high since some of the fibers responsible for the rewarding effect of stimulation at the two sites are the same. In the absence of collision it is also possible that summation levels can be high ie. when the outputs of different fibers within a bundle converge or different bundles converge on a common final pathway. In Shizgal et al.'s (1980) study, many rats with unilateral LH/VTA electrodes did not show collision-like effects; paired pulse effectiveness remained constant with C-T interval. The rewarding effects of stimulation showed reasonably good summation (75-80%). Figure 4 provides an explanation for this (from Shizgal et. al, 1980).

Assume that two electrodes are located in a single

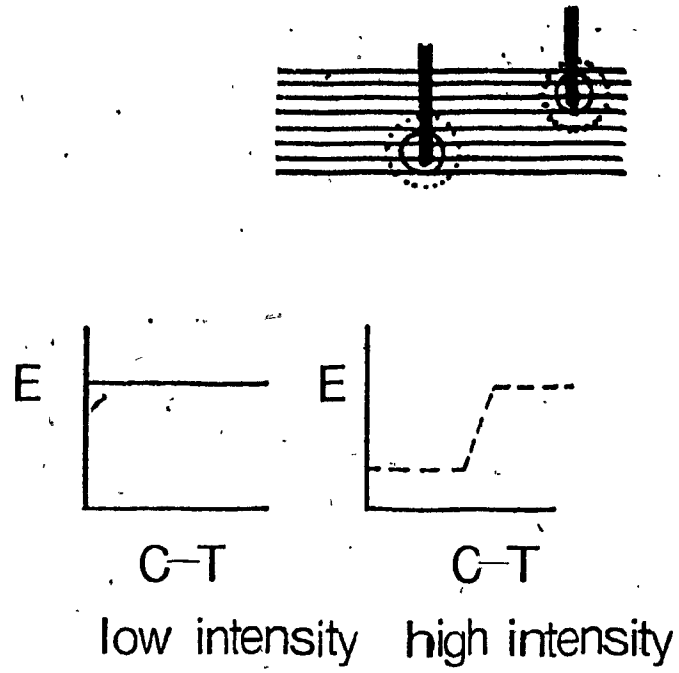

Figure 4 Misaligned electrodes within a fiber bundle. When low currents are used, the effective regions of excitation (solid circles) do not share reward relevant fibers. The effectiveness vs. C-T interval function is flat showing no evidence of collision. As current is increased, fibers that are common to both stimulation fields are recruited and collision is observed.

fiber bundle but that the current through each electrode is sufficient to activate only a subset of fibers within the bundle. The solid circles drawn around the electrode tips in Figure 4 represent this effective area of excitation. As can be seen, there is no overlap between the stimulated fibers at the two sites. In this situation, collision is not expected since stimulation through the two electrodes does not activate any of the same fibers.Increasing the effective stimulation field (by increasing current intensity, for example) may recruit fibers that pass through both stimulation fields (denoted by the broken circles in Figure 4). In this manner, it may be possible to observe collision at high current intensities but not at low ones. This is usually the case for placements that show collision-like effects (Bielajew & Shizgal, personal communication; Shizgal et. al, 1980).

Spatial summation of the outputs of different fiber bundles can also be inferred from two electrode experiments.The notion of summation in the absence of collision is exemplified by the interactions between the substrates for LH and central gray self-stimulation (Bielajew et al., 1981). Rats with unilateral electrodes were tested in the collision paradigm using high current intensities. No evidence of collision-like effects between the rewarding effects derived from stimulation of these two sites was observed although summation levels were quite high. It is still possible that common reward relevant

fibers link these two stimulation sites, but this would require that in all cases, the electodes were misaligned so that the two stimulation fields did not share common fibers within the same bundle. The most parsimonious explanation of the data is that the two electrodes stimulated different reward related fibers that converged at some later stage in the reward substrate.

Applications of the collision experiment

The collision technique allows one to determine whether some of the fibers subserving BSR at two different sites are activated by both electrodes. If so, then the conduction velocity of these common fibers can be estimated. In the absence of collision, the amount of temporal summation provides an estimate of the degree of convergence between the outputs of two different first-stage (directly stimulated) fibers.

The collision technique has also been applied to investigate the trajectories of fibers subserving the different behavioral effects of stimulation through a given electrode. Initially rewarding stimulation of the LH and VTA, becomes aversive over time; rats will perform operant responses to terminate the stimulation. Some investigators have attributed the escape responses to adaptation of the same cells that subserve the rewarding effects of stimulation at these sites (Deutsch and Hawkins, 1972). That is, the rat terminates the stimulation so that it can reinitiate it. Others (Shizgal & Mathews, 1977; Margules,

1966; Skelton & Shizgal, 1980) have attributed the escape responses to an aversive effect that builds gradually during the stimulation and is due to the activation of a population of cells distinct from the reward substrate. In a most elegant test of these two possibilities, Bielajew and Shizgal (1980) failed to observe collision-like effects for escape from LH and VTA stimulation using the same electrodes and current intensities that produced collision for BSR. This implies that different neurons subserve the rewarding and aversive effects of stimulation at these two sites.

A comparison between the substrates for BSR and stimulation-induced exploration has yielded similar results (Miliaressis, personal communication). Collision in the BSR paradigm can be observed when stimulating cells at some LH/VTA electrode placements in the absence of collision in the stimulation induced-exploration paradigm. In other subjects, the opposite is also observed. Hence, it seems that the technique is also powerful in differentiating fibers subserving different behaviors elicited via stimulation through the same electrodes.

The combination of refractory period and collision/ summation tests is a powerful means of determining characteristics of the neural substrate for BSR. Yet another set of characteristics has been inferred from the trade-off between current intensity and pulse duration, which is covered in the next section.

STRENGTH-DURATION EXPERIMENTS

The threshold current required for excitation declines as a function of pulse duration (see Figure 1). The threshold current decreases rapidly at first and then approaches a minimum value as duration is further increased. The current threshold at infinitely long pulse durations is called the rheobasic current. This value is influenced by such factors as electrode size and the spatial relationship between the electrode and the substrate (Rushton, 1927; Grundfest, 1932).

A convenient means of describing the shape of the strength-duration function is to determine the duration corresponding to twice rheobasic intensity. This measure, termed chronaxie (Erlanger and Gasser, 1937) is believed to be less dependent than the rheobase on electrode characteristics. For example, the angle of current flow does not effect chronaxie but does influence rheobase (Rushton, 1927).

In some cases the strength-duration curve for single neurons has been best fit by an exponential function while in others, a hyperbolic function provides a better fit (Matthews, 1978). At least four independent variables, the membrane time constant, the geometry of the cell, the process of accomodation and the non-linearity of the current/ voltage curve (Noble and Stein, 1966) will influence the rate at which the current threshold decreases with pulse duration. Therefore, it is not surprising that one cannot predict, a priori, which function will account

for strength-duration data of a given cell. The effect of these variables on the strength-duration curve will be examined in the next sections.

Membrane time constant

In predicting subthreshold behavior, an excitable membrane can be successfully modelled as a parallel resistor/ capacitor (R/C) network (Hodgkin and Rushton, 1946). In an RC circuit, the voltage changes exponentially as a function of the time since the onset of a step-like input. Hence, it has been suggested (Hill,1936) that the strength-duration function of a cell exposed to rectangular depolarizing pulses will also be exponential. The membrane time constant is equal to the product of the specific resistance and capacitance of the membrane and, in the exponential model, accounts for the time course of charge integration in the membrane.

This model can account for changes in voltage with time only in uniformly polarized cells (Noble and Stein, 1966). For example, different functions relate the membrane potential to the time since the onset of the stimulation in cylindrical versus spherical cells. In spherical or uniformly polarized cells, the internal resistance can be treated as negligible when compared to the membrane resistance. Consequently, the cytoplasm can be treated as equipotential. The equivalent electrical circuit consists of parallelled R/C units with each individual R/C representing

a patch of membrane. Hence, this circuit can be reduced to a single R/C unit where R is the parallelled resistance and C is the parallelled capacitance of the patches. The strength-duration curve in such a circuit will follow the exponential model. In contrast, the internal resistance of a long cylindrical process (like an axon) cannot be disregarded. In the equivalent electrical circuit there are resistors inserted between each parallelled R/C unit. Current injected at a point in the circuit will initially flow through the nearest R/C and after a delay will enter R/C units located further away. The strength-duration curve in this circuit will deviate from the exponential model (Hodgkin and Rushton, 1946; Betz, Note 6). If one normalizes the charging curves for the two cell types, the percentage of the final voltage that is achieved in one time constant is higher for cylindrical or point-polarized cells (84%) than for spherical or uniformly polarized cells (63%) (Betz, Note 6). Therefore, for a single homogeneous axon stimulated at a point, the change in voltage with time will not be described by an exponential function. More complicated behavior will arise if the electrical characteristics of the axon vary systematically along the path in which current is flowing.

Accomodation

Yet another membrane property that can contribute to the strength-duration function is the process of

accomodation. This process occurs as a long duration pulse is applied to an axon. The threshold to fire the neuron decreases during long duration anodal pulses and increases during cathodal pulses. The time course for accomodation is expected to be substantially slower than that for excitation since if the two occurred with the same speed, no change in membrane potential could cause the neuron to reach the always changing threshold. The effect of the accomodation process on the strength-duration curve is to shift the required current towards higher values as duration is increased (Nobel and Stein, 1966).

The influence of the accomodation process can be seen in the phenomenon of anode-break excitation (Matthews, 1978). Hyperpolarization via long duration anodal pulses may result in accomodation of the firing threshold to a new, more negative, membrane potential. At the end of the anodal pulse, the membrane potential will rapidly return to its resting level at a rate determined by the membrane time constant (Hill, 1936). The accomodated threshold may be passed, and if so, the neuron will generate an action potential at the offset of the anodal pulse.

Non-linearity of the current/voltage relation

During subthreshold depolarization the membrane conductance increases. That is, the membrane R/C values will decrease. The membrane time constant will therefore vary during the pulse. The exponential model assumes that R/C

will be invariant. Since in all excitable cells the current/voltage relation is non-linear, deviations from the exponential model will occur.

The shape of the strength- duration function in uniformly polarized cells that do not exhibit accomodation and have linear current/voltage relations is expected to be exponential. However, excitable cells are generally elongated and not uniformly polarized by stimulating currents, undergo accomodation and have non-linear current/voltage relations. Therefore, all strength-duration curves should deviate from the exponential to some extent unless the deviations produced by any one factor or combination of factors compensate for the deviations produced by another factor or factors. One reason why the hyperbolic function may best fit strength-duration data (Matthews, 1978) is that the accomodation process elevates the threshold for excitation at the longer pulse durations thereby causing the curve to approach assymptote more slowly than an exponential function.

Some uses of strength-duration functions

The differentiation of neural substrates on the basis of chronaxie and rheobasic current was pioneered by Lucas (1917). He was able to demonstrate two distinct neural mechanisms for closing the crab claw; one mechanism controls the twitch-like closure while the other is responsible for the slow contraction. The rheobasic current for the twitch response was approximately three times the threshold for the

slow contraction. This difference in threshold intensity allowed for the selective elicitation of either (a) the slow contraction or (b) the slow contraction plus twitch. Similarly, differences in chronaxie for slow-contraction and twitch strength-duration functions allowed Lucas to conclude that two distinct motor neuron substrates were being stimulated, one leading to a slow contraction of the muscle and one leading to a fast contraction.

The strength-duration function has been applied to characterize a neuronal population as well as single fibers. This classical neurophysiological procedure has been applied using multipulse stimuli and neural population response measures. Abeles (1967) was able to differentiate the EEG activation neurons of the thalamic projection system and the mesencephalic reticular formation (both with chronaxie of .3 msec) from those of the nucleus reticularis pontis caudalis and solitary tract (both with chronaxie of .2 msec). No differences in chronaxie were obtained for the EEG synchronization or desynchronization substrates, but in the region of the solitary tract the rheobasic current for the former was four times that required for the latter.

Studies on BSR

The commonly used method for measuring strength-duration relationships for BSR is to determine the current required to maintain a criterial level of behavior at a given pulse duration (Figure 1 depicts this method). The required current has been defined as the current

corresponding to the locus of rise in the function relating running speed in an alley to current intensity (Matthews, 1977; Gallistel. 1978) or that intensity necessary to maintain a particular rate of lever pressing in a Skinner box (Milner, Note 2; Horrell, 1975; White, 1976).

The earliest study of this nature determined the strength-duration relation for septal self-stimulation (Ward,1959). Ward showed that the trade off between pulse duration (.01 - 10 msec) and current (plotted on log/log co-ordinates) was fairly linear and was not influenced, to any great degree, by pulse frequency. Although long pulse durations were tested (>5 msec), the rheobase was not empirically obtained.

Wetzel (1971) also attempted to determine strength-duration relations of the LH self-stimulation substrate. She measured response rates for different combinations of pulse duration and intensity. As the current intensity was doubled (between 20 and 80 uA) and the pulse duration halved, the same rate of responding was attained; ie. these two parameters traded off in a scalar fashion (within these limits). Due to the nature of the experimental design, it is impossible to obtain rheobase and chronaxie estimates from Wetzel's data.

Unfortuately, constant current pulses were not employed in Ward's or Wetzel's experiments. As pulse duration is increased, the impedence of the electrode/brain interface also increases. Therefore, current varies during each pulse.

More gradual changes in the electrode/brain interface may also occur making it difficult to compare data from different trials.

The first attempt to behaviorally measure chronaxie using constant current stimulators was made by Horrell (1975). He determined strength-duration relations in a manner analogous to the classical neurophysiological method using medial forebrain bundle self-stimulation current threshold as the measure of excitation. The chronaxie estimates were not influenced by frequency manipulations (25-100 Hz) and were fairly consistent within and across rats. The range of chronaxie estimates was .11 to .52 msec, within the range of those reported in the neurophysiological literature for somata and fine fibers (Ranck, 1975).

Matthews (1977) compared strength-duration characteristics for BSR (chronaxie =.85-3.0 msec) and stimulation induced motor effects (chronaxie=.15-.48 msec) using the same stimulating electrode. The observed chronaxie difference shows that the substrates for stimulation induced behaviors can be differentiated on the basis of the strength-duration curve. The relatively long chronaxies for the BSR substrate reported by Matthews have also been reported by Gallistel (1978) and Shizgal & Schindler (Note 3).

One factor that may influence the description of the strength-duration curve is the means by which chronaxie and rheobase are derived. As mentioned earlier, the relation

between current strength and pulse duration has been fit by both the exponential and hyperbolic equations. These two functions are difficult to distinguish at short pulse durations but diverge as the duration increases. If the rheobasic current is not empirically obtained, then it must be estimated by fitting a function (eg.,exponential or hyperbolic) to the data.This method is error-prone since the rheobase is estimated via extrapolation well beyond the data, and the chronaxie estimate is based on the rheobase.

Milner (Note 2) has plotted the required current as a function of the reciprocal of the duration. When plotted in this manner, a hyperbolic function becomes a straight line; the current intercept is equal to the rheobase and the slope of the line is equal to the product of the rheobase and the chronaxie. Milner observed that when only short duration pulses were tested, the data were fit well by a single straight line. When both long and short duration data were included, two intersecting lines were required to produce an acceptable fit. If a single straight line was forced through the data points (by fitting an hyperbola) the current intercept (rheobase) predicted using the long duration data points was lower than when these points were excluded from the analysis. This finding indicates that strength-duration data obtained for MFB self-stimulation do not fit an hyperbolic function perfectly; the inclusion of longer duration pulses has the effect of decreasing the predicted rheobase and, hence, increasing the predicted chronaxie.

Since Matthews has tested the longest duration pulses to date in strength-duration experiments, this may explain why his chronaxie measures are so much longer than Horrell's.

Even though Matthews (1977) used very long pulse durations (up to 15.0 msec) in his experimental design, the required current continued to drop as pulse duration was increased. Two possibilities have been offered to explain this finding.

First, it is possible that, since one is measuring a population response, the stimulated neural elements comprise a heterogeneous group with regard to their chronaxies. The inferred chronaxie is therefore a weighted average of a population that include some long chonaxie elements.

The other explanation that Matthews (1977) proposed was that multiple firings contributed to the continued decline in required current at long pulse durations. That is, the maintained depolarization may produce not one impulse but a train of impulses in a neuron. This would be equivalent to increasing the frequency of stimulation, a manipulation that allows the same behavioral output to be produced at lower currents. Some brainstem units directly excited by medial forebrain bundle stimulation fire multiply during stimulation pulses longer than 2.0 msec (Matthews, 1978). However, the number of multiple firings did not increase as duration was further increased and therefore, such neurons cannot account for all the behavioral data.

One may get the impression that strength-duration

functions for BSR are not very useful for inferring physiological characterisitics due to the difficulty in empirically obtaining rheobase and chronaxie measures. However, even in the absence of a well defined rheobase, the behaviorally derived strength-duration function can place quantitative constraints on the neural population that subserves a behavioral phenomenon and guide the interpretation of electrophysiological data. For example, the substrate for BSR must include some elements with long time constants or some elements that fire multiply during long-duration cathodal pulses. The ratio of anodal to cathodal required currents provides an additional characteristic of the substrate for a stimulation-produced behavior that can guide the interpretation of electrophysiological data.

The mechanisms of anodal and cathodal stimulation are analogous. During extracellular stimulation, current enters the neuron near the anode and exits near the cathode. It is the exiting current that depolarizes and can thus cause the neuron to fire. The onset of an anodal pulse excites neurons by pushing out current at some point remote from the stimulating electrode. This phenomenon has been called anode-make excitation (Matthews, 1977). With short duration pulses (durations shorter than the time at which the accomodation process influences stimulation effectiveness), cathodal and anodal stimulation of fibers should produce parallel strength-duration curves since anode-make

stimulation excites in the same way as cathodal stimulation. The only difference is that for anode-make excitation to occur, more stimulation should be required since the effective current flows through a larger area.

Matthews compared anodal and cathodal strength-duration functions for medial forebrain bundle self-stimulation. He found that the two curves were roughly parallel up to pulse durations of 5.0 msec.; approximately twice as much anodal current as cathodal current was required for equivalent performance. At longer pulse widths, the required current for anodal pulses dipped sharply, suggesting that the stimulation had become more effective. This was interpreted as the beginning of anode break excitation.

Matthews's study exemplifies the use of strength-duration functions to characterize and differentiate the substrates for different stimulation-induced behaviors. Although the chronaxie of the strength-duration curve for the BSR substrate has not been well defined, it is clear that this substrate is different from that for stimulation-produced motor twitch, a behavior for which the rheobase of the strength-duration curve is well defined and hence the chronaxie can be accurately estimated.This interpretation of the cathodal strength-duration curves is strengthened when one includes the differences in the time course for the accomodation process and the different anode/cathode ratios for the two stimulation-induced behaviors.

TEMPORAL AND SPATIAL INTEGRATION IN THE REWARD SUBSTRATE

In a previous section, the collision technique was shown to be one way to assess the efficiency of summation in the reward system. The efficiency of summation can also be assessed by use of the trade-off between temporal (frequency) and spatial (current intensity) parameters. The effects of these two parameters are believed to be integrated at some point beyond the directly stimulated substrate (Gallistel, 1974). This aspect of the substrate has been termed the integrator and its function has been likened to a counter (Gallistel, 1978). The integrator's proposed function is to count reward related impulses that arrive from preceding stages in the neural network. A decision is then made as to whether the number of reward related impulses that arrived within a given period of time is sufficient to sustain reward-related behavior. Assuming that each pulse in a train produces a single action potential per stimulated neuron, the number of reward relevant action potentials reaching the integrator will be a function of the number of neurons stimulated and the stimulation frequency. The number of stimulated neurons is believed to be a scalar (Gallistel, 1978) or linear (Shizgal and Schindler, Note 3) function of current intensity. If current is increased, a constant level of neuronal firings will arrive at the integrator only if the number of firings per neuron is decreased in a compensatory fashion. It follows from these arguments that the number of stimulating pulses required to hold behavioral output constant should be

a scalar or linear function of stimulation intensity at least over a limited range of currents and frequencies.

The frequency/intensity trade-off

It has been argued that the shape of the trade-off between frequency and intensity can provide information concerning the geometry of the fiber bundle that carries the reward signal (Yeomans, Pearce and Wen, 1982). It also can provide an index of the temporal and spatial limits of summation of reward effects at some site distal to the stimulating electrode. The relationship between the reciprocal of the required number of pulses and current predicted by Gallistel at al. (1981) is depicted in Figure 5. Empirical data fit the theoretical predictions quite well (Gallistel, 1978; Shizgal and Schindler, Note 3)

The procedure for determining the data points in this experiment is methodologically similar to the procedure shown in Figure 1 for the trade-off between current strength and pulse duration. For the 1/N vs. I trade-off, response rate/ frequency functions are obtained for a series of intensities. The number of pulses per stimulation train required to maintain half maximal response rates is then interpolated from each curve. The reciprocal of this value (1/N) is then plotted against the current.

The 1/N versus intensity curve can be divided into three sections. The center portion (B) represents the range of currents that trade off fairly linearly with 1/N. This

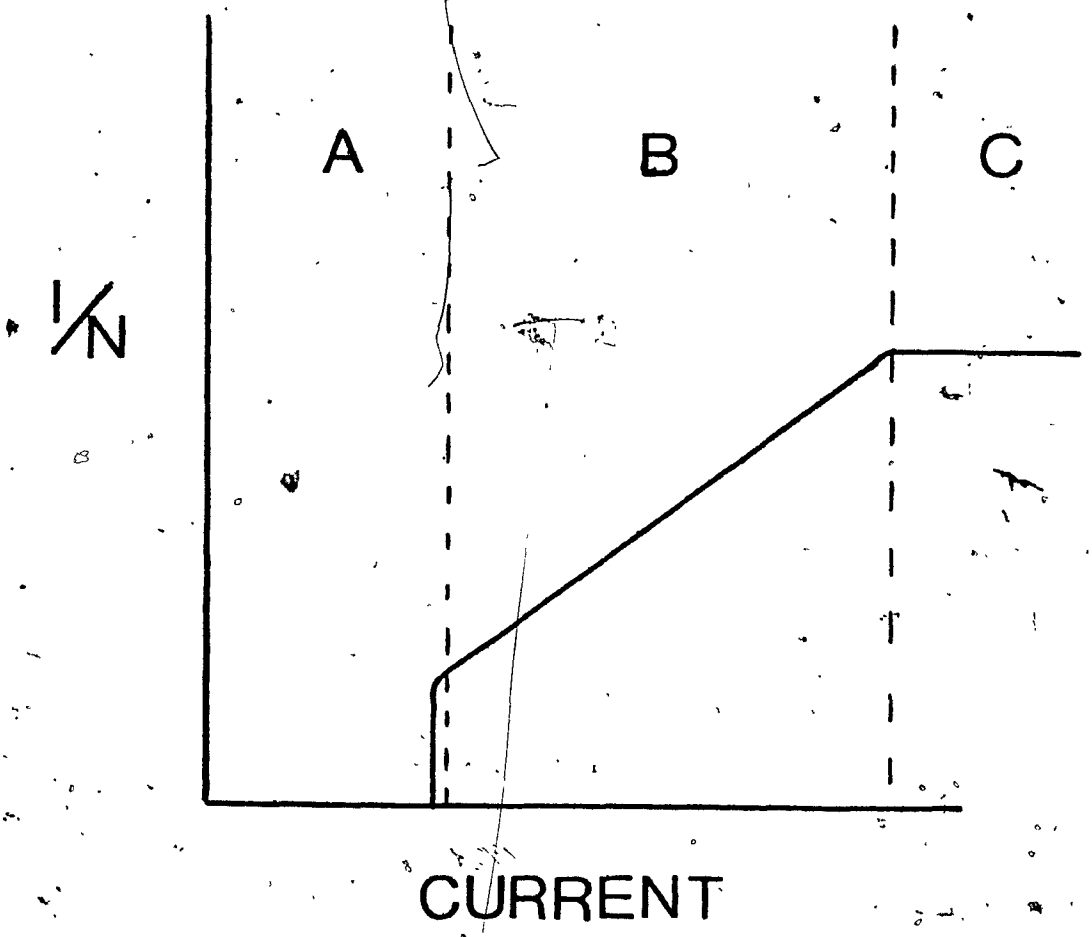

Figure 5 Hypothetical 1/N vs. intensity trade-off. The curve is divided into 3 sections. Section A represents currents that are too low to support self-stimulation. Section B shows the range of currents that trade-off linearly with 1/N. In section C asymptotic 1/N values are depicted.

portion of the curve is sandwiched between two extreme current ranges that mark the breakdown of the linearity of the trade-off. Section A represents current intensity values that are too low to support responding, regardless of stimulation frequency and slightly higher current intensities at which the required number of pulses is above the linear range. Section C represents currents that are either ineffective or less effective in increasing the magnitude of the reward effect.

The value of the minimum current that will support responding for BSR at maximal frequencies is influenced by several factors. The ability of the substrate to follow high frequency stimulation will have an effect on this value (Shizgal and Schindler, note 3; Shizgal, note 5). The spatial distribution of the substrate relative to the stimulating electrode will also influence this value. For example, if the electrode is situated within a fiber bundle, lower currents will be required to recruit the nearest reward relevant neurons than if the electrode is off target. This value will also be influenced by the integrator criterion, i.e., if a large number of reward impulses are required by the integrator, then high currents will be needed. The same will be true for a spatially diffuse system.

The range of currents that trade-off linearly with 1/N will be influenced by the geometry of the reward relevant substrate and the current/distance relationship. If the

bundle is large, then the range of effective currents will be large. The criterion of the integrator will influence the slope of this section of the trade-off. As the criterion is increased, the slope of the function will become less steep. Since the number of reward relevant neurons stimulated by a given amount of current is determined, in part, by the current/ distance relationship and by the packing of the neurons (spatial distribution) these values will influence both the range of effective currents and the slope of the 1/N vs. I trade-off (Gallistel et al., 1981; Shizgal and Schindler, note 3).

The trade-off function breaks down at the high end of the intensity scale presumably due to the geometry of the bundle. Further increases in current do not result in an increase in 1/N which may indicate that the increased current fails to recruit additional reward fibers. Alternatively, the breakdown of the linearity of the trade-off may indicate that the effectiveness of the stimulation declines at low frequencies.

The frequency/intensity trade-off may provide a means of testing the interpretation of collision/summation and refractory period data. The basis of the scaling formula used in both of these tests is derived from the counter model. Therefore, the linearity of the frequency/intensity trade-off tests the basic assumption that the rewarding effects of stimulation are integrated regardless of their spatio-temporal pattern of input. Although deviations from

linearity may stem from sources other than violations of the counter model (e.g. the particulars of the spatial distribution of the directly stimulated cells), an approximately linear trade-off does demonstrate that the rewarding value of stimulation increases steadily with the frequency.

THE PREFRONTAL CORTEX AS A REWARD SUBSTRATE

The PFC is defined as the cortical projection site of the dorso-medial thalamus (Leonard, 1969). In the rat, the PFC is divided into two components; the sulcal aspect forms the dorsal bank of the rhinal sulcas and the medial aspect forms the medial boundary of the hemisphere rostral and dorsal to the genu of the corpus callosum. Self-stimulation of both of these loci has been reported (Routtenberg & Sloan, 1972).

The rewarding effects of PFC stimulation have been attributed to activation of cells that project to the LH (Routtenberg & Sloan, 1972). Indeed, there is ample evidence for reciprocal connections between these two sites. Both regions of the PFC that support BSR send long axons caudally to intermingle with the MFB at the level of the LH (Leonard, 1969). Further evidence comes from a series of neurophysiological studies showing that LH self-stimulation causes the activation of PFC neurons (Rolls & Cooper, 1973; 1974), although the relevance of these neurons to reward is unknown. Bilateral anesthetization of the sulcal prefrontal cortex (but not the medial) with procaine hydrochloride attenuates or blocks LH self-stimulation (Rolls & Cooper, 1974), which has been interpreted to suggest that the PFC reward substrate may share common fibers with the LH reward substrate.

In contrast, there are several converging lines of evidence that suggest that the anatomical connections between the PFC and the LH may not be reward-related. The

results of psychopharmacological experiments have been interpreted to suggest that dopamine does not play a major role in mediating the rewarding effects of PFC stimulation whereas this transmitter appears to modulate the rewarding effects of LH stimulation (Wise, 1978). For example, 6-hydroxy-dopamine lesions fail to produce a long- lasting loss of PFC self-stimulation. The authors concluded that the rewarding effects of stimulation do not depend on the presynaptic release of dopamine in this brain site (Gerfen & Clavier, 1981). Amphetamine, a potent dopamine agonist, is ineffective in enhancing PFC self-stimulation (Goodall & Carey, 1975; Carey, Goodall & Lorens, 1975). On the other hand, many dopamine receptor blockers attenuate responding for PFC stimulation (Mora, Rolls, Burton & Shaw, 1976; Mora, Alba, Sanguinatti, Rodriguez & Vives, 1980) although it is difficult to determine the extent to which the decreased response rates produced by these manipulations reflect a drug-induced motor impairment rather than a decreased reward effect. Due to the methodological problems associated with the use of response rate measures to assess drug- or lesion-induced changes in the rewarding effect of stimulation (Valenstein, 1964; Edmonds and Gallistel, 1974), the results of such studies cannot be unambiguously interpreted. In order to establish the role of dopamine in PFC self-stimulation, the studies must be conducted using the more rigorous techniques that have been developed to study the neuropharmacology of MFB self-stimulation (e.g.,Edmonds

and Gallistel, 1974; Fouriezos and Wise, 1976).

There are also differences between the interaction of LH and PFC self-stimulation with conventional rewards. LH self-stimulation is enhanced by food deprivation (Hoebel, 1969) a finding that has been cited in support of the hypothesis that food reward and BSR share a common neural substrate. In contrast, food deprivation has little effect on PFC self-stimulation (Carey et. al, 1975; Goodall and Carey, 1975). Whereas forebrain neurons that are activated by an LH self-stimulation electrode respond to the presentation of food (Rolls et. al, 1976), neurons in the PFC do not (Mora, Avrith and Rolls, 1980).

Electrophysiological data are also inconsistent with the view that the LH and PFC share a common reward substrate. PFC cells are fired with latencies that are inconsistent with behaviorally derived estimates of conduction velocity and refractory periods in LH reward related neurons. The former have refractory periods in the range of 1.2-2.5 msec (Rolls & Cooper, 1973), values considerably longer that the behaviorally derived estimates of 0.4-1.2 msec. for the directly stimulated neurons mediating LH self-stimulation (Yeomans, 1975; Bielajew & Shizgal, 1982; Rompre & Millliaressis, 1980).

Although methodological problems plague many of the relevant studies, the data generally suggest that there may be different substrates for LH and PFC self-stimulation, a view recently expressed by Robertson, Laferriere and

Franklin (1981). The present thesis explores this possibility in detail through experiments designed to measure neurophysiological properties of the directly stimulated reward substrate in the PFC. Two experiments compare behaviorally derived refractory period and strength-duration characteristics of the substrate for LH and PFC self-stimulation. The collision technique is used to determine whether the two substrates are linked by reward relevant fibers. Finally, the linearity of the trade off between stimulation frequency and intensity is tested.

EXPERIMENT 1

Anatomical, neurochemical and electrophysiological data have suggested that different neurons may mediate LH and PFC self-stimulation. If so, the directly stimulated neurons in the two substrates may have different refractory periods. The first experiment investigates this possibility.

METHOD

Subjects

Subjects were 6 male hooded Long Evans rats (labelled C) and 2 male Sprague-Dawley rats (labelled H) weighing 350-400 grams at the time of surgery. The animals were individually housed in wire mesh cages on a 12 hr. light/dark cycle and given ad lib. access to Purina rat chow and water.

Surgery

Under sodium pentobarbital anesthesia (60 mg/kg, i.p.) two stimulating electrodes were stereotaxically aimed at the PFC and the LH. The de Groot co-ordinates were: PFC:4.5 mm. rostral to bregma; 0.7 mm. lateral to the saggital suture; and 2.5 mm. below the dura; LH: 0.4 mm. caudal to bregma; 1.7 mm. lateral to the saggital suture; and 8.0 mm. below the dura. Electrodes were 254 um stainless steel wire insulated with Formvar to within 0.5 mm. of the rounded tip. A flexible stainless steel wire wrapped around 4 stainless steel skull screws served as the current return. The assembly was secured to the skull with dental acrylic.

Procedure

After several days recovery from surgery, the subjects were trained to self-stimulate at both electrode placements. The testing chamber was a wooden box (25X25X70 cm) with a Plexiglas front and a grid floor. Depression of a lever mounted in one corner of the box resulted in the delivery of a 0.5 sec train of rectangular cathodal pulses, 0.1 msec in duration. Stimulation parameters were controlled by integrated circuit pulse generators and constant current amplifiers (Mundl, 1980).

Once the subjects learned to self-stimulate, a series of stabilization tests was begun. In these tests the number of pulses per train was decreased after each 1 minute trial in 0.1 log steps from the value that produced maximal responding to a value for which the animal failed to respond. The required number was defined as the number of pulses that supported a one-half maximal rate of responding and was determined by graphical interpolation. The criterion for stability was a range of required numbers not greater than 0.15 log units during a stabilization test. Stabilization was carried out for both electrode placements. Current intensities were then adjusted so as to match the required numbers at the two sites. (The rationale for this procedure is explained in Experiment 2).

Following stabilization, refractory period tests were begun. In these tests, trains of pulse pairs were used with both the C- and T-pulses delivered to the same electrode. The C-T interval was varied from 0.15 to 20.0 msec. Every 4

determinations a baseline test was conducted during which trains of single pulses were delivered. In a given session, the average required number from these single pulse tests served as a comparison for the 10-15 pulse pair tests.

Scaling

The effectiveness of the T-pulse was assessed by comparing the required number of pulse pairs at each C-T interval to the average of the required numbers for all of the single pulse determinations carried out on that test day. Effectiveness ratios (E-values) were calculated according to Yeomans' (1975) formula:

$$E=(Nsp/Npp)-1$$

where

E=T-pulse effectiveness

Nsp=single pulse required number

Npp=paired pulse required number

If, for example, the T-pulse is ineffective because it falls within the absolute refractory period, the required number of pulse pairs will be maximal. The scaling formula will then generate a minimal T-pulse effectiveness value. If the T-pulse fires the same number of reward related neurons as the C-pulse, the required number of pulse pairs will be half the required number of single pulses and the effectiveness value will be 1.0.

Histology

At the completion of the experiment the subjects were administered an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and stored in 10% formalin for at least 1 week. The tissue was then frozen and sliced in 40um sections. Every fifth section was mounted and stained with formal-thionin. Histological data for this, and all subsequent experiments are presented in the Results section of the final experiment

RESULTS

All 8 rats self-stimulated at the PFC electrode site. The amount of time required to shape the rats to the lever varied from 1 day to 2 weeks. The rate of responding for most rats increased steadily over the two week shaping period until it stabilized at 20-50 responses per minute. In contrast, all animals except for C9 were trained to self-stimulate at the LH electrode site in one testing session. The rates of lever pressing for LH stimulation stabilized, in the first testing session, at 40-150 responses per minute.

Figure 6 presents the average T-pulse effectiveness as a function of C-T interval and electrode placement for each subject. The characteristics that are of interest in comparing the LH and PFC reward substrates are: (1) the C-T interval at which recovery begins; (2) the rate of recovery; and (3) the C-T interval at which recovery is complete. To

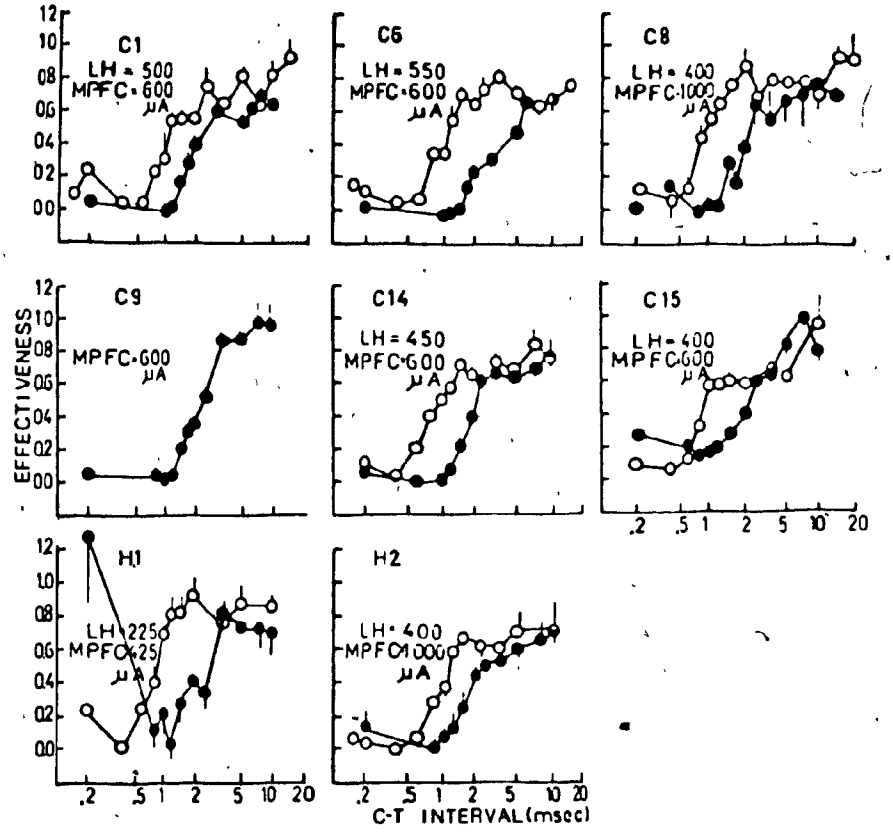

Figure 6 Refractory period data for the PFC (●---●) and LH (O---O) sites.

determine the C-T interval at which recovery is complete, a rule of thumb has been developed (Bielajew & Shizgal,1982). Starting at the longest C-T interval, the mean effectiveness value (E value) and its associated standard error of the mean (SEM) were compared to the E value and SEM for the next shortest C-T interval. If the SEMs overlapped, these data were pooled and a new mean and SEM calculated. These pooled values were then compared in an analogous fashion to the mean E-value and SEM for the next shortest C-T interval. In this way, the shortest C-T interval for which the SEMs were shown to overlap was determined. This interval was defined as the C-T interval at which recovery was complete.

It can be difficult to determine the C-T interval at which recovery from refractoriness begins because of the overlap of local potential summation effects with the onset of recovery. The decay of local potential summation has not been successfully fit by a simple function (Yeomans, 1979). Therefore, there is no obvious way to mathematically remove these effects from pulse pair data. Instead, curve fitting methods were used to estimate the onset of recovery.

First an attempt was made to define the lower limit of the recovery curve using the iterative method described below. This lower limit represents the C-T interval at which little or no recovery has occurred.

A weighted regression analysis was performed on all pairs of E-values and C-T intervals below and including the C-T interval at which recovery was defined to be complete.

Initially, the 4 longest C-T intervals were subjected to weighted linear regression. An E-value and its associated 95% confidence interval was predicted for the next lowest (fifth) C-T interval. If the actual E-value for this C-T interval was included in this 95% confidence interval, the regression procedure was repeated, including this pair of scores. This procedure was repeated until an E-value was found that fell above the 95% confidence interval around the predicted E-value for that C-T interval. The divergence of this E-value from the regression line was viewed as reflecting local potential summation and/or absolute refractory period contributions. Hence, the C-T interval associated with the shortest E-value to fall within the 95% confidence interval was viewed as the longest interval at which no recovery from refractoriness was evident.

In order to obtain a measure that would reflect the onset of recovery in a meaningful proportion of the stimulated fibers, the C-T interval corresponding to an E-value of 0.2 was estimated using the weighted regression parameters. These values are presented in Table 1.

A t-test for independent samples confirmed that recovery began earlier at the LH sites than at the PFC sites ($t(.01,12)=4.57, p<.01$). Similarly, recovery approached asymptote at a shorter C-T interval for the LH placements than for the PFC placements ($t(.01,13)=5.54, p<.01$).

All data bounded by the upper and lower limits described above were analyzed by regression methods based on

the analysis of variance (Neter and Wasserman, 1974). This analysis determines whether the recovery curve for each subject is best described by one regression line fit to the pooled LH and PFC data or by two lines, one fit to the data from each placement. The variability accounted for by the regression lines fit to the pooled data and by those fit to the individual data sets were statistically compared. In all 7 subjects, the variability accounted for by the individual regression lines was significantly greater that the variability accounted for by the regression line fit to the pooled data (Table 2). The slopes of the individual curves were then analyzed by determining 95% confidence intervals about the differences between the slopes of the PFC and LH regression lines (Neter and Wasserman, 1974). The slopes of the individual curves and the 95% confidence intervals for the slope differences are presented in Table 3. Only in one subject did the confidence interval for the difference include 0. In the rest of the subjects, the differences in slopes indicated that T-pulse effectiveness increased more quickly as a function of C-T interval at the LH placement than at the PFC site.

Subject	LH	PFC
C1	.78	1.65
C6	.70	2.58
C8	.60	1.50
C9	---	1.54
C14	.64	1.49
C15	.63	1.11
H1	.69	1.52
H2	.57	1.29

Table 1 C-T interval (msec) corresponding to an E value of .20 (based on weighted regression analysis described in the text).

Subject	Upper Asymptote PFC	Upper Asymptote LH	Lowest Point PFC	Lowest Point LH	F
C1	3.0	1.2	1.0	0.6	19.11
C6	6.0	1.5	1.0	0.4	96.44
C8	2.5	1.5	0.8	0.4	34.26
C9	3.5	---	1.0	--	-----
C14	2.5	1.2	1.2	0.4	30.25
C15	3.5	1.0	0.8	0.4	12.09
H1	3.5	1.2	0.8	0.4	33.74
H2	3.5	1.5	0.8	0.4	43.37

Table 2 C-T intervals (msec) for upper and lower limits of data points used in the regression analysis of refractory period estimates for the PFC and LH substrates. The F ratios from the analysis of variance approach to linear regression are presented in the right-most column. All of these ratios are significant ($p<.01$), ie. the PFC and LH data are best fit by two regression lines, one fit to each set of data, rather than one line fit to the pooled data.

Subject	Slope PFC	Slope LH	95% confidence interval about the slope diff.
C1	.315	.810	.1744-1.3106
C6	.126	.680	.4247-0.9706
C8	.390	.661	-.4286-0.9706
C14	.405	.720	.0812-2.2041
C15	.205	.807	.2648-0.9392
H1	.247	1.011	.4278-0.9965
H2	.207	.669	.2920-0.6320

Table 3 Slopes and 95% confidence intervals for the difference in slopes for the regression lines fit to the PFC and LH refractory period data. Slope difference intervals that do not include 0 indicate that the slopes of the best fitting regression lines are significantly different.

DISCUSSION

The results in Figure 6 reveal marked differences in the time course of recovery from refractorines in the PFC and LH reward substrates. The recovery process appears to begin earlier, finish earlier and span a shorter time interval in the LH substrate.

The substantial difference in the C-T interval at which recovery appears to begin suggests that the largest PFC reward neurons have longer absolute refractory periods than the largest reward-related neurons activated by LH stimulation. The slow rate of recovery in the PFC substrate can be interpreted in two ways. It is possible that both the relative and absolute refractory periods of reward related PFC neurons are longer than their LH counterparts. Alternatively, the relative refractory period may contribute little to the time course difference which may instead reflect differences in the means and ranges of the absolute refractory period distributions for the two substrates. The unequal pulse technique (Yeomans, 1979; Bielajew et al., 1982) will help to choose between these two interpretations.

The differences in refractory period estimates for the reward related neurons in the LH and PFC are consistent with the idea that the reward substrates are different. Further, the PFC estimates suggest that small fibers may subserve the rewarding effects of stimulation at this site. However, it is also possible that the same descending axons are stimulated at both sites and that a segment of these axons between the two sites was smaller in diameter than the

portion of the axons between the LH site and the terminals, since it is the orthodromic action potentials that are believed to be responsible for the rewarding effects of stimulation and the LH refractory period is shorter than the PFC refractory period. The second experiment further investigates this interpretation.

EXPERIMENT 2

The first experiment suggested that different caliber fibers may mediate LH and PFC self-stimulation. In this experiment, the existence of a reward related anatomical link between the LH and PFC is investigated. In the absence of such a linkage, the degree to which the rewarding effects of stimulation at these two sites summate should reflect the extent to which the outputs of these distinct fibers converge.

METHOD

Subjects

Six of the eight subjects from the first experiment were used. All current intensities were the same as in the first experiment except for subject C1. The current intensity used at the PFC site for this rat had to be lowered from 600 to 300 uAmps in order to keep the required numbers at the two placements equal.

Procedure

The procedure for determining collision effects was analogous to the procedure for refractory period[9] tests with the exception that each pair of pulses was divided between the two electrodes. The C-T interval was varied from 0.4 to 25.0 msec under two conditions. In the antero-posterio (AP) condition, the anterior electrode (the PFC) received the C-pulse and the posterior electrode (the LH) rceived the T-pulse. In the postero-antero (PA) condition, the LH

received the C-pulse and the PFC the T-pulse. The order of presentation of the C-T intervals was counterbalanced across the 4-6 replications per condition.

A gating circuit in the amplifier output stage disconnected the unused electrode during delivery of a pulse to the second electrode. In the absence of a pulse in either channel, the cathode and anode were connected through a 1k resistor to allow the electrode/brain interface to discharge.

Scaling

The effectiveness of paired pulse stimulation was assessed by means of a weighted scaling formula:

$$E=(Nsp/Npp)-1)) \times Nsph/Nspl$$

where

E=paired pulse effectiveness

Nspl=lower required number of single pulses

Nsph=higher required number of single pulses

Npp=paired pulse required number

The weighting is necessary in order to offset the underestimation of E-values that would otherwise result from differences in the required number of single pulses for the two sites. The counter model (Gallistel, 1980) predicts that when the required numbers at the two placements are exactly the same, double pulse stimulation at long C-T intervals will be twice as effective as single pulse stimulation. However, if there is a difference in the required numbers at

the two sites,the maximum effectiveness of paired pulse stimulation will be the ratio of the lower and higher single pulse required numbers. The corrected scaling formula is equivalent to Yeomans's (1979) formula for pulses of unequal amplitude.

RESULTS

Figure 7 shows changes in paired pulse effectiveness as a function of C-T interval and condition for each subject. All 6 functions are relatively flat, showing no systematic changes in effectiveness as a result of changing C-T interval. There is also little difference between the AP and PA conditions. This was confirmed statistically by use of a repeated measures analysis of variance (condition X C-T interval) of the results from each rat. There were neither significant effects of condition nor interactions between the effects of condition and interval. In five of the six subjects, there was also no main effect of C-T inteval. The analysis of the results for C6, however, indicated that there was a significant change in effectiveness as a function of C-T interval ($F(8,24)=2.56$, $p<.05$). Tukey post-hoc tests confirmed that the main effect was due to the significant difference between the effectiveness at 0.8 and 5.0 msec. and 0.8 and 25.0 msec (HSD= .149, $p<.05$). There was substantial across subject variability in the magnitude of the summation between the rewarding consequences of LH and PFC stimulation. Collapsed across C-T interval, the average summation levels vary between .05 and .40 (Table 4).

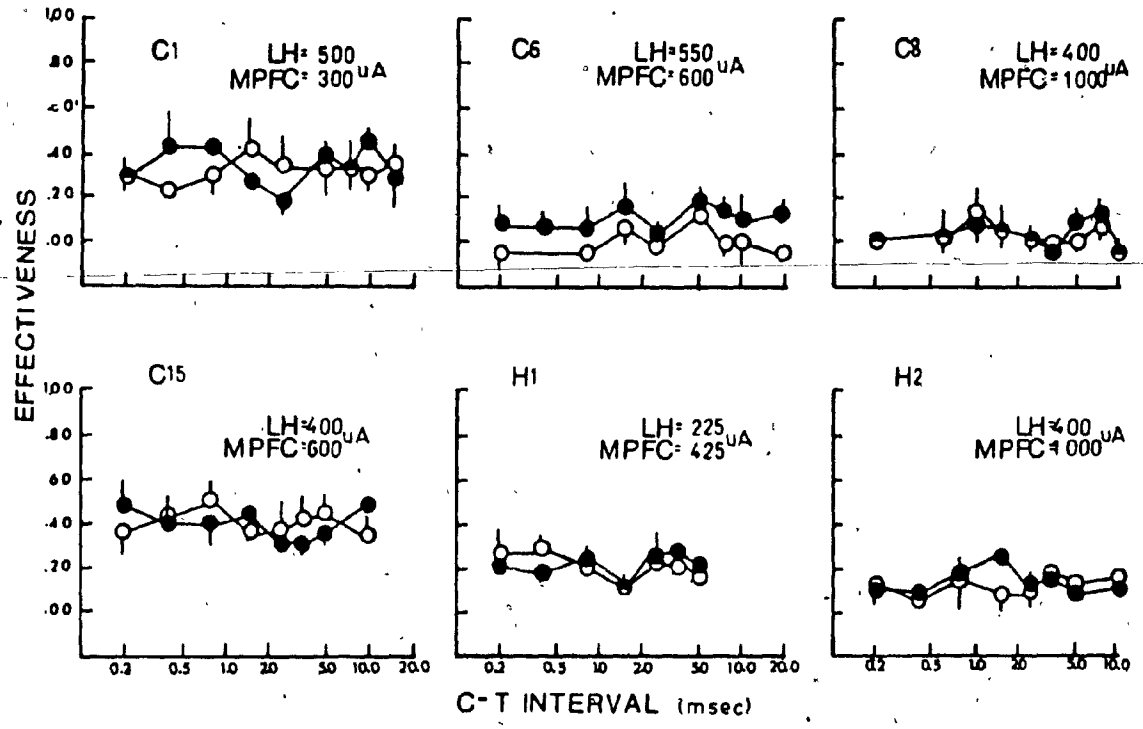

Figure 7 Colliaion/summation data for LH and PFC sites (AP= 0---0; PA= ●---●)

Subject	Summation
C1	0.33
C6	0.06
C8	0.05
C15	0.40
H1	0.21
H2	0.13

Table 4 Summation levels collapsed across C-T interval and condition.

DISCUSSION

No systematic changes in effectiveness as a function of C-T interval were observed. This finding is in sharp contrast to the step-like increases in effectivness that may be seen when collision tests are conducted with LH and VTA electrodes (Shizgal et. al, 1980; Bielajew & Shizgal, 1982). It is possible, but unlikely, that collsion effects might have been observed in the present study had longer C-T intervals been tested. In order for such long collision intervals to be obtained the conduction speeds of the stimulated fibers would have to be much slower than the available estimates for reward related fibers coursing through the LH (Bielajew & Shizgal, 1982).

Flat functions relating paired pulse effectiveness to C-T interval can be interpreted in two ways. It is possible that the stimulation fields at the two sites are misaligned so that they do not include any of the same behaviorally relevant fibers. Flat functions may also be anticipated if two fiber bundles with converging outputs are stimulated. In both cases, the E-values will reflect the efficiency of spatial summation.

Neither of these interpretations adequately explains all of the flat functions obtained in this study. In the subjects in which summation levels are near 0, almost the same number of pulse pairs as single pulses are necessary to maintain a half maximal rate of responding. That is, adding stimulation of the second site does not appear to increase the rewarding effects produced by stimulation of a single

site. This would suggest that there is little functional relationship between the stimulated substrates.

This arguement is weaker for those placements that show greater summation levels. It appears that for these placements there is some integration of the rewarding effects derived from LH and PFC stimulation. Summation levels for these subjects (C1 and C15) are nonetheless lower than those that have generally been oserved in bilateral LH (Shizgal et. al, 1980) LH and central gray (Bielajew et. al, 1981) and LH and VTA (Shizgal et. al, 1980; Bielajew & Shizgal, 1982) tests. At present, I cannot offer an explanation for the across subject variance in summation levels.

EXPERIMENT 3

If, as the refractory period estimates and collision data suggest, the directly stimulated PFC substrate is comprised of different fibers than the LH substrate, then it is possible that the chronaxie and rheobase of the cathodal strength-duration function also differ at the two sites. Further, anodal strength-duration estimates may indicate whether the accomodation process in these two substrates follows a similar time course. Finally, the ratio of anodal/cathodal currents provides an additional means of comparing these two substrates. Due to the difficulty associated with curve fitting procedures that was indicated in the introduction, long pulse durations were tested (up to 15.0 msec) in the hope of empirically obtaining the rheobasic intensity.

METHOD

Subjects

Eight male hooded rats of the Long Evans strain (Canadian Breeding Farms Ltd., St. Constant, Quebec) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). A stimulating electrode was stereotaxically aimed at the PFC or LH using the same co-ordinates as in experiment 1. The rats weighed 350-400 grams at the time of surgery and were maintained on a 12 hour light/dark cycle. Purina rat chow and water were freely available.

Monopolar electrodes were made from 90% platinum/10% iridium wire. The 254 um diameter wire was cut flush and

insulated with glass to the tip; only the cross section of the tip remained uninsulated. Current return was via 6 stainless steel skull screws.

Apparatus

The apparatus was the same as that described in Experiment 1.

Procedure

Pre-training: Once the rats were trained to self-stimulate, the required number of pulses was determined in the same manner as in the first experiment. Once the required numbers were stable for a given rat both within and across test sessions, the strength-duration determinations began.

Experimental sessions: The frequency of stimulation was set at either 25, 19 or 15 hz and the train duration was fixed at 500 msec. Within each testing session, the pulse duration was varied from 0.5 to 15.0 msec and required currents determined. Starting at values that supported maximal responding, the current was decreased in 0.05 log steps until the rat failed to respond. Through graphical interpolation, the required current was determined as the current intensity necessary to maintain a half maximal rate of lever pressing at a given pulse duration (see Figure 1). Both anodal and cathodal strength duration functions were determined for each rat.

Rat NM ceased bar pressing after the cathodal curves had been obtained. It was therefore impossible to obtain anodal curves for this rat.

RESULTS

Figure 8 presents the anodal (closed circles) and cathodal (open circles) strength-duration curves for the 6 PFC rats (A-F) and 2 LH rats (G-H) plotted on log/log co-ordinates. The characteristics of the curves that are of interest are (1) rheobasic current for the cathodal curves; (2) the chronaxies of the anodal and cathodal curves; and (3)anode/cathode current ratios. These features will be compared for the LH and PFC substrates.

Cathodal curves: Data from individual rats were analyzed using a non-linear least squares approach (Cuthbert and Wood, 1980; Tee, Note 5). The best fitting hyperbolic

$\log(I)=\log(Ir(1+C/d))$ where I=intensity

Ir=rheobasic intensity

C=chronaxie

d=duration

and exponential

$\log(I)=\log(Ir/(1-\exp(-d/k))$ where k= time constant

functions were determined by finding the values of Ir and C or k that provided the minimum residual sum of squares. The value of k can be tranalated into a chronaxie estimate by solving the exponential function for I=2Ir. The formula then reduces to C=.693k. Since the standard error of the current values tends to be a constant proportion of the average

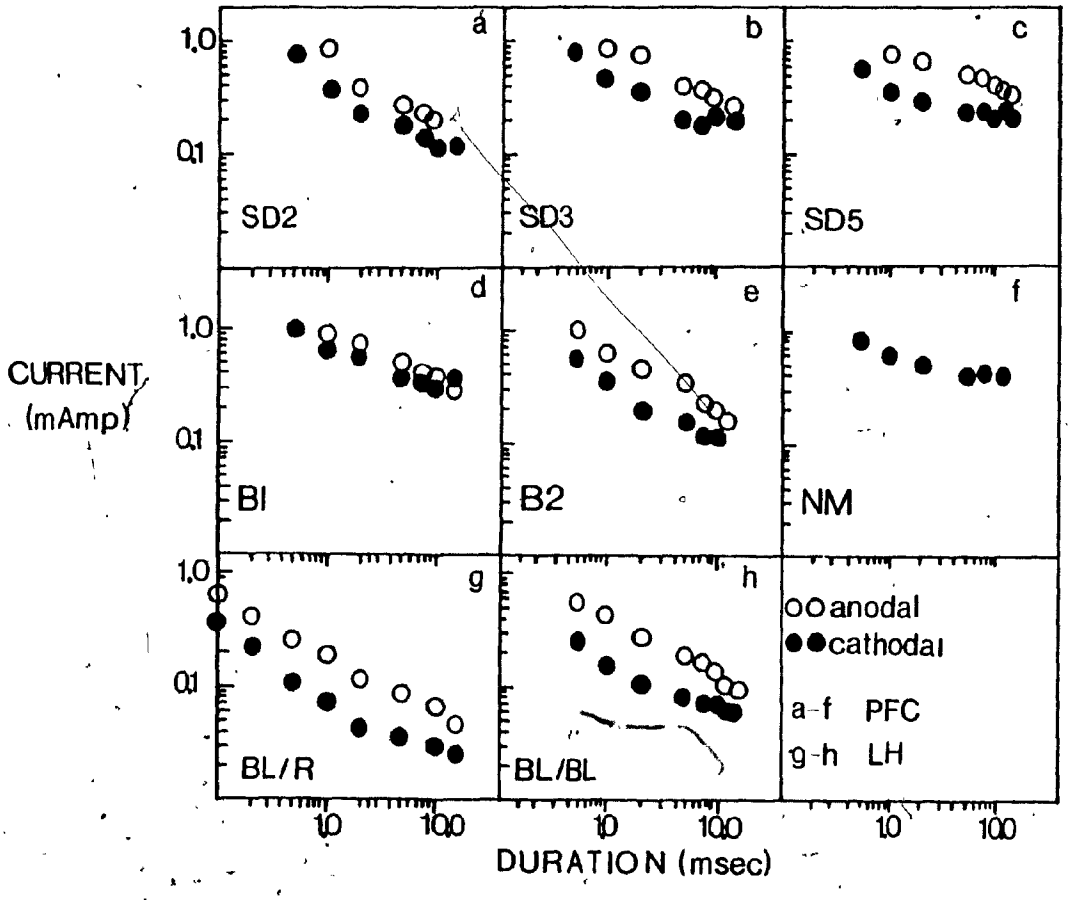

Figure 8 Anodal and cathodal strength-duration data for PFC (a-f) and LH (g-h) sites.

intensity, the log of the average current values was used in the curve- fitting procedure so as to equate the standard error of the current across pulse duration. In all cases except for one PFC site (rat SD3) the hyperbolic equation fit the data better than did the exponential function. For rat SD3 there was little difference between the goodness of fit for the exponential and hyperbolic equations. Table 5 presents the best estimates of Ir and C and the value of the sum of squares residual for the hyperbolic and exponential fits derived by using this curve-fitting procedure. The range of chronaxie (C) values for the PFC site is .689-3.462 msec., with a median value of 2.839 msec. There is considerable overlap between these values and those estimated for LH sites.

The two LH sites were tested at frequencies of 19 Hz (B/B) and 24 hz (B1/R). The frequencies tested at all PFC sites, except for SD3 were equal to or higher than these. Nonetheless, the Ir values are substantially higher at the PFC sites than at the LH sites (Table 6).

Anodal curves: The anodal curves for the LH and PFC substrates have much the same features (Figure 8). For both sites, the required current continues to decline at long pulse durations. In one PFC site (Figure 8C), the anodal strength-duration curve crosses the cathodal curve at long durations.

In order to compare the chronaxies for the anodal and cathodal strength- duration functions, the data for pulse

	HYPERBOLIC				EXPONENTIAL		
PFC	Ir	C	SSr	Ir	k	C	SSr
SD3	158.48	2215.98	1.056	194.39	2091.22	1449.07	1.036
NM	369.90	689.29	0.674	406.53	867.57	601.22	0.824
SD5	200.87	885.59	0.260	225.64	1048.31	726.48	0.309
B2	83.62	2861.39	1.010	110.06	2398.03	1661.83	1.060
SD2	90.25	3462.86	0.909	117.01	2976.79	2062.92	1.020
B1	298.27	1256.52	0.511	342.69	1387.08	961.25	0.638
LH							
BR	24.70	1551.00	0.225	29.60	1432.05	992.41	0.456
B/B	57.10	1742.79	0.217	67.80	1744.19	1208.72	0.343

Table 7 Chronaxie and rheobase estimates derived from the non-linear least squares regression analysis. Values are presented for the hyperbolic and exponential functions. The residual sum of squares for each fit are presented in the right-most columns for each function.

	CATHODAL		ANODAL			
Subject	Ir	C	Ir	C	F	p
PFC						
SD3	160.84	2089.27	224.85	3711.42	2.21	.0700
NM	369.90	689.29	------	--------		
SD5	205.28	759.28	348.07	1392.33	7.54	.0004
B2	83.62	2861.39	179.52	2449.53		
SD2	84.06	4332.25	174.27	2454.47		
B1	289.95	1454.02	289.22	2516.56	5.05	.0013
LH						
BR	24.70	1551.00	58.80	1420.12	2.48	.0475
B/B	57.10	1742.79	93.49	3146.64	11.67	.0001

Table 6 Comparison of the anodal and cathodal rheobase and chronaxie measures for the best- fitting hyperbolic equations. Currents used in the analysis included those that corresponded to durations that were common to both the anodal and cathodal curves. At the right are the F-values and probability levels for the interaction between pulse duration and polarity as determined by a 2-way ANOVA.

durations that were common to both sets of curves for each placement were analysed using the least squares regression method described previously. In all cases the data were best fit by the hyperbolic equation. The anodal and cathodal chronaxie and rheobasic intensity values for the data used in this regression analysis are presented in Table 6. For 3 PFC sites (SD3, SD5 and B1) and 1 LH site (B/B) the anodal chronaxies are substantially longer than the cathodal ones. The anodal and cathodal chronaxies are roughly equal at 1 PFC site (B2) and 1 LH site (B1/R). At the remaining PFC site (SD2) the cathodal chronaxie exceeds the anodal one. To further investigate possible differences between the shapes of the anodal and cathodal strength- duration curves, individual data sets that included anodal and cathodal required currents at 15 msec. durations (SD3, SD5, B1, BR, B/B) were analyzed using a 2-way ANOVA (duration X polarity). Only data from rats that had been run at the longest durations were included since the curves appeared to converge at durations greater than 5.0 msec. The F-ratios for the interaction effect and their associated probability levels are presented in Table 6. The interaction between duration and polarity was significant [redacted] all placements except SD3. This confirms that the two curves begin to converge at long pulse durations.

A salient characteristic of Mathews's (1977) anodal data for LH sites was the abrupt decrease in required anodal current as pulse duration was increased beyond 5.0 msec. This is not as apparent in the LH or the PFC curves of the

present study.

Anode/cathode ratios: In order to determine an average anode/cathode ratio for each rat, the mean cathodal and anodal required currents were compared at each pulse duration. The current ratios were then averaged across pulse duration. All ratios at the PFC site were between 1.0 and 2.0 (mean= 1.54) whereas for the two LH sites, the anode/cathode ratios exceeded 2.0 (mean=2.2).

DISCUSSION

The chronaxie values for the LH and PFC cathodal curves overlapped, suggesting that the shape of the best fitting strength-duration curves for the two sites were not consistently different. These values tended to be long compared to data from individual axons (Ranck, 1975). If one interprets these curves as Matthews (1977) has, there are two possible explanations for the long chronaxies. It may be that the neural elements subserving self-stimulation fire repetitively during long-duration pulses. This development should appear to the integrator as an increase in stimulation frequency. If so, the current required to maintain a criterial level of neural firings arriving at the second stage in the neural network can be reduced in a compensatory fashion (Gallistel, 1978). An alternative explanation is that long chronaxie elements represent a significant part of the reward substrate.

It is interesting to note that all the PFC curves tend to level out as pulse duration is increased beyond 10 msec.

This is not apparent in the LH curves. In Matthews's (1977) strength-duration data for the rewarding and priming effects of LH stimulation, only 2 of 11 curves exhibit a levelling off in required current at long durations. Since the chronaxie values for the LH and PFC sites overlap, there do not appear to be statistical grounds for differentiating the long-duration portion of the curves obtained at the two sites. The failure to observe consistent differences between the LH and PFC curves may be due to the small sample of points run beyond 10.0 msec durations and the relatively large variance. A follow-up study should be designed in such a manner as to include a larger number and range of pulse durations greater than 5.0 msec run with more replications. The chronaxie values should then reflect differences in the shape of the long-duration portion of the curves..

The anodal PFC curves differ from Mathews's (1977) LH data. Whereas he reported a sharp decrease in required current with long duration anodal pulses applied to the LH, the PFC data do not reveal such an abrupt increase in stimulation effectiveness. Rather, the required anodal current continues to decrease at aproximately the same rate (in logarithmic space) at the longer pulse durations as at the short ones. For the sites at which the cathodal required currents level off at long durations and at which 15 msec anodal pulses were run (SD3, SD5 and B1) the anodal chronaxies exceed their cathodal counterparts. For two of these three rats (SD5 and B1) there was a significant interaction between pulse duration and polarity. The results

for the third placement (SD3) approached the .05 significance level. These findings support the notion that the anodal curves, at these sites, begin to converge with the cathodal ones, and suggests that the accomodation process is a factor that influences the effectiveness of long duration anodal pulses. It may be that a heterogeneous population of neurons subserves the rewarding effects of PFC stimulation, a notion supported by the findings of Experiment 1. If so, the gradual rather than abrupt decrease in required currents may represent a weighted average of the time course of the accomodation process in the different subpopulations.

Another explanation for the differences between Matthews' LH data and the present PFC data rests on the procedural differences between the two studies. In the former, the runway paradigm was used whereas the present study employed a Skinner box. The two dependent measures are therefore somewhat different, a factor that White (1976) has suggested can influence strength duration data. This possibility is unlikely since there is good agreement between the cathodal curves of the present study and those of Mathews's study. It may be necessary to run longer durations to clearly observe a break firing effect. The abrupt decrease in intensity that Matthews attributed to break firing was most clearly observed with anodal pulses exceeding 15 msec durations. Since the LH curves of the present study do not exhibit this same effect, it may be necessary to test longer durations.

The anode/cathode ratios for the PFC substrate were consistently smaller than the ratios for the LH sites. This finding may prove useful for differentiating the reward substrates in electrophysiological studies. Further, these ratios may provide an indication as to the nature of the directly stimulated elements. If unmyelinated fibers alone constituted the directly stimulated substrate, larger anode/cathode ratios than those observed here might be expected (Ranck,1975). In contrast, cell bodies would be easier to stimulate anodally than axons when the point of current exit during anodal stimulation was at the initial segment. It is therefore possible that the site of stimulation in some components of the PFC reward substrate, is at or near the initial segment.

An interesting observation is that the absolute current required at the different pulse durations as well as the estimated Ir is much higher for PFC than for LH self-stimulation. This finding can be interpreted in three ways. First, it is possible that the criterion of the reward integrator is higher for PFC than LH stimulation. Second, the spatial distribution of the reward-related substrate may be more diffuse at PFC sites. Finally, smaller, less excitable neurons may subserve the rewarding effects of PFC stimulation, an hypothesis consistent with the interpretation of the refractory period data. This issue is further investigated in the last experiment.

EXPERIMENT 4

In the present experiment, the spatio-temporal integration of rewarding impulses derived from stimulation is examined by trading off the stimulation frequency and the current. This experiment was designed with several aims in mind. First, the linearity of the function may provide an index of the geometry of the fiber bundle that carries the reward signal (Yeomans, 1982; Shizgal and Schindler, note 3). Second, the minimum current that will maintain criterial behavior is related to the excitability of the directly stimulated substrate. For example, the less excitable the directly stimulated cells, the higher the minimum required current and the more gradual the slope of the frequency/ intensity trade-off (Shizgal, note 5). That the directly stimulated substrate for PFC self-stimulation is comprised of less excitable fibers is consistent with the findings of the first and third experiments. Provided that differences in the integrator's criterion or the spatial distribution of the directly stimulated cells do not compensate for excitability differences, the minimum current and slope of PFC and LH frequency/ intensity curves should differ.

The third purpose of this experiment is to further investigate the hypothesis (Robertson et. al, 1981; Goodall and Carey, 1975) that current increases do not enhance the rewarding effects of PFC stimulation. There are several possible explanations for the finding that performance for PFC stimulation was not enhanced by increasing the current. For example, a small, well-defined fiber bundle may mediate

the rewarding effect of PFC stimulation and the lowest currents used recruited all reward-relevant neurons. If so, then one would expect the frequency/intensity trade-off at this site to rise steadily over a relatively narrow range of currents and then to flatten out.

METHOD

Subjects:

Four male Long Evans rats and three male albino Sprague-Dawley rats from the previous experiments were used.

Apparatus:

Apparatus and training procedure were the same as in Experiment 3.

Procedure:

To obtain frequency/intensity trade offs, the required number of pulses was determined for various current intensities. For most rats the currents tested ranged from the minimum current required to maintain behavior up to 1200 uA. For all points on the curve, the current was randomly chosen and required numbers determined as in Experiments 1 and 2. The reciprocal of the average required number at each current was then plotted. This procedure was replicated 3-6 times per rat.

The minimum current that will support PFC self-stimulation marks the break-down of the frequency/intensity trade-off. In order to obtain the minimum required current, the procedure was slightly modified. Rather than fixing the current and determining the

required frequencies, the frequency was set at 252, 318 or 400 Hz. and the required current determined in the same manner as in Experiment 3. The difference between adjacent current values was either .05 or .10 log units. The required current was averaged across these three frequencies for each rat yielding the minimum current that would maintain self-stimulation.

RESULTS

Figure 10 presents the reciprocal of the number of pulses required to maintain half maximal responding as a function of current intensity for each rat. Linear regression was performed on all data points. The resulting correlation coefficients and slopes are presented in Table 7. As current is increased, there is an initial range over which the number of pulses decreases (1/N increases) in a fairly linear fashion. For some subjects (H1, B1), the linear trend appears to be interrupted for spans of up to 200 uA during which 1/N and current no longer trade off. That is, reciprocity between current and frequency seems to break down within these limits and then to resume as current is further increased. The 1/N values appear to approach an asymptote at the highest currents tested.

For all rats except for B1 the minimum required currents were determined. These values are presented in the left-most column of Table 7. The remaining columns in this table present parameters of the straight line fit to the remaining 1/N vs current data.

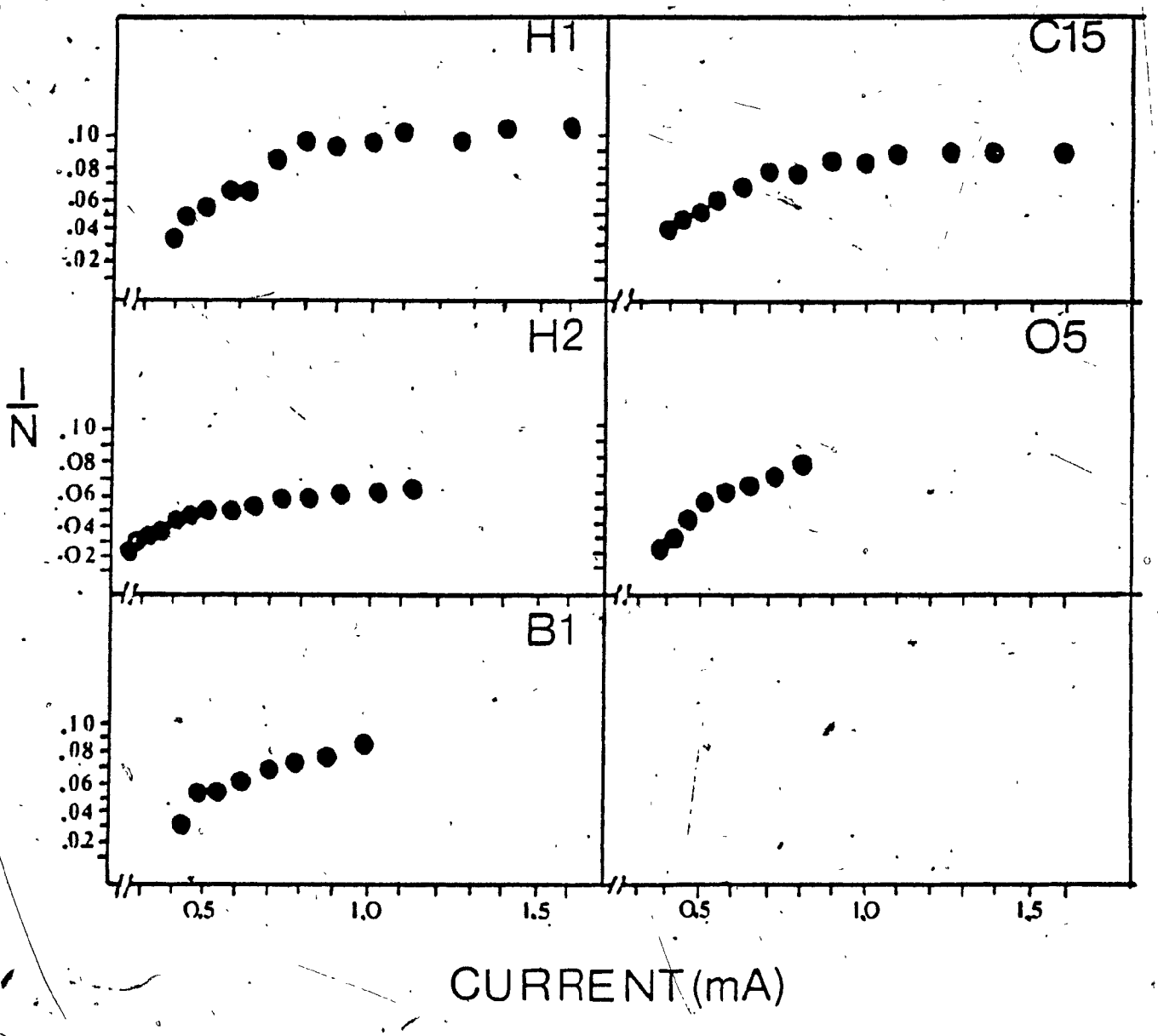

Figure 9 1/N vs. I trade-offs for the PFC sites.

Subject	Imin	r	r^2	slope
C15	378	.68	.46	.00016
O5	334	.98	.96	.00013
H1	352	.85	.72	.000052
H2	230	.95	.91	.000062
B1	---	.80	.64	.000074

Table 7 Average minimum required currents for PFC self-stimulation at 252, 318 and 400 Hz. The correlation coefficient (r) and the coeffiecient of determination (r^2), and the slope of the best fitting straight line through the data are also presented.

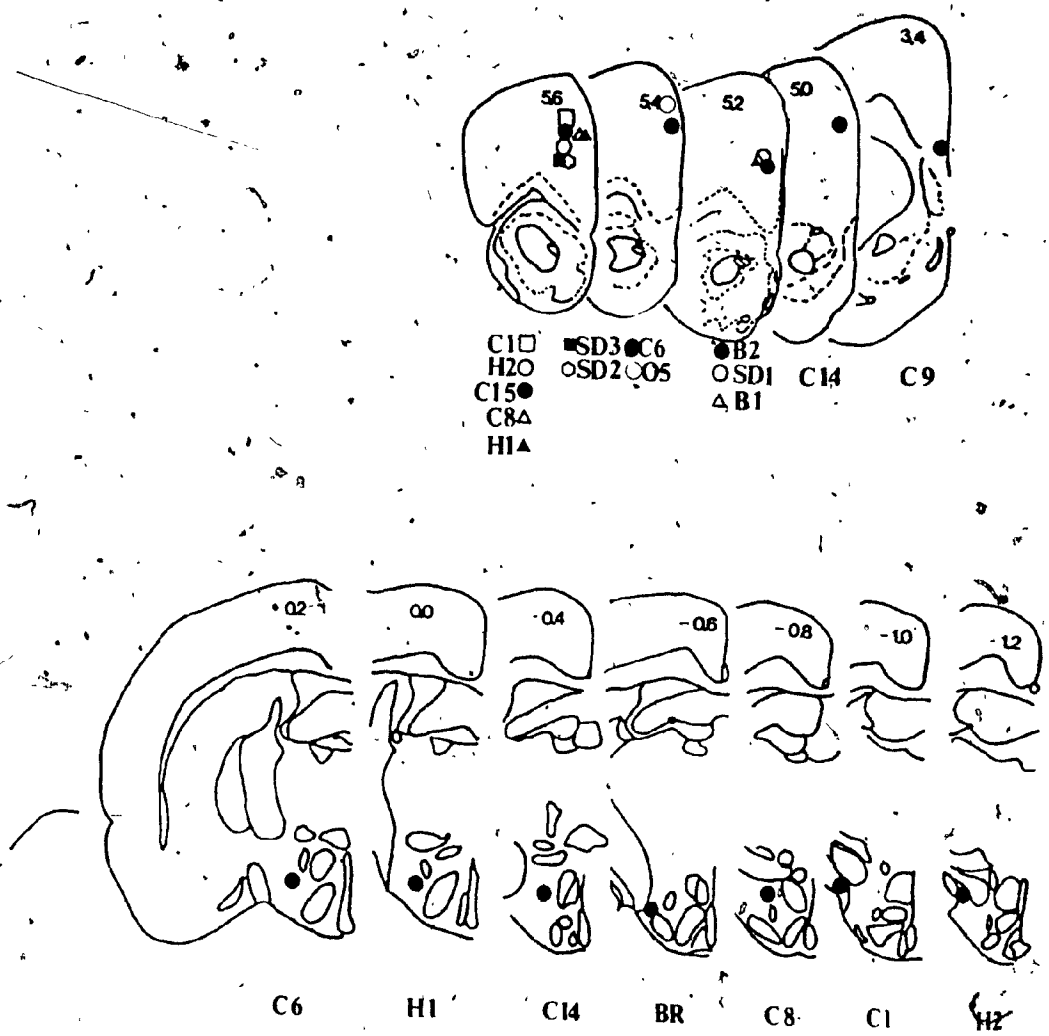

Figure 10 Tracings from the Pellegrino et al., (1979) atlas. The symbols represent the electrode tips for the PFC and LH placements.

The average minimum current, collapsed across placements is 323.5 uA. The data from individual placements were analyzed to determine whether the slopes of these functions differed significantly from 0. A 95% confidence interval was placed around the average slope for each function. The confidence interval for all of the trade-offs except for B1 did not include 0, indicating that the slopes of the best fitting straight line were reliably positive.

Histology

The atlas drawings (Pellegrino, Pellegrino and Cushman, 1979) corresponding to sections containing the electrode tips for the placements in all subjects used in Experiments 1 to 4 were traced and are presented in Figure 10. The sections containing the LH electrode tips in subjects C15 and B/B and the PFC tips in subjects NM and SD5 were not available. All PFC tips except for that of subject C9 were located in the medial aspect of the PFC rostral to the genu of the corpus callosum. The placement in C9 was located caudal to the genu of the corpus callosum, in the prefrontal cortex dorsal to the hippocampus. The tips of the posterior electrodes for C6, H1, and BR were located in the LH within the medial forebrain bundle. The posterior placements in C14 and C15 were located in the LH medial and dorsomedial to the fornix respectively. The tips for C1 and H2 were located in the zona incerta.

DISCUSSION

The minimum current that is capable of maintaining

self-stimulation with 500 msec train durations and comparable frequencies is substantially higher at PFC than at LH sites (Gallistel, 1978; Shizgal & Schindler, note 3). This finding is consistent with the view suggestd by the findings of Experiments 1 and 3, that the substrate for PFC self-stimulation is comprised of less excitable fibers than the substrate for LH self-stimulation. It is also possible that the high currents required to maintain PFC self-stimulation reflect the spatial distribution of the substrate relative to the stimulating electrode rather than or in addition to the physiological characteristics of the constituent neurons. That is, the PFC substrate may be more diffusely organized so that larger currents are required to recruit the minimum number of reward relevant fibers required to maintain behavior. Alternatively, the integrator criterion for the reward effects of PFC stimulation may be higher than the criterion of the LH integrator.

The slopes of the PFC curves were generally more shallow than those obtained at LH stimulation sites (Shizgal and Schindler, note 3), although a statistical comparison awaits more detailed analysis of the LH data.

That these curves have a non-zero slope contradicts Robertson et. al's (1981) assertion that current increments do not enhance the rewarding effects of PFC stimulation. As current was increased in the present study, the required number of pulses decreased, suggesting that the stimulation becomes more rewarding as the current is raised. Perhaps the

frequency chosen by Robertson et. al corresponded to a flat spot in the frequency/current trade-off ie., one at which larger current increases are necessary to observe a trade-off between these parameters. Had a number of frequencies been tested, the enhanced responding with current that is characteristic of LH self-stimulation may have been observed. Also, the shape of the function that maps reward value into response rate for PFC stimulation on the VI schedule used by Robertson et al. is not known. If this function were step-like, an increase in reward value produced by raising the current might be masked by a response ceiling. A more compelling means of assessing the rewarding value of PFC stimulation would be to measure the rat's preference between low and high currents that had previously been shown to correspond to different 1/N values.

GENERAL DISCUSSION

The results reported in this thesis are all consistent with the notion that different substrates subserve the rewarding consequences of PFC and LH stimuation. Recovery from refractoriness in the PFC substrate begins later and spans a longer interval than recovery in the LH substrate. These refractory period estimates are among the longest reported to date for BSR, matched only by the estimates obtained at dorso-medial thalamic sites (Bielajew et.al, Note 1).

That the substrates are comprised of different fibers is most strongly supported by the failure to observe collision or strong summation between the effects of concurrent stimulation of these two sites.

Strength-duration characterisitcs of the two sites were similar but not identical. Although chronaxies of the cathodal curves did not consistently differ, the PFC curves tended to flatten out at long durations, a feature that was not apparent in the LH curves. Further experiments, concentrating on increasing the resolution at the long durations may enable one to differentiate the LH and PFC cathodal curves. At both sites the chronaxies were long (up to 3.6 msec at the PFC site; 1.7 msec at the LH site) suggesting that both short- and long-chronaxie elements subserve the rewarding effects of stimulation and/or that the directly stimulated elements fire multiply during long duration pulses.

The anode/cathode required currents were lower at the PFC sites than at the LH sites providing an additional characteristic that can guide the electrophysiological identification of these cells.

Neither the LH nor PFC anodal strength-duration curves provided dramatic evidence of anode break excitation. Nonetheless, the anodal curves continued to decline over durtions at which the cathodal curves tended to flatten. One explanation for this is that the substrate is comprised of a heterogeneous population of neurons with different accomodation time constants. The gradual rather than abrupt decrease in currents at long durations may represent the sequential onset of break firing in these different subpopulations.

The largest difference between the PFC and LH strength-duration curves was the substantially higher rheobase of the cathodal PFC curves. An explanation of this finding that is consistent with the findings of the first experiment is that less excitable fibers subserve the rewarding effects of PFC stimulation. This hypothesis was supported by the relatively large currents required to maintain self-stimulation at high frequencies (Experiment 4). It is also possible that the PFC substrate is more diffusely organized and/or that the integrator criterion is higher than in the LH substrate.

The LH substrate appears to include small, myelinated fibers with conduction velocities ranging from 1.0-7.8 m/sec

(Shizgal et al., 1980; Gallistel, Shizgal & Yeomans, 1981; Bielajew & Shizgal, 1982). The directly stimulated sites in the PFC substrate, are more likely to be comprised of smaller, more slowly conducting fibers and/or initial segments.

These results suggest that the axonal connections between the LH and PFC (Leonard, 1969) are not reward-related but rather may subserve some other function. The failure for reversible procaine lesions of the medial PFC to attenuate LH self-stimulation (Rolls & Cooper, 1974) or for massive electrolytic lesions of the LH to attenuate PFC self-stimulation (Corbett, Laferierre & Milner, 1980) supports this interpretation of the data.

If the PFC is not part of the MFB reward system, then to where do its reward related neurons project? A fruitful avenue of inquiry will likely be the interactions between the rewarding effects of stimulating the medial PFC and sulcal PFC. Robertson (1982) has shown that experimenter delivered stimulation of the sulcal PFC potentiates acquisition of medial PFC self-stimulation. Electrolytic lesions of the sulcal PFC also disrupt medial PFC self-stimulation (Corbett et al., 1980). Further investigation may reveal reward related fibers that directly link these sites.

Bielajew et. al (Note 1) have recently reported refractory period estimates for the dorso-medial thalamic reward substrate that are in the range of those reported

here for PFC self-stimulation. When taken together with the strong neuroanatomical connections between these sites (Leonard, 1969), this finding suggests that the dorso-medial thalamus may be an important aspect of the cortical reward system.

In summary, the data reported here constrain the neurophysiological characteristics of the medial PFC reward substrate. These characteristics appear to differ from those of the LH reward substrate suggesting that different neurons subserve the rewarding effects of stimulation at these two sites. For the electrophysiologist interested in studying reward circuitry, the results of this thesis will provide criteria for distinguishing reward related cells from other neurons activated by PFC stimulation.

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