LH and PAG stimulation are mediated by different pathways that ultimately converge.

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Introduction

The observation that electrical stimulation of selective neural structures can reinforce operant behavior (Olds & Milner, 1954; Olds & Olds, 1963) has led to a plethora of studies examining the physiological basis of reward and motivation. The initial demonstration of self-stimulation in the rat has since been extended to many other species including the gold fish (Boyd & Gardner, 1962), cat (Roberts, 1958), dog (Stark & Boyd, 1963), monkey (Porter, Conrad & Brady, 1959), dolphin (Lilly & Miller, 1962), and man (Bishop, Elder & Heath, 1963). Although diverse neural structures support the behavior, it is most dramatically obtained from medial forebrain bundle (MFB) stimulation. The MFB is an extensive fiber tract that links many midbrain and forebrain structures and includes both ascending and descending fibers (Nauta & Haymaker, 1969).

For several reasons, self-stimulation has proven to be a valuable tool in studying reward mechanisms. Generally, the behavior is extremely persistent, extinguishes rapidly, and possesses many of the properties of conventionally reinforced behaviors. In addition, self-stimulation experiments allow one to tap directly into structures involved in reward. This method for selectively activating discrete regions of neural tissue

offers a "window" on brain mechanisms that underlie appetitively motivated behaviors.

Given this rationale, investigators have been interested in tracing and identifying the neural substrate for self-stimulation and determining the organizing principles used by these neural networks. One difficulty in attempting to delineate reward pathways is that brain stimulation often has several behavioral consequences. For example, feeding (Hoebel & Teitelbaum, 1962), drinking (Mendelson, 1967), copulatory behavior (Caggiula & Hoebel, 1966), exploration (Rompré & Miliaressis, 1980), escape (Bower & Miller, 1958), and self-stimulation may be elicited from MFB stimulation. Do many functions share a common substrate or are different critical fibers intermingled anatomically? The use of psychophysically based measurement techniques has, in several cases, supported the latter possibility (Bielajew & Shizgal, 1980; Miliaressis & Rompré. 1980; Skelton & Shizgal, 1980).

The psychophysical approach is based on the logic developed in sensory research in which trade-off functions are used to determine quantitative properties of sensory channels. Trade-off functions map the combinations of two stimulus variables that produce the same effect. For example, the scotopic spectral sensitivity curve describes the different combinations

of values of wavelength and intensity that produce a constant level of brightness. It is remarkable that this trade-off function is nearly identical to the in situ absorption spectrum of rhodopsin (Cornsweet, 1970), despite the fact that the first function is based on the verbal response of a human subject and the second function is based on direct measurements of light absorption by photopigments. There is good reason to believe that this similarity is not a mere coincidence.

The isomerization of rhodopsin is known to be the first stage in scotopic vision. How is it possible for the relationship between wavelength and intensity at this stage to be replicated at the behavioral level? Assume that all intervening stages between isomerization of rhodopsin (input) and behavioral response (output) were monotonic. In a monotonic system, a given value of the output of any stage is obtained as a result of one and only one input. If the final output of such a system were held constant, the outputs of all preceeding stages would necessarily be held constant as well. For example, if the output of the nth stage is held constant, then the output of the n-1st stage is also held constant because the input of the nth stage is the output of the n-1st stage, and only one input can correspond to a given output in a monotonic system.

Since the output of the n-1st stage is constant, and it's input is the output of the n-2nd stage, then the output of the n-2nd stage must also be held constant. In this manner, constancy is propogated all the way back to the output of the initial stage. If the visual system behaves in this manner, then two combinations of wavelength and intensity that produce the same level of brightness must also have produced the same amount of isomerized rhodopsin.

Gallistel and Norman (cited in Gallistel, Yeomans & Shizgal, in press) have shown that monotonicity can be easily demonstrated in a system that comprises many stages, without any direct observation of the intervening stages. This can be accomplished by showing that a monotonic increase in one of the input variables produces a corresponding monotonic change in the final output. If a given value of the output of any intervening stage were produced by more than one value of the input, then the relationship between initial input and final output would be nonmonotonic. Nonmonotonicities cannot be removed at later stages because information has been lost; there is no way of knowing which one of several input values produced a given output. Therefore, in order for the output of the final stage to change monotonically in response

to a monotonically changing input applied to the initial stage, all intervening stages must be monotonic. The monotonicity of the relationship between brightness and light intensity thus demonstrates the monotonicity of all stages in the scotopic system in response to single spots of light.

These arguments can be applied directly to studies of brain-stimulation reward (Gallistel et al., in press). As in the scotopic system, all stages between the input, the neurons directly activated by the electrode, and the output, the behavioral response of the organism, can be shown to be monotonic over a wide range of inputs. Current intensity and stimulation frequency are parameters that trade-off against each other (Gallistel, 1980; Shizgal, Howlett & Corbett, Note 1). Within a broad range, a monotonic increase in either parameter will result in a monotonic increase in behavior, e.g., running speed in an alley. This demonstration implies monotonicity of all stages, and provides the logical justification for using behaviorally derived trade-off functions to characterize the reward substrate. This justification is valid as long as performance conditions, e.g., task difficulty, fatique of the subject, etc., are held constant.

The psychophysical approach has been used in

self-stimulation experiments to examine the characteristics of rewarding sites along the MFB, and has provided information regarding the connectivity of such sites (Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1980), excitability cycles (Miliaressis & Rompré, 1980; Rompré & Miliaressis, 1980; Schenk, Shizgal & Bielajew, 1980; Shizgal, Bielajew & Yeomans, 1979; Yeomans, 1975, 1979), strength-duration properties (Gallistel, 1978; Matthews, 1977), temporal integrating characteristics (Gallistel, 1978; Milner, 1978; Shizgal & Matthews, 1977; Shizgal & Schindler, Note 2), conduction velocity (Shizgal, Bielajew & Yeomans, 1979), and directionality (Shizgal, Bielajew & Kiss, 1980). Moreover, the results of these studies have provided a basis for interpreting electrophysiological recordings from structures activated by MFB stimulation. The psychophysically derived characteristics define a set of criteria that an MFB neuron must posses in order to be classified as reward related. There is growing evidence of consistency between electrophysiologically and behaviorally derived properties (Gallistel, Rolls & Greene, 1969; Kiss, Shizgal & Rosen, Note 3).

The studies reported in this thesis use the approach just described to examine some of the neurophysiological features of reward neurons in

the periaqueductal gray (PAG). This midbrain structure was chosen because the properties of neurons that support self-stimulation at this site are unknown, self-stimulation obtained from activating PAG reward neurons resembles that seen in the MFB, and there are known anatomical connections between the PAG and MFB (Robertson, Lynch & Thompson, 1973).

The experiments presented here are concerned with three specific questions. First, what are the refractory periods of reward-relevant neurons in the PAG; second, do these characteristics differ from the well-documented refractory period properties of lateral hypothalamic (LH) reward neurons; and third, in what manner, if any, do reward fibers link the LH and PAG.

Excitability Cycles of Single Neurons

The pulse-pair technique used in behavioral studies has its roots in classical neurophysiological recording of peripheral nervous system activity. Helmholtz, in 1854, first demonstrated the phenomenon of refactoriness from a frog nerve-muscle preparation (cited in Gallistel, 1973). Pairs of electric shocks were applied to a nerve and the amplitude of the resultant muscle twitch was examined. It was found that 1.6 msec was the minimum interval at which the second shock produced an increase in the amplitude of the muscle twitch. Intervals of

less than 1.6 msec produced a twitch that was comparable to that obtained from single shocks.

The implications of these findings were not made clear until a series of experiments (Adrian & Lucas, 1912; Boycott, 1899; Bramwell & Lucas, 1911; Gotch & Burch, 1899) in which the neural refractory period was demonstrated directly by electrophysiological recording of the nerve innervating the muscle. These studies showed that the same refractory period was obtained whether recorded directly in the nerve or indirectly, using the behavior of the muscle as an index of excitation.

The invention of the oscilloscope and the vacuum tube amplifier made possible the detailed study of the neural refractory period. Erlanger and Gasser's (1937) classic monograph describes fully the excitability cycle in peripheral nerve. Although our understanding of the mechanisms underlying refractoriness has changed substantially since that time, the accepted description of the events comprising the excitability cycle has not. In addition, the picture obtained in the peripheral nervous system seems to generalize quite well to central neurons (Swadlow & Waxman, 1978). The basic excitability cycle is outlined in the following section.

A chain of events ensue during and after electrical stimulation of an axon. If a less than adequate (subthreshold) stimulus is applied, a local perturbation in the membrane potential occurs and then quickly subsides. The return of the membrane to original conditions depends on the time constant of the membrane. A suprathreshold stimulus has quite different consequences for the axon; an action potential is produced which initiates the first event in the excitability cycle, the absolute refractory period. During this period, a second action potential can never be elicited no matter how intense the stimulus. Following the absolute refractory period is the relative refractory period, so named because the membrane is now somewhat excitable but requires a higher than threshold stimulus for an action potential to occur. During the fairly long supernormal period that may follow the relative refractory period, the nerve appears to be hyperexcitable; the threshold for the action potential is reduced. There is evidence that the length of this period depends on the type and state of the fiber (Graham, 1934). Some classes of fibers do not have a supernormal period (Eyzaguirre & Fidone, 1975). A subnormal period lasting up to 100 msec is the final event in the excitability cycle. In some neurons,

it seems to be of little consequence, reducing membrane excitability by only .2% (Gasser & Grundfest, 1936).

Earlier it was stated that a subthreshold stimulus will not produce an action potential. However, if a second subthreshold stimulus closely follows the first, the local potential will not decay much, and the sum of the two depolarizations may reach threshold. This phenomenon, termed local potential summation or latent addition was first demonstrated by Helmholtz (cited in Gallistel, 1973) and later quantified by Lucas (1910).

When dealing with pairs of electrical stimulating pulses, the following convention is used: the first pulse is called the C pulse (conditioning pulse) because it establishes the state of the membrane which is to be tested by applying the second pulse, called the T pulse (test pulse). Accordingly, the interval between the two pulses is called the C-T interval. Plotting the inverse of the T-pulse threshold as a function of C-T interval produces a post-stimulation excitability curve.

To this point, the excitability cycles of single neurons have been discussed. Since two of the studies reported in this thesis are concerned with estimating the refractory period of a population of neurons, the next description of double-pulse stimulation must be

generalized to include the case when many fibers are stimulated. For the purpose of this discussion, it is assumed that all relevant neurons have the same excitability characteristics and the tissue resistance is homogeneous.

Since it is thought that current density decreases as the square of the distance from the electrode tip (Ranck, 1975), there will be an area surrounding the tip where the current density will be above the firing threshold for the population of neurons included in that region. This area will be defined as the suprathreshold region. The neurons closest to the electrode tip will fire once for every C pulse and will fire a second time when out of their absolute refractory periods. Neurons further away from the tip, but still within the suprathreshold region, will be exposed to a lower current density. These neurons will also fire to the C pulse but may not fire a second time until they are out of their relative refractory periods. Beyond the suprathreshold region is the local potential summation region. The current reaching this area will be below threshold; therefore neurons in this region will not fire to a C pulse. However, if the C pulse produces a depolarization that brings the neurons in this region more than halfway to threshold, and the

T pulse is presented before much of the C-pulse depolarization has decayed, the summation of the two depolarizations should result in action potentials.

The post-stimulation excitability curve will depend on both the spatial distribution and excitability characteristics of the neurons under investigation. If a large proportion of the relevant neurons are distributed close to the electrode tip, the number of firings produced by the T pulse will begin to increase as soon as the absolute refractory periods of the neurons are exceeded. Alternatively, if most of the relevant neurons are distant from the electrode tip, an increase in the number of firings will not occur until a longer C-T interval, i.e., when the relative refractory periods of the neurons are exceeded. The significance of local potential summation will also depend on the ratio of the number of reward-relevant neurons in two regions. A large effect is predicted if a relatively large number of relevant neurons are in the subthreshold region of the stimulation field. For a detailed discussion of the the effects of double-pulse stimulation, see Yeomans, (1975, 1979) and Yeomans, Matthews, Hawkins, Bellman and Doppelt (1979).

Behavioral Derivation of Excitability Cycles

Deutsch (1964) first adapted the pulse-pair

technique to derive post-stimulation excitability characteristics of reward-related neurons. He found that sharp increases in the rate of responding occurred as the C-T interval increased from 0.8 to 1.1 msec. Deutsch reasoned that the accelerated performance observed in this range of C-T intervals reflected the recovery from refractoriness in neurons subserving reward.

Plotting the change in behavior as a function of C-T interval has been the strategy used by other investigators to determine the refractory periods of neurons involved in stimulation-produced feeding (Hawkins & Chang, 1975; Rolls, 1973), drinking (Hu, 1973; Rolls, 1973), activity (Rolls & Kelley, 1972), and escape from aversive stimulation (Schmitt, Sandner & Karli, 1976).

Yeomans (1975) demonstrated that a serious problem existed with these early studies. He argued that the use of rate as a measure of reward is based on the unwarranted assumption that the level of responding is linearly related to the total excitation in the neural substrate. In most of these studies, the stimulation parameters were arbitrarily chosen and dictated the refractory period function obtained. Usually a frequency X was chosen such that 2X produced high rates of responding while X produced only baseline responding

levels. Yeomans showed that when rate vs C-T interval curves were derived at nine different stimulation frequencies, nine different refractory period estimates resulted! With extreme frequencies, there was virtually no effect of C-T interval, since initial responding was near floor or ceiling levels.

A new method of scaling was proposed based on a constant behavioral output. This notion rests on the assumptions that behavioral output is monotonically related to the total level of excitation, and that the behavioral weight of each pulse is invariant over the range of frequencies tested. Recent evidence (Gallistel, 1978; Hawkins, Roll & Yeomans, Note 4; Shizgal, Howlett & Corbett, Note 1) has provided support for these assumptions.

The "counter model" (Gallistel, 1975) is the basis for the logic underlying Yeomans' procedure. The model states that the magnitude of the reward producing by a train of fixed duration is proportional to the total number of firings the train elicits in the directly stimulated reward neurons. Gallistel (1978) developed a minimal model that incorporates the simplest assumptions that can link a train of stimulation pulses to a change in performance. The counter model is incorporated into the minimal model and defines the

second stage, those neurons that are responsible for integrating the incoming impulses over time and space. The output of the integrator is the reward signal. The first stage, termed the cable, comprises the directly stimulated reward-relevant neurons; it is assumed that the number of stimulated neurons is a linear function of the intensity (Gallistel et al., 1981; Shizgal, Howlett & Corbett, Note 1). In the second stage, the behavioral weights assigned to each impulse are the same or vary randomly over time and space. Provided that the train duration is constant, the impact on the integrator is identical whether twenty neurons receive five impulses each or fifty neurons receive two impulses each. Any pattern of stimulation that produces the same number of firings within a given duration will result in the same integrator output. If performance conditions are not altered, a constant behavioral output will ensure that the output of the integrator is fixed.

In refractory period experiments, the number of pulse pairs is traded off against the C-T interval. At C-T intervals within the refractory range, T pulses generate fewer action potentials than C pulses. Therefore extra pulse pairs must be added to maintain the same integrator output. If the assumptions to the counter model are correct, then the number of pulse

pairs added is directly proportional to the number of action potentials lost due to refractoriness.

From these principles, Yeomans (1975) devised a scaling formula to quantify the effectiveness of T pulses in refractory period experiments. For a homogeneous population of neurons, T-pulse effectiveness expresses the number of reward-related neurons fired by the T-pulse as a proportion of the number of such neurons fired by the C pulse. In a non-homogeneous population, the size principle (Davis, 1971; Henneman, Carpenter & Sonjeman, 1965) may operate and the behavioral impact of neural firings may be correlated with fiber diameter. If so, T-pulse effectiveness expresses the behavioral weight of the firings elicited by the T pulse as a proportion of the behavioral weight of the firings elicited by the C pulse.

How confident can one be that the behavioral post-stimulation excitability curves truly reflect the weighted contribution of the excitability characteristics of the directly stimulated neurons? There are several reasons to think that axonal rather than synaptic changes are being expressed. The order of events followed by the behaviorally derived curves simulates that found in individual neurons. Local potential summation, a membrane phenomenon, is seen at

pulse-pair intervals too short to be accounted for by synaptic temporal summation. In addition, the entrainment phenomenon (Kocsis, Swadlow, Waxman & Brill, 1979) suggests that the membrane characteristics of the axons directly beneath the electrode tip are being measured. Entrainment occurs when the second pulse is presented during the relative refractory or supernormal periods. The conduction velocity during these parts of the excitability cycle either decreases or increases as spikes are propogated down the axon. If the axon is of sufficient length, the interspike interval is locked to a constant "entrainment" and no longer mimics the C-T delay. Therefore it is unlikely that the behaviorally derived curves reflect the properties of synapses or axons belonging to cells other than the directly stimulated ones. Finally, changes in the order of .05 to .1 msec C-T interval measurably affect the excitability curves. Chemical synapses, being rather labile, would be unable to accurately transmit such precise temporal information. Taken together, the above arguments suggest that the behaviorally derived curves express the membrane recovery characteristics of directly stimulated neurons.

Behavioral Inference of Anatomical Linkage

The final experiment in this thesis makes use of a technique for drawing anatomical inferences about neural pathways mediating the behavioral effect of brain stimulation. As in the first two experiments, the technique arises from early neurophysiological work and is based on a well-known phenomenon, the conduction block caused by the collisions of orthodromic and antidromic action potentials in an axon. Neurophysiologists routinely use a related procedure to determine if a stimulating electrode directly activates the axon of a cell that is under observation via a recording electrode in or near the soma.

The original application of the method (Lucas, 1913) was to establish the conduction velocity of axons in a known bundle. This was accomplished by inferring from behavior the time it took for action potentials elicited by one electrode to travel past the second electrode. Working with a nerve-muscle preparation, Lucas determined the stimulation parameters that would produce no observable muscle twitch from a single shock and a detectable twitch when two shocks were delivered in close succession. This procedure was used to determine the shortest interval between shocks that produced a twitch when a pair of shocks were applied

to a single electrode or concurrently through two electrodes spaced along the nerve. Lucas found that in the latter case, the required interval was longer. He reasoned that the difference between the two intervals was the conduction time between the two electrodes. While Lucas used this procedure to determine the conduction velocity of an excised nerve, an analogous procedure is employed in this study to determine if direct anatomical linkage exists between self-stimulation loci.

The inference of collision from behavioral data is analogous to the procedure used in behavioral refractory period experiments. The question addressed in both cases is whether each pair of stimulation pulses produces one or two action potentials in behaviorally relevant axons. The excitation produced by one member of each pulse-pair will be reduced due to collision and the magnitude of the blocking effect can be assessed by determining how many additional pulses are needed to maintain performance at the criterial level.

This strategy was used (Shizgal et al., 1980) to test whether reward fibers are among the long axons thought to link the LH and ventral tegmental sites (Nauta & Haymaker, 1969). Concurrent ipsilateral LH

and ventral tegmental area stimulation produced results consistent with a collision block (Shizgal et al., 1980).

In the first study reported here, the paired-pulse paradigm was employed to obtain a refractory period estimate of reward neurons activated by PAG stimulation. The second study was designed to directly compare the refractory period profiles of the LH and PAG reward substrates within the same subject. In the last experiment, the collision technique was used to determine if reward fibers directly link the LH and PAG sites.

EXPERIMENT 1

In this study, Yeomans' (1975) scaling method was used to behaviorally estimate the refractory periods of neurons subserving PAG self-stimulation. This information provides a basis for comparison of PAG reward neurons to neurons subserving self-stimulation at other sites. In addition, it will be useful in interpreting the electrophysiological responses to stimulation of PAG reward sites.

Method

Subjects

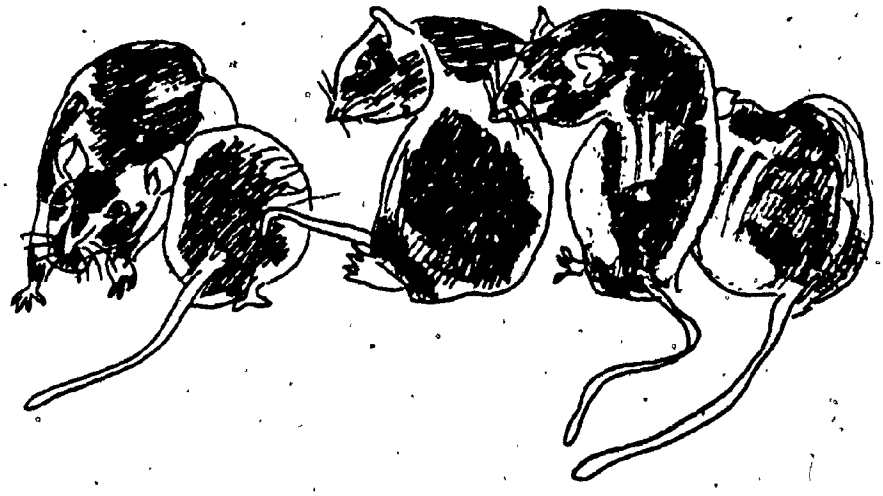
The subjects were five male hooded rats (Figure 1) of the Royal Victoria strain (Canadian Breeding Farms and Laboratories) weighing between 300 and 400 g at time of surgery. They were individually housed in wire-mesh cages and maintained on a 12 hr light/dark cycle. Free access to Purina Rat Chow and water was available in the home cage.

Electrode Implantation

Electrodes were 254 µm stainless steel wires insulated with Formvar to within .25 mm of the rounded tip. Standard stereotaxic procedures were used to aim the electrodes at the following coordinates: anterior/

FIGURE 1

The five male hooded rats in Experiment 1 before surgery.



posterior = bregma -6.0 mm; lateral = 0.0 mm; and ventral = 6.0 mm below the dura. With the incisor bar set at +5.0 mm, these coordinates correspond to the location of the dorsal raphé nucleus in the Pellegrino, Pellegrino and Cushman (1979) atlas. Sodium pentobarbital (60 mg/kg) was used as the anaesthetic. A flexible stainless steel wire, wrapped around four stainless steel skull screws, served as the current return, and the whole assembly was secured to the skull with dental cement.

Apparatus.

The test chamber was a wooden box, 25 cm x 25 cm x 70 cm high with a grid floor and Plexiglas front. A Lehigh Valley rodent lever protruded into the right corner of the chamber, 6 cm above the floor. The test chamber was located in a separate room from the programming equipment, and the animals' behavior was continuously monitored with video equipment.

Stimulation parameters were controlled by integrated circuit pulse generators and constant-current amplifiers (Mundl, 1980). The outputs of each channel of the dual constant-current amplifiers were shorted through 1KΩ resistors in the absence of a pulse on either channel. Current intensity was monitored on an oscilloscope by reading the potential difference across a precision 1KΩ resistor in series with the rat.

Procedure

Stabilization. Following several days recovery from surgery, the subjects were screened for self-stimulation using a CRF schedule and conventional shaping procedures. Stimulation trains were 0.5 sec long and consisted of rectangular, monophasic, cathodal pulses, 0.1 msec in duration. Only animals yielding high rates of responding (> 30 responses/min) were included in the study. Stimulation intensities were chosen to produce vigorous responding without severe motoric side effects and then kept constant throughout the experiment.

Self-stimulation performance was stabilized by repeatedly determining the number of stimulation pulses required to maintain bar pressing at a half-maximal rate. After a brief warm up with parameters that supported vigorous responding, the number of pulses per train was decreased every trial (trial = one minute) in 0.1 $\log_{10}$ steps until less than five responses/min occurred. The required number, defined as the number of pulses that would support a half-maximal rate of responding, was calculated by interpolation. Determinations of the required number were performed 5-10 times per session. The criterion for stability was established as a less than 0.1 $\log_{10}$ change in the required number across a session. Stability was generally achieved after three

sessions.

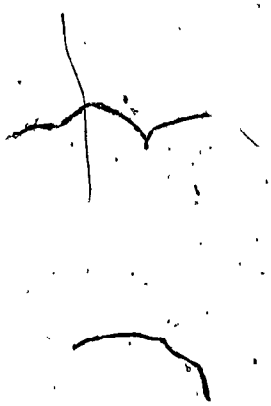
Refractory Period Test. The refractory period test consisted of a series of required-number determinations. In the first and last determinations, trains of single pulses were delivered. The last determination was carried out in order to permit the detection of fatique or sensitization effects. In the remaining determinations, trains of pulse pairs were delivered. Both the first pulse of each pair (the C pulse) and the second pulse (the T pulse) were applied to the same electrode. The interval between the two pulses (the C-T interval) was varied from 0.15 msec to 25.0 msec, and presented in a random order, so that each double-pulse determination was based on a different C-T interval. The longest C-T interval tested could never exceed half the C-C interval, e.g., if the C-C interval was 50 msec, then the maximum C-T interval was $\leq$ 25 msec. This procedure is illustrated in Figure 2. In the single-pulse condition (Figure 2a), only C pulses are delivered and the pulses are separated from each other by a constant interval (C-C), while in the double-pulse condition (Figure 2b), a T pulse follows each C pulse. The determination of the required number in the double-pulse condition is analogous to the procedure used in the single-pulse case except that pulse pairs are counted instead of single

FIGURE 2

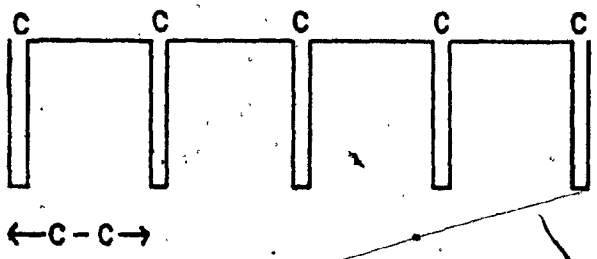
An illustration of the relationship between the single- and double-pulse conditions.

(a) Trains of single pulses (C pulses) are presented. Since the train duration is always held constant, there is a reciprical relationship between the number of pulses and the interval between pulses (C-C interval), i.e., an increase in the number of pulses in a train results in a decrease in the C-C interval.

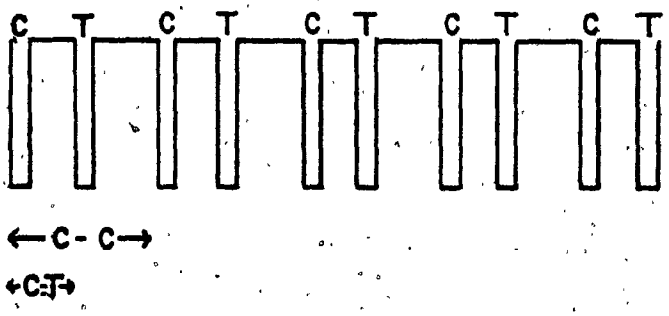
(b) For the double-pulse condition, each C pulse is followed by a T pulse of the same amplitude.



(a) SINGLE-PULSE CONDITION
C PULSES



(b) DOUBLE-PULSE CONDITION
C & T PULSES



pulses. The refractory period test was repeated from 4-6 times in each animal.

Data Format. The effectiveness of the T pulse was assessed by comparing the conditions when T pulses were present to the condition in which they were absent, i.e., the required number of pulse pairs for a given C-T interval was compared with the required number for the single-pulse condition. The contribution of the T pulses to the rewarding effect was scaled using the following formula (Yeomans, 1975):

$$E = \frac{RN_{sp} - RN_{C-T}}{RN_{C-T}}$$

where E = effectiveness of the T pulse,

RN_{sp} = average of the single-pulse required number for that session,

RN_{C-T} = required number of pulse-pair stimulation.

An E of 1 is obtained when the total number of pulses required to produce criterial performance with pulse-pair stimulation does not differ from the total number required to meet criterion in the single-pulse condition. This indicates that the T pulse is as effective as the C pulse, or to give a numerical example, 25 pulse pairs are behaviorally equivalent to 50 single

pulses. An E of 0 is obtained when twice as many pulses are required to meet criterion in the double-pulse condition, indicating that the T pulse has no behavioral effect. In this case, 50 pulse pairs are behaviorally equivalent to 50 single pulses.

Data Analysis. The E values that were obtained from the refractory period tests were plotted as a function of C-T interval. Typically, at short pulse-pair intervals, moderate E values are obtained (Yeomans et al., 1979). As the C-T interval increases, a rapid decline in the E values occurs. In the MFB, the lowest E value is usually seen at 0.4 or 0.6 msec. This initial decline is due to the decaying of local potential summation and is discussed in detail below. As the pulse-pair interval is further lengthened, an increase in the C-T interval typically results in an accompanying increase in E. Eventually the E value reaches an asymptote and no significant increases in this value are seen at longer C-T intervals. If the transition from the rising portion of the curve to plateau is abrupt, then the time (C-T interval) at which the plateau is reached can be estimated by visual inspection. However, in many cases, the slopes of the refractory period curves fall off gradually as C-T interval increases, making it difficult to visually estimate the point at which the curve

approaches asymptote.

A rule of thumb was developed so that the point at which these curves approach asymptote could be estimated in a consistent manner. It was assumed that this point corresponded to a near complete recovery from refractoriness. The initial step was to compare the standard error of the mean E value for the longest C-T interval with that of the preceeding C-T interval. If the standard errors overlapped, then the E values obtained for those two C-T intervals were pooled, a new standard error and mean were calculated, and these new values were then compared with the values for the next shorter C-T interval. This procedure was repeated until a C-T interval was found such that the standard error for its associated mean E value did not overlap with the standard error of the pooled E values. Once this interval was found, the smallest C-T interval to contribute to the pooled standard error (the next longest C-T interval) was designated as the asymptote, that is, the point beyond which no significant recovery from refractoriness occurred. This test, performed on the results from each animal, will hereafter be called the asymptote test.

Histology. At the end of the experiment, all subjects were given a lethal dose of sodium pentobarbital and perfused intracardially with physiological saline

followed by 10% formalin. The brains were removed and stored in 10% formalin for at least 24 hours. The tissue was sliced in 40 µm sections in a cryostat and stained with thionin. Location of electrode tips was verified using the Pellegrino et al. (1979) atlas. The atlas drawings corresponding to the critical sections were traced.

Results and Discussion

Histology

All electrode tips were located in or near the ventral portion of the PAG (Figure 3). Two electrode tips were found in the dorsal raphé nucleus (animals CJ-13 and CJ-14), two others were located immediately below the floor of the aqueduct (CJ-1 and CJ-14), and the electrode tip of the remaining subject (JFE-7) was situated in the fasciculus longitudinalis medialis, just ventral to the PAG.

Refractory Period Data

Figure 4 shows the refractory period results from each subject in this experiment. Yeomans' (1979) study suggests that the rising portion of the E vs C-T interval curves reflects recovery from refractoriness in directly stimulated reward-relevant neurons. All subjects in the current study show gradual increases in E as the C-T interval is increased beyond the values at which local

FIGURE 3

Tracing of pertinent sections from the Pellegrino et al. (1979) atlas. The circled arrow tips indicate the location of the electrode tips. The atlas plate numbers appearing at the top of each section refer to the distance (mm) of the section from bregma. The alphanumeric located below each section refers to the identity of the subject.

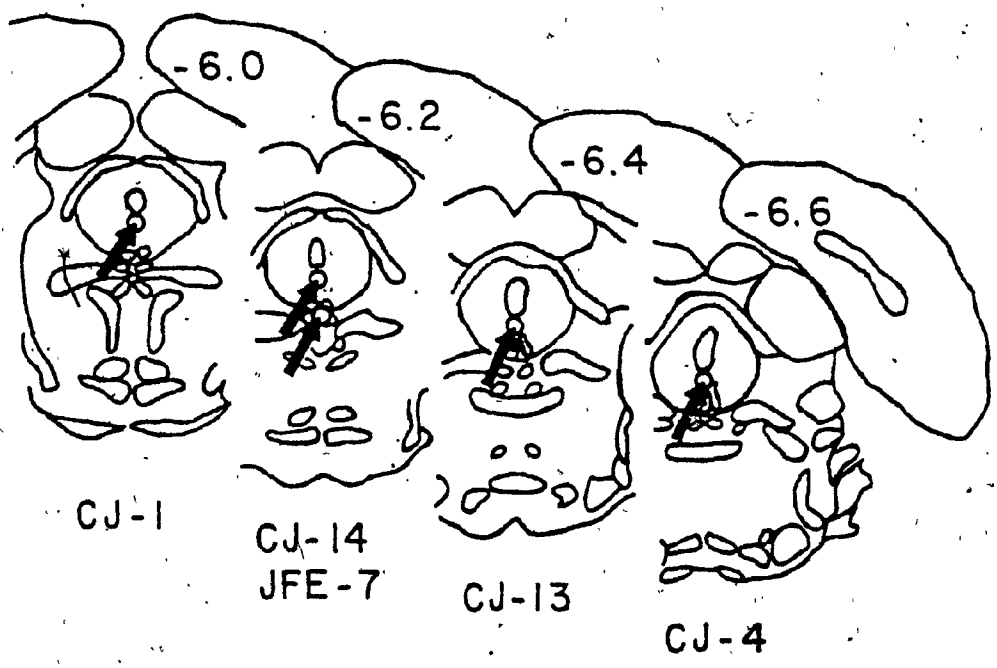
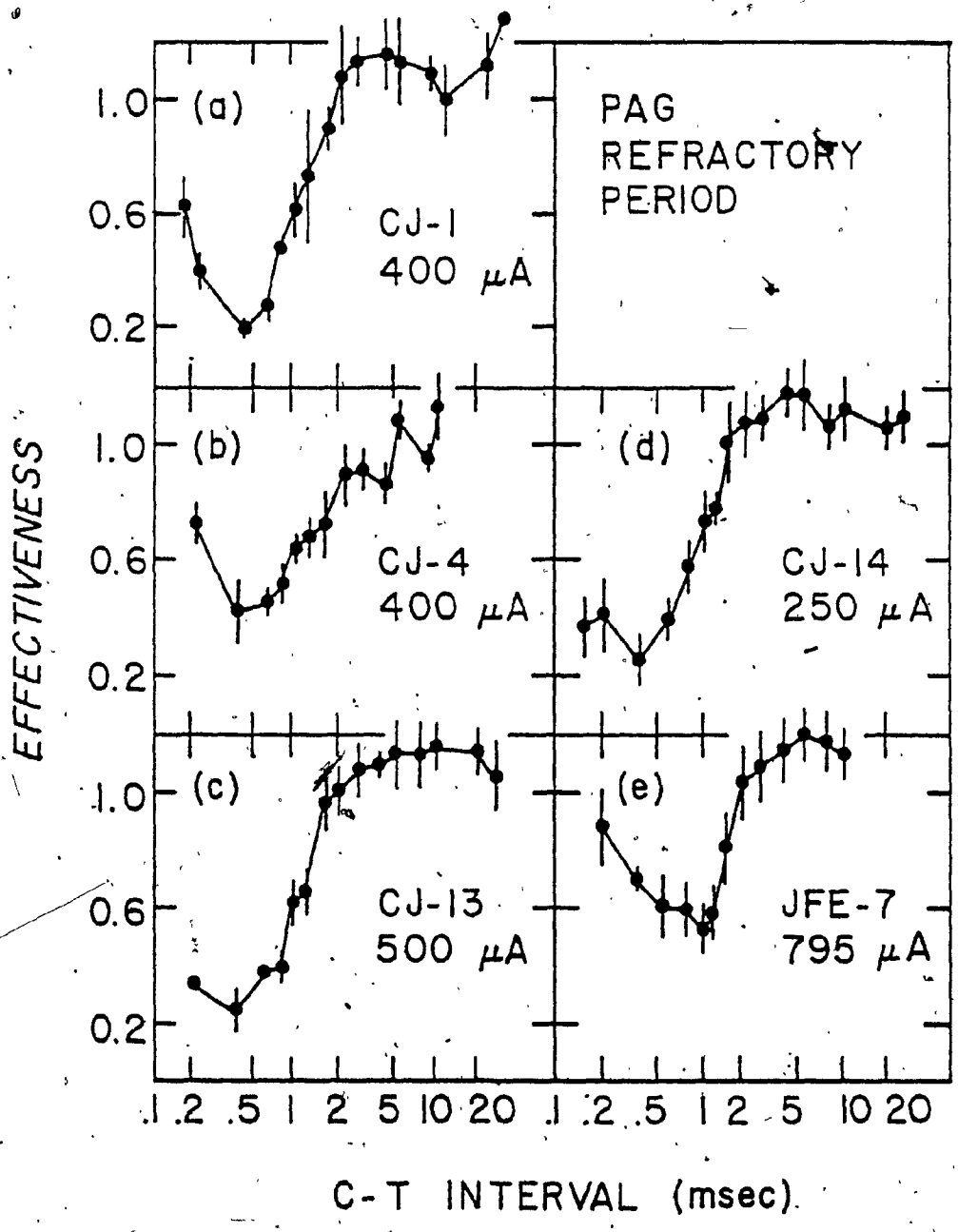

-6.0
-6.2
-6.4
-6.6
CJ-1
CJ-14
JFE-7
CJ-13
CJ-4

FIGURE 4

The PAG refractory period results from each subject. Changes in T-pulse effectiveness are plotted as a function of C-T interval. The letter-number combination appearing in each box identifies the subject. The current intensity at which each subject was tested is recorded below the animal number.


(a)
CJ-1
400 μA
PAG
REFRACTORY
PERIOD
(b)
CJ-4
400 μA
(d)
CJ-14
250 μA
(c)
CJ-13
500 μA
(e)
JFE-7
795 μA
1.0
0.6
0.2
EFFECTIVENESS
.1 .2 .5 1 2 5 10 20
C-T INTERVAL (msec)

potential summation is observed. (The initial decline in E values at short C-T intervals which is thought to be due primarily to the contribution of local potential summation (Yeomans et al., 1979) will be discussed later.) In four out of five subjects (Figure 4a, b, c, d), recovery from refractoriness appears to begin between 0.4 and 0.6 msec, whereas the curve for the remaining subject (Figure 4e) starts to rise between 1.0 and 1.2 msec.

The curves in Figure 4 all rise gradually and meet the asymptote criterion at 1.5 to 5.0 msec. The rate of recovery from refractoriness in these subjects is more gradual than that reported for self-stimulation sites along the MFB (Yeomans, 1975); the refractory period curves for LH stimulation approach asymptote by approximately 1.2 msec. The C-T interval at which the asymptote criterion was met for each subject is presented in Table 1.

There is a large across-subject variation in the time course of recovery from refractoriness, despite the fact that, excluding subject JFE-7, there was little across-subject variation in the location of the electrode tips. However, the histological analysis used in this study could provide only a rough approximation of the electrode tip location and no information about the size or shape of the effective

Table 1

C-T interval corresponding to maximum recovery from refractoriness.

Subject	C-T Interval (msec)
CJ-1	2.0
CJ-4	5.0
CJ-13	2.5
CJ-14	1.5
JFE-7	2.0

stimulation field. Thus, subtle differences in electrode placement could account for the wide range of estimates observed. If the reward substrate in the PAG consists of a heterogeneous population of neurons with different refractory periods, then the recruitment of any particular subpopulation will depend heavily on the exact location of the electrode.

It is interesting to note that recovery from refractoriness is apparent no later than a C-T interval of 0.6 msec in all subjects, with the exception of animal JFE-7 (Figure 4e). The curve from this subject starts to rise between C-T intervals of 1.0 and 1.2 msec. In addition, four out of five electrode tips were closely distributed in the PAG. Again, the single exception was that of JFE-7. The tip from this subject was located below the PAG.

Three factors may account for the delayed start of recovery seen in this subject. First, perhaps only neurons with long absolute refractory periods support self-stimulation at this electrode placement. Second, recovery from refractoriness may actually begin earlier but may be obscured by the large local potential summation effect. Although local potential summation in the MFB is generally at a minimum at C-T intervals of 0.4 and 0.6 msec (Yeomans et al., 1979), this

phenomenon has not been studied in detail in the PAG. Third, the displacement to the right of JFE-7's refractory period curve may reflect a relative dearth of reward-related neurons near the electrode tip and a relatively higher density in the relative refractory period region of the effective stimulation field. Recall that neurons distant from the electrode tip are exposed to a lower current density than neurons surrounding the tip, and thus will be unlikely to fire in response to the T pulse until their relative refractory periods are surpassed. Although this third interpretation is consistent with the large local potential summation effect observed at this placement, initial screening of this subject suggested that there were reward-relevant neurons close to the electrode tip. Behavior was obtained at a much lower intensity than that used in this study.

As stated earlier, the refractory period curves represented in Figure 4 recover more slowly than those reported for MFB stimulation (Yeomans, 1975). This apparent slope difference suggests that the substrate for self-stimulation in the PAG and MFB differ at the level of the directly stimulated neurons. The equal-pulse technique used in this study cannot assess the degree to which such slope differences reflect the contributions of absolute or relative refractory

periods. The use of the unequal-pulse technique (Yeomans, 1979) could more precisely document the source of the disparity in recovery from refractoriness between LH and MFB self-stimulation neurons.

The remainder of this section will deal with the results obtained at short pulse-pair intervals where it is expected that the directly stimulated neurons are in the absolute refractory state. How then, can one account for the fact that the lowest E value seen was .20 (animal CJ-1)? These non-zero E values are probably due to local potential summation (Yeomans et al., 1979). This phenomenon occurs when neurons just beyond the border of the effective stimulation field are fired as a result of the addition of subthreshold depolarizations caused by the C and T pulses. The factors that determine the magnitude of the local potential summation effect are a) the size of each local potential, b) the time constant of the membrane, c) the pulse-pair interval, and d) the proportion of the behaviorally relevant fibers in the subliminal region relative to the effective stimulation field. It is likely that there will be large differences across animals in the degree of local potential summation since the anatomy of the structure as well as electrode placement will dictate the size of the effect. In Figure 4, this effect

ranges from .36 (animal CJ-13) to .92 (animal JFE-7).

To summarize, the results of this experiment provide an estimate of the refractory periods of PAG reward-related neurons. Furthermore, the time course of recovery in the PAG appears to be longer than that previously observed in the MFB (Yeomans, 1975). While recovery from refractoriness in the MFB quite consistently reached a maximum between 1.2 and 1.5 msec, the PAG sample reported here ranged from 1.5 - 5.0 msec. This wide range of PAG values may reflect the recruitment of subpopulations of relevant neurons with long absolute refractory periods at some PAG placements and not others, and/or differences in the contributions of the relative refractory period. In the following experiment, both PAG and MFB refractory period curves are obtained from the same subjects so that direct within-subject comparisons between the excitability characteristics of MFB and PAG reward neurons can be made.

EXPERIMENT 2

The procedure used in Experiment 1 was similar but not identical to that used by Yeomans (1975). In order to rule out procedural factors as the source of the difference between my PAG results and Yeomans' MFB results, a second experiment was conducted in which within-animal comparisons were drawn. This second experiment allowed quantitative comparisons to be made between MFB and PAG results.

Method

Subjects

Five male hooded rats of the Royal Victoria strain weighing approximately 350 g each were individually implanted with electrodes aimed at the PAG and the LH. The coordinates for the PAG were the same as those used in Experiment 1. The LH coordinates were: anterior/posterior = bregma -0.4 mm; lateral = bregma +1.7 mm; and dorsal/ventral = -8.0 mm below the dura. The incisor bar was set at +5.0 mm. Surgical and histological procedures are described in Experiment 1.

Procedure

The procedure was analogous to that employed in Experiment 1. Within each subject, the current

intensities for each electrode were individually adjusted with the aim of equating the required number of single pulses. The refractory period tests for the two electrodes were conducted in an alternating fashion and repeated 4-6 times. The effectiveness of double-pulse stimulation was computed using the scaling formula described in the first experiment.

Data Analysis

The LH and PAG refractory period curves for each subject were analyzed in the following manner. First, the C-T interval associated with asymptotic recovery from refractoriness was determined using the test described in Experiment 1. Then each curve was transformed in order to facilitate comparison of the time course of recovery following stimulation of the two sites. The transformation consisted of normalizing the rising portion of each curve so that it spanned the range of E values from 0 to 1. The rising portion of the curve was defined as that portion that lay between the C-T interval that yielded the lowest E value and the C-T interval designated as the asymptote. The purpose of this transformation was to reduce the number of extraneous factors contributing to the slope of recovery. These factors include the amount of local potential summation and the E values obtained at C-T intervals

beyond and including the asymptote. In MFB results (Yeomans et al., 1979), local potential summation effects often do not decay to zero before recovery from refractoriness begins. Hence, at certain C-T intervals the E values reflect both local potential summation and recovery. In order to study the time course of recovery from refractoriness, it would be useful to reduce the contribution of local potential summation. This is accomplished by the transformation outlined above but at a small cost. Fixing the lowest E value at zero may eliminate not only the effect of local potential summation but also the contribution of recovery in the neurons with the shortest refractory periods. It is likely however, that the amount of recovery from refractoriness at C-T intervals where the lowest E value was obtained in this study (0.4 - 0.6 msec) is small. For example, I know of no evidence that any CNS neurons have refractory periods as short as 0.3 msec.

At C-T intervals longer than the value at which the asymptote is reached, an E value of 1.0 is expected. In theory, an E value of 1.0 indicates that all the neurons contributing to the rewarding effect at a given current intensity have recovered from refractoriness. E values of 1.0 are rarely observed when testing MFB sites. This phenomenon may be peculiar to the MFB

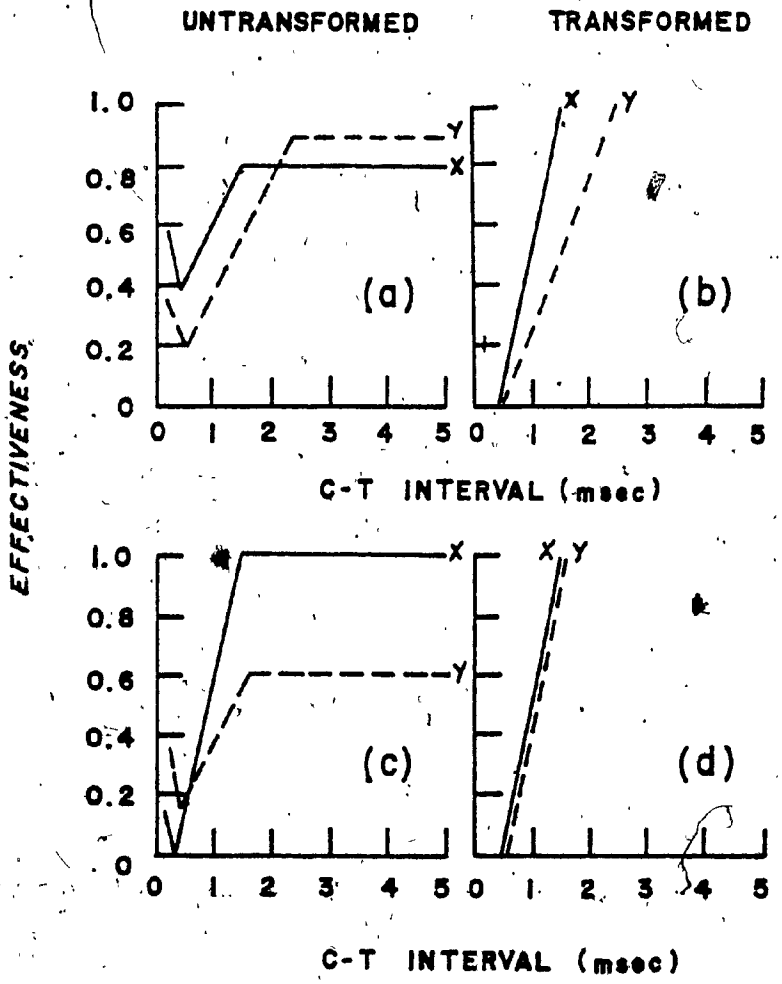
(Schenk, Shizgal & Bielajew, 1980; Shizgal, Jordan & Bielajew, Note 5) and is not understood.

If the E values for one placement level off at 1.0 and the E values for a second placement level off at a lower value, then the slope of recovery will appear to be different for the two sites even if the curves begin to rise at the same C-T interval and level off at the same C-T interval. As illustrated in Figure 5, the transformation removes this effect and reduces the influence of local potential summation on the slope of recovery from refractoriness.

The two hypothetical untransformed curves in Figure 5a have identical slopes although curve X reaches asymptote at a C-T interval of 1.5 msec while curve Y continues to rise well past 1.5 msec and only levels off at 2.4 msec. Had curve X begun to rise at the same time (0.4 msec) but at an initially lower E value, the resulting slope would have been much steeper. Alternatively, if the maximum E value of curve X were greater than 0.8, the slope would again appear steeper. The transformation (Figure 5b) forces the rising portion of each curve to lie between E values of 0 and 1. As a result of the transformation, it is clear that the proportional rate of recovery is greater for curve X. In addition, it can be more clearly seen in the

FIGURE 5

Hypothetical examples of the effects of differences in local potential summation and maximum E values on the slope of the refractory period function. The transformation reduces the first confound and eliminates the second, thus facilitating comparison of the time courses of recovery.


UNTRANSFORMED
TRANSFORMED
EFFECTIVENESS
1.0
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5
X
Y
(a)
(b)
(c)
(d)
C-T INTERVAL (msec)

transformed functions that curve X reaches asymptote at a shorter C-T interval than curve Y, but that recovery begins at the same time for both curves.

Figure 5c shows how the differences in the slopes of untransformed curves might lead one to a questionable conclusion about differences in the time course of recovery. When the curves in Figure 5c are transformed (Figure 5d), there is clearly little difference in the proportional rate of recovery, the C-T interval at which recovery begins, or the C-T interval at which recovery ends.

The transformation was achieved using the following formula:

$$E_{transformed} = \frac{E_{C-T} - E_{min}}{E_{asymptote} - E_{min}}$$

Where $E_{transformed}$ = the effectiveness of double-pulse stimulation adjusted to a scale of E values between 0 and 1,

E_{C-T} = the E value in its original form associated with a given C-T interval,

E_{min} = the lowest E value obtained,

$E_{asymptote}$ = the maximum E value evaluated by the asymptote test.

The results of each of the 4-6 refractory period tests performed at each electrode site in this experiment were subjected to the above analysis.

Results and Discussion

Histology

The Pellegrino et al. (1979) atlas drawings corresponding to sections containing the electrode tip were traced and are shown in Figure 6.

The anterior electrode placements (upper sections) were all localized in the LH, generally just ventral to the zona incerta. One electrode tip (CJ-11) was found in a more ventral portion of the LH, at the level of the fornix. The PAG placements (lower sections) were widely distributed in the anterior/posterior plane ranging from 5.6 to 6.6 mm behind bregma. Two electrodes were localized in the dorsal raphé nucleus (animals CJ-7 and CJ-8), while the remaining tips were situated immediately below the floor of the aqueduct.

Refractory Period Data

The individual subject refractory period results for each stimulation site are presented in Figure 7 with changes in E plotted as a function of C-T interval.

FIGURE 6

Tracings of Pellegrino et al. (1979) atlas drawings corresponding to pertinent sections. The circled arrow tips denote the location of the electrode tips. The letter-number combination identifies the subject. The sections above and below a subject number refer to the anterior and posterior placments of that subject respectively. Each subject had electrodes aimed at the LH and dorsal raphé nucleus.

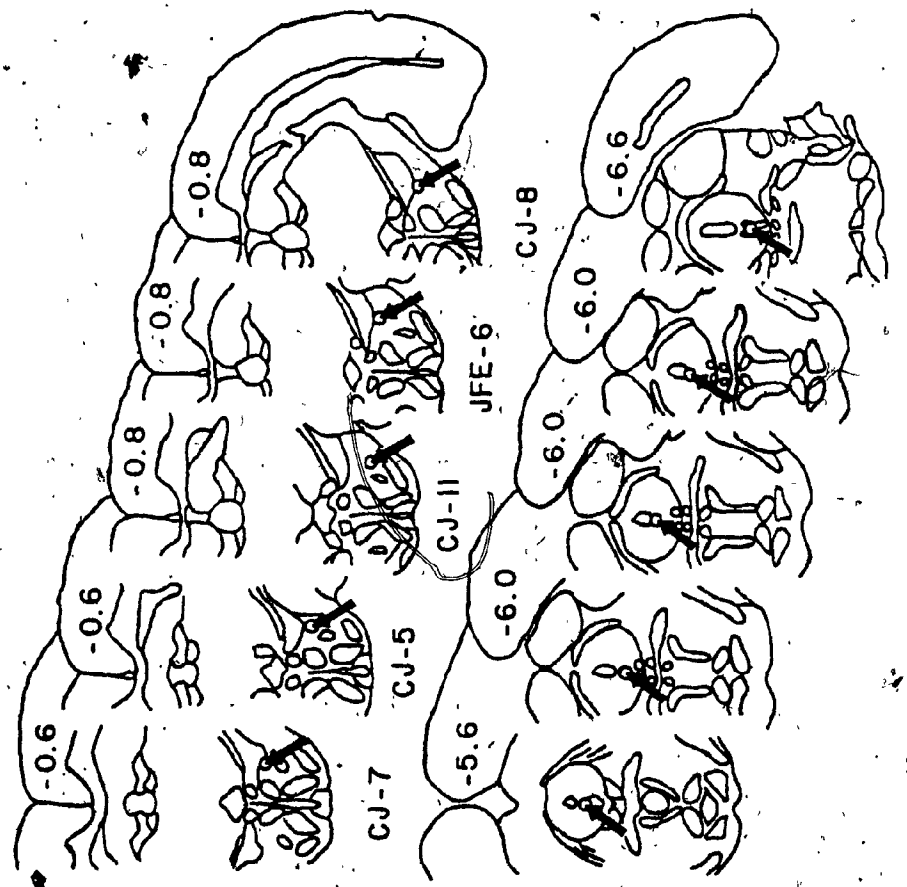
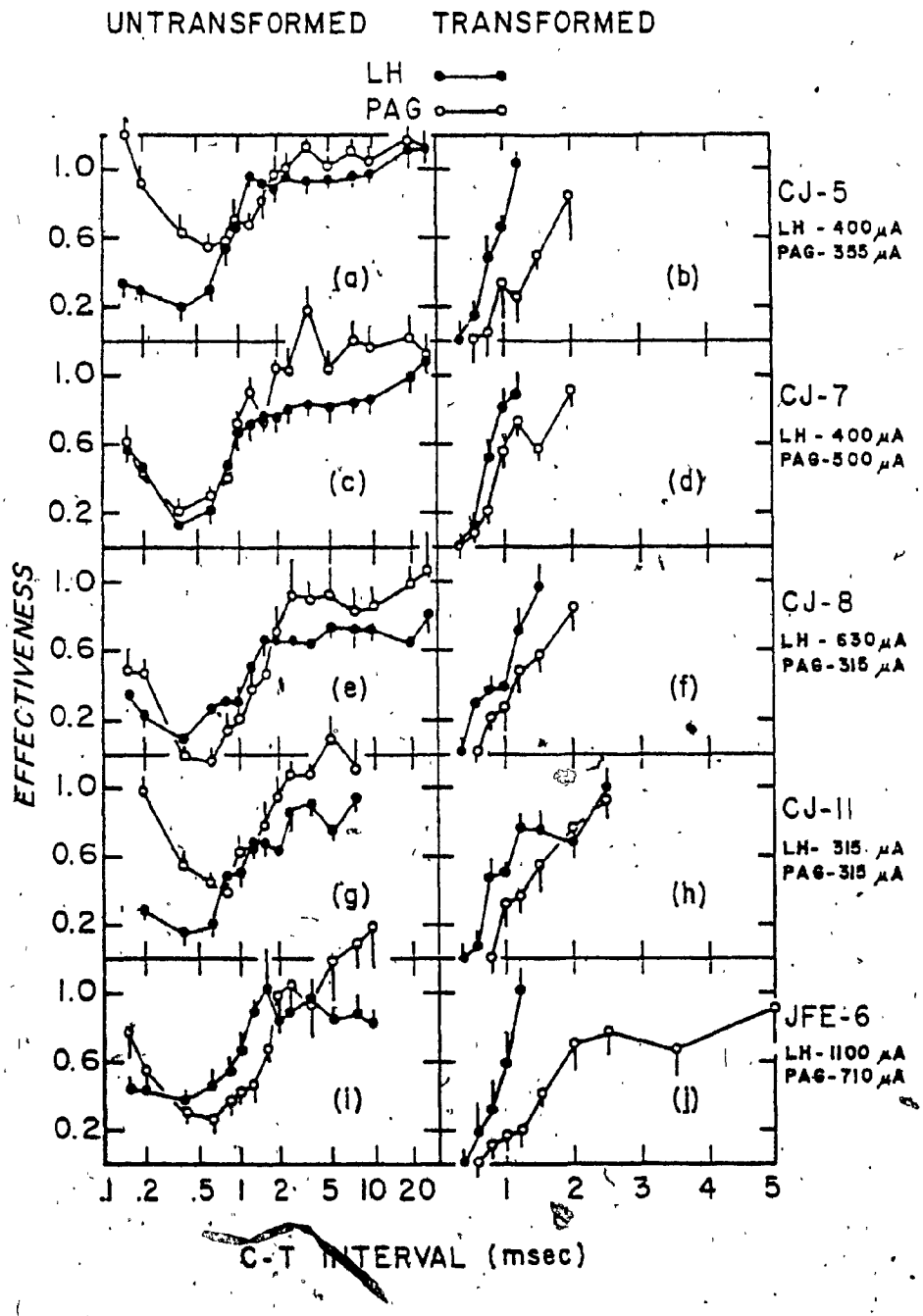

-0.8
-0.8
-0.8
-0.6
-0.6
CJ-7
CJ-5
CJ-11
JFE-6
CJ-8
-6.6
-6.0
-6.0
-6.0
-5.6

FIGURE 7

The results of the refractory period tests for each subject. Changes in T-pulse effectiveness are plotted as a function of C-T interval. The LH data are represented by filled circles; PAG data by open circles. The boxes forming the left column contain the untransformed data; the transformed data are contained in the right boxes. The graphs in the left column are semilogarithmic plots while the coordinates in the right column are linear. Semilogarithmic plots were used for the untransformed data because of the large range and uneven spacing of the C-T intervals tested. The alphanumeric listed on the extreme right of each pair of boxes refers to the subject. The current intensity is listed below that subject.


UNTRANSFORMED
TRANSFORMED
LH
PAG
EFFECTIVENESS
1.0
0.6
0.2
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
CJ-5
LH - 400 μA
PAG- 355 μA
CJ-7
LH - 400 μA
PAG-500 μA
CJ-8
LH - 630 μA
PAG-315 μA
CJ-11
LH- 315 μA
PAG-315 μA
JFE-6
LH-1100 μA
PAG-710 μA
.1 .2 .5 1 2 5 10 20
1 2 3 4 5
C-T INTERVAL (msec)

The untransformed data for each subject are located in the left panels, while the right panels give the transformed results. Note that the untransformed results are plotted on semilogarithmic coordinates while the transformed results are plotted on linear-linear coordinates.

The changes in E seen in these behaviorally derived curves (Figure 7a, c, e, g, i) are consistent with axonal excitability changes. The E values quickly decline at very short pulse-pair intervals (local potential summation), and thereafter rise steadily as the C-T interval is increased. At long C-T intervals, the E values approach asymptote; further increases in C-T interval result in little or no increase in the E value, except in the case of the PAG curve for JFE-6.

I. Statistical Analysis:

a) Transformed data. The question addressed in this experiment was whether the reward-relevant neurons directly activated by LH and PAG stimulation could be distinguished on the basis of their refractory periods. Therefore, the statistical analysis focused on the time course of the rising portion of the LH and PAG curves within each subject. The analysis consisted of two parts. First, the analysis of variance approach to regression analysis (Neter & Wasserman, 1974) was

used to determine if more variance is explained by fitting separate regression lines to the rising portion of the curves for each placement than by fitting one regression line to the pooled results from both placements. If the data are better represented by two lines, this shows that the refractory period functions for the two placements are different. When such differences were obtained, the second part of the analysis was applied. This consisted of testing for significant differences between slopes. The second test made it possible to distinguish cases where the rates of recovery at the two placements were the same but recovery was delayed at one site from cases where the rates of recovery were different.

The F ratios computed in the first test were significant for each set of curves in Figure 7b, d, f, h, j and are reported in Table 2. The source tables are presented in Appendix I.

As can be seen from Table 2, the rising portions of the LH and PAG refractory period curves from each subject cannot be collapsed and treated as one line. The significant F ratio in each case indicates that the linear component of recovery at the two sites differes.

The results of the t tests for slopes differences are shown in Table 3. The level of significance for all t tests was set at .05 (1-tail).

A significant t score was obtained in three

Table 2

Results of test determining equality of regression lines

Subject	F ratio	df	Significance Level
CJ-5	17.065	2/62	$p = 1.2 \times 10^{-6}$ *
CJ-7	17.980	2/68	$p = 5.4 \times 10^{-7}$ *
CJ-8	6.612	2/49	$p = 2.9 \times 10^{-3}$ *
CJ-11	3.732	2/84	$p = .028$ *
JFE-6	6.356	2/70	$p = 2.9 \times 10^{-3}$ *

*significant ($p < .05$)

Table 3

Results of significance test for LH and PAG slopes of recovery functions

Subject	LH Slope	PAG Slope	df	t Score	Significance Level
CJ-5	1.3061	0.6009	62	2.9119	$p = .002$ *
CJ-7	1.2196	0.5684	68	4.3793	$p = 2.1 \times 10^{-5}$ *
CJ-8	0.7482	0.5786	49	1.2003	$p = .118$
CJ-11	0.4207	0.4942	84	0.7517	$p = .227$
JFE-6	0.7727	0.2031	70	2.7584	$p = .004$ *

*significant ($p \leq .05$, 1-tail)

subjects (Figure 7b, d, j). The results for these subjects clearly show that the LH slope is steeper than the PAG slope, suggesting that, at least in these subjects, the rate of recovery from refractoriness in LH reward neurons is more rapid than that in the PAG. Let us now examine the results of the remaining subjects.

Since the t test for slope was not significant for subject CJ-8 (Figure 7f), the difference in the LH and PAG curves for this subject must be due to intercept differences or to a combination of small slope and intercept differences. The LH curve rises between 0.4 and 1.5 msec while the PAG curve rises between 0.6 and 2.0 msec. The curves appear to be roughly parallel at the short C-T intervals and begin to diverge towards the longer C-T intervals. Although there is not much difference in the rate of recovery in the LH and PAG curves for subject CJ-8, all points on the PAG curve fall below the corresponding points on the LH curve. The recovery for the PAG curve appears to be shifted to the right, i.e., delayed.

The interpretation of the regression analysis is more complex when the recovery curves are highly nonlinear. In such cases, the linear regression line is a poorer representation of the overall shape of the curve and an analysis of local portions of the curve is worthwhile. In animal CJ-11 (Figure 7h), no significant slope

difference was found. Nevertheless, if one looks closely at the LH curve, it can be seen that 75% of recovery occurs by a C-T interval of 1.2 msec. The remaining 25% recovers between 2.0 and 2.5 msec, perhaps due to the influence of a subpopulation of reward-relevant neurons with long refractory periods. Most LH refractory period curves level off after 1.2 msec (Yeomans, 1975). Given that most of the recovery in CJ-11's results occurred by this time, it seemed worthwhile to inquire whether the slope of this early component of recovery in the LH differed from the slope of the smoother, more continuous curve for the PAG. This time, the points contributing to the later stages of recovery in the LH (C-T intervals greater than 1.2 msec) were omitted from the analysis. The asymptote test was repeated to verify that the LH curve approached a local plateau of a C-T interval of 1.2 msec. (The asymptote results for all subjects' data are reported in the next section.) Since the asymptote had been earlier estimated to occur at 2.5 msec, the second asymptote test was conducted from the next smallest C-T interval (2.0 msec). Therefore, the second analysis of CJ-11's data dealt with a comparison of the regression lines based on C-T intervals of 0.4 to 1.2 msec in the LH and C-T intervals of 0.8 to 2.5 msec in the PAG. The F

test confirmed that the data could still be represented by two lines ($F = 8.719$; $df = 2/66$; $p = 4.4 \times 10^{-4}$). The difference between the slopes of these two lines was significant ($t = 2.495$; $df = 66$; $p = .008$), suggesting that initially the LH substrate recovers more rapidly than the PAG substrate. The refractory period curves in this subject converge at 2.0 msec and the remaining 25% recovery occurs at roughly the same rate in both placements.

Major nonlinearities are also evident in CJ-7's data (Figure 7d). Although a highly significant slope difference was found, the PAG curve for this subject is largely recovered (70%) at 1.2 msec. For this reason, the slopes were once again compared, considering only the recovery between 0.4 and 1.2 msec in the PAG. The asymptote test confirmed the C-T interval of 1.2 msec to correspond to an asymptote. A significant F ratio was obtained nonetheless ($F = 4.564$; $df = 2/56$, $p = .015$), implying that over this range of C-T intervals, PAG recovery is delayed when compared to the LH. No significant slope difference was found ($t = 1.430$, $df = 56$, $p = .079$). Since the intercept values are fairly similar, and no significant difference in slope was obtained, perhaps the combination of small differences in both intercept and slope contributed to the

significant F ratio.

b. Across-placement comparison of asymptote values. As defined by the asymptote test, the PAG curves reach an asymptote between C-T intervals of 2.0 msec (Figure 7a, c, e) and 5.0 msec (Figure 7i). These PAG values are comparable to the results of Experiment 1 and are presented in Table 4. For purposes of comparison, the asymptote values obtained in Experiment I and the LH values from this experiment are included in this table.

There was no significant difference between the PAG mean asymptote values obtained in the two experiments ($t = 0.1176$; $df = 8$; $p = .455$). Thus this finding appears to be robust; recovery from refractoriness at the PAG site is relatively slow in both experiments 1 and 2. In contrast, the LH curves from Experiment 2 rise more quickly and level off between 1.2 msec (Figure a, c, i) and 2.5 msec (Figure 7g). This difference in the C-T interval at which the LH and PAG curves approach a plateau was significant ($t = 1.864$; $df = 8$; $p = .05$). The across-subject comparison is consistent with the within-subject test which showed that the LH and PAG substrates are represented by different refractory period functions. In addition, the LH results from Experiment 2 are in good agreement with data from another laboratory (Yeomans, 1975).

Table 4

C-T interval (msec) corresponding to asymptote at each stimulation site

Subject	PAG	LH
	Experiment 1	
CJ-1	2.0	
CJ-4	5.0	
CJ-13	2.5	
CJ-14	1.5	
JFE-7	2.0	
	Experiment 2	
CJ-5	2.0	1.2
CJ-7	2.0	1.2
CJ-8	2.0	1.5
CJ-11	2.5	2.5
JFE-6	5.0	1.2

These findings suggest that, in general, the reward-relevant neurons activated by PAG stimulation are more heterogeneous than MFB reward neurons. While only one of the MFB curves (Figure 7g) deviated from the pattern generally observed at that placement (Schenk et al., 1980; Shizgal et al., 1979, 1980; Yeomans, 1975), there were large differences across subjects in the PAG refractory period profiles.

The principal findings can be summarized as follows: The results of the F test for each subject indicate that the LH and PAG reward substrates can be differentiated on the basis of refractory period. There are clear overall slope differences in the data from subjects CJ-5 and JFE-6, with the LH curve recovering more rapidly than the PAG curve; one subject (CJ-8) shows similar rates of recovery in the LH and PAG, although the recovery curve at the PAG site is delayed. For the remaining two subjects, the LH and PAG curves are similar over some C-T intervals and different over others. The C-T intervals at which asymptotic E values are reached are significantly longer for the PAG curves than for the LH curves. In no case did a PAG curve begin to rise earlier, level off earlier, or rise significantly more rapidly than an LH curve. Only one of the 34 PAG E values exceeds the corresponding LH

value.

c. Conclusions. The observation that recovery from refractoriness in the LH and PAG appears to begin at the same time suggests that some of the neurons at each site have similar, short refractory periods. The possibility of a direct axonal link is explored in the next experiment, in which a procedure is employed to test if there are reward-related fibers common to the LH and PAG.

The continued recovery seen in most PAG placements after the LH curves have levelled off may be due to the activation of subpopulations of neurons, not present in the LH, with long absolute refractory periods. Slope differences between the two sites would then be interpreted as differences in the mean and range of absolute refractory periods. Alternatively, PAG stimulation may also recruit a population in which relative refractory period contributions are more pronounced than in the LH. Finally, it is conceivable that the same reward-related neurons course through both stimulation fields. The longer recovery seen in the PAG would then reflect structural differences between LH and PAG axon segments. For example, axon diameter may be smaller in the PAG than in the LH, provided that the fibers are ascending. In

descending fibers such an arrangement should yield identical refractory periods for both sites since the action potentials triggered by the LH electrode would have to pass through the PAG "bottleneck".

The remaining two sections describe observations that are not directly related to the main findings of this study, but nevertheless merit acknowledgement. Since I believe that these observations have little bearing on the interpretation of the refractory period results, they have been included in a separate section.

II. Further Observations on the Untransformed Data. At C-T intervals where all behaviorally relevant neurons have recovered from refractoriness, an E value of 1.0 is predicted because double-pulse stimulation should be twice as effective as single-pulse stimulation. E values in the LH did not consistently reach a maximum value of 1.0. The lowest maximum E value obtained was .70 in subject CJ-8 (Figure 7e). This finding is congruent with other LH data previously reported (Yeomans, 1975). At the longest C-T intervals tested (20.0 and 25.0 msec), a slight increase was seen at some LH placements (Figure 7a, c), but these results were not uniform across subjects.

At all PAG placements, the refractory period curves did attain a plateau of 1.0 and in a few

subjects, overshot this value (Figure 7c, i). The importance of these overshoots is not clear due to the large variability of many of the relevant points.

Local potential summation contributions are evident at both sites at short C-T intervals. The magnitude of this effect ranges across subjects from .28 to .56 in the LH and .49 to 1.20 in the PAG. Earlier it was argued that the spatial distribution of behaviorally relevant neurons will affect the magnitude of the local potential summation effect. The median value found in the LH was .35 (animal CJ-5) and .80 (animal JFE-6) in the PAG, suggesting that relative to the effective stimulation fields, the proportion of distant neurons was greater at the PAG placements than at the LH placements.

III. An Incidental Observation. Another distinction between performance for LH and PAG stimulation concerns the long-term stability of the required number. Animals with electrodes in the PAG, but not the MFB often show changes across days in the required number of pulses in the single-pulse condition. These changes may occur in either direction. Within--session shifts at the two placements were rarely observed but have not been rigorously compared.

F tests for homogeneity of variance were conducted

between the four to six sets of LH and PAG single-pulse required-number values for each animal and are presented in Table 5. (These data are drawn from both Experiments 2 and 3.)

Results were significant in four out of five animals indicating greater variability in the required-number values from the PAG than from the LH.

The refractory period estimates reported here appear to be unaffected by these shifts. The shape of the T-pulse effectiveness versus C-T interval curves usually remains stable despite concurrent shifts in the required number. In the refractory period tests, a new single-pulse required number value is established at the start of each testing session and generally remains stable over the entire four-hour session. It is to this required number that each double-pulse required number from that session is compared. Thus, across-session shifts in the single-pulse required number do not affect within-session scaling.

A possible explanation of this shift concerns the location of the PAG electrodes. The procedure used for implanting electrodes in these studies was such that the electrode was driven through the aqueduct in order to reach the target placement. Damage to the ventral portion of the aqueduct wall may have allowed cerebral

Table 5

Comparison between LH and PAG required number of single pulses

Subject	LH	PAG	F Ratio	df	Significance Level
CJ-5	30.6±0.59	29.3±0.57	1.09	35/35	$p = .400$
CJ-7	34.7±0.78	51.9±1.37	3.04	35/35	$p = 7.2 \times 10^{-4}$ *
CJ-8	56.1±0.38	36.6±0.96	6.78	29/27	$p = 1.7 \times 10^{-6}$ *
CJ-11	18.2±0.35	18.1±0.83	6.14	29/27	$p = 4.6 \times 10^{-6}$ *
JFE-6	54.2±0.97	49.9±1.62	2.60	32/30	$p = .005$ *

*significant ($p \leq .05$)

spinal fluid (CSF) to leak along the electrode scar. It is possible then, that some of the stimulation current was shunted through the low resistive CSF (Ranck, 1975). This would increase the number of pulses required to maintain self-stimulation. A lower required number might result if neurogliosis formed over time at the injured aqueduct wall, allowing less CSF seepage. Bidirectional shifts in the required number may have been due to changes over time in the structure of the aqueduct wall. The seal between the electrode and wall may have been unstable, resulting in more or less CSF seepage from day to day.

If these factors do, in fact, account for the shift in the required number at PAG sites, the problem could be circumvented by implanting electrodes aimed at structures below the aqueduct at an angle of at least 30° off the midline, thus avoiding the aqueduct. During stimulation, continuous monitoring of the electrode/tissue interface impedence is also suggested, in order to detect changes in the resistivity of the surrounding tissue.

EXPERIMENT 3

The results of the previous experiments suggest that some of the reward-relevant neurons activated by LH and PAG stimulation begin to recover from refractoriness at roughly the same time. The aim of this third study was to determine if such neurons directly link the LH and PAG. The collision technique was used to test this hypothesis. The technique is based on the conduction failure caused by the collisions of orthodromic and antidromic action potentials. Collision-like effects have been previously demonstrated from rewarding stimulation of two MFB sites (Shizgal et al., 1980).

Concurrent stimulation through two electrodes might produce several effects. If the two electrodes are located in the same axon bundle, collision block is predicted at C-T intervals equal to or less than the sum of the conduction time and refractory period. Given that axons can conduct in two directions, the collision block should be the same regardless of which electrode receives the C pulse and which receives the T pulse. The rewarding effects of stimulation at the two sites should summate when the C-T interval exceeds this critical value. If the two electrodes are located in different but converging axon bundles, no collision

effects should be observed. Since each axon is stimulated by only one electrode, only summation effects are expected.

The presence or absence of collision block is inferred by determining whether the number of pulse pairs required to maintain behavior remains constant when the C-T interval is varied (no block), or decreases abruptly when the C-T interval exceeds some critical value (block). The decrease in the required number is presumed to reflect an increase in the effectiveness of the pulse pairs due to the recovery from collision block. Effectiveness is scaled in a manner analogous to that used in Experiments 1 and 2.

Method

Subjects

The same subjects and current intensities were used as in Experiment 2.

Procedure

In the first two experiments, pulse pairs were delivered to the same electrode; in this case, pulse pairs were applied in alternating fashion to the PAG and the LH. The anterior-posterior test consisted of delivering the C pulse to the LH and the T pulse to the PAG; in the posterior-anterior test, the C pulse was

delivered to the PAG, and the T pulse to the LH. The C-T interval was varied from 0.15 to 25.0 msec. The order of presentation of C-T intervals was randomized. Each test was run 4-6 times per animal, and interdigitated with the refractory period tests in Experiment 2.

Data Format

The effectiveness of paired-pulse stimulation was assessed using the following formula:

$$E = \frac{RN_{SP_L}}{RN_{C-T}} - 1 \div \frac{RN_{SP_L}}{RN_{SP_H}}$$

where E = effectiveness of paired-pulse stimulation,

RN_{SP_L} = lower of the two single-electrode required numbers,

RN_{C-T} = required number of pulse pairs,

RN_{SP_H} = higher of the two single-electrode required number values.

This formula is equivalent to Yeomans' (1979) effectiveness statistic for pulses of unequal amplitude. The current intensities for each electrode within a given subject were selected so that the single-pulse required numbers were as similar as possible. Thus, in the ideal case, the right-hand term of the above equation would equal 1.0.

and the formula would reduce to that used in the first two experiments. However, as discussed earlier, the single-pulse required numbers for the PAG (see Table 5) tended to shift over time. The use of the present formula compensated for the resultant imbalances in the LH and PAG single-pulse required numbers.

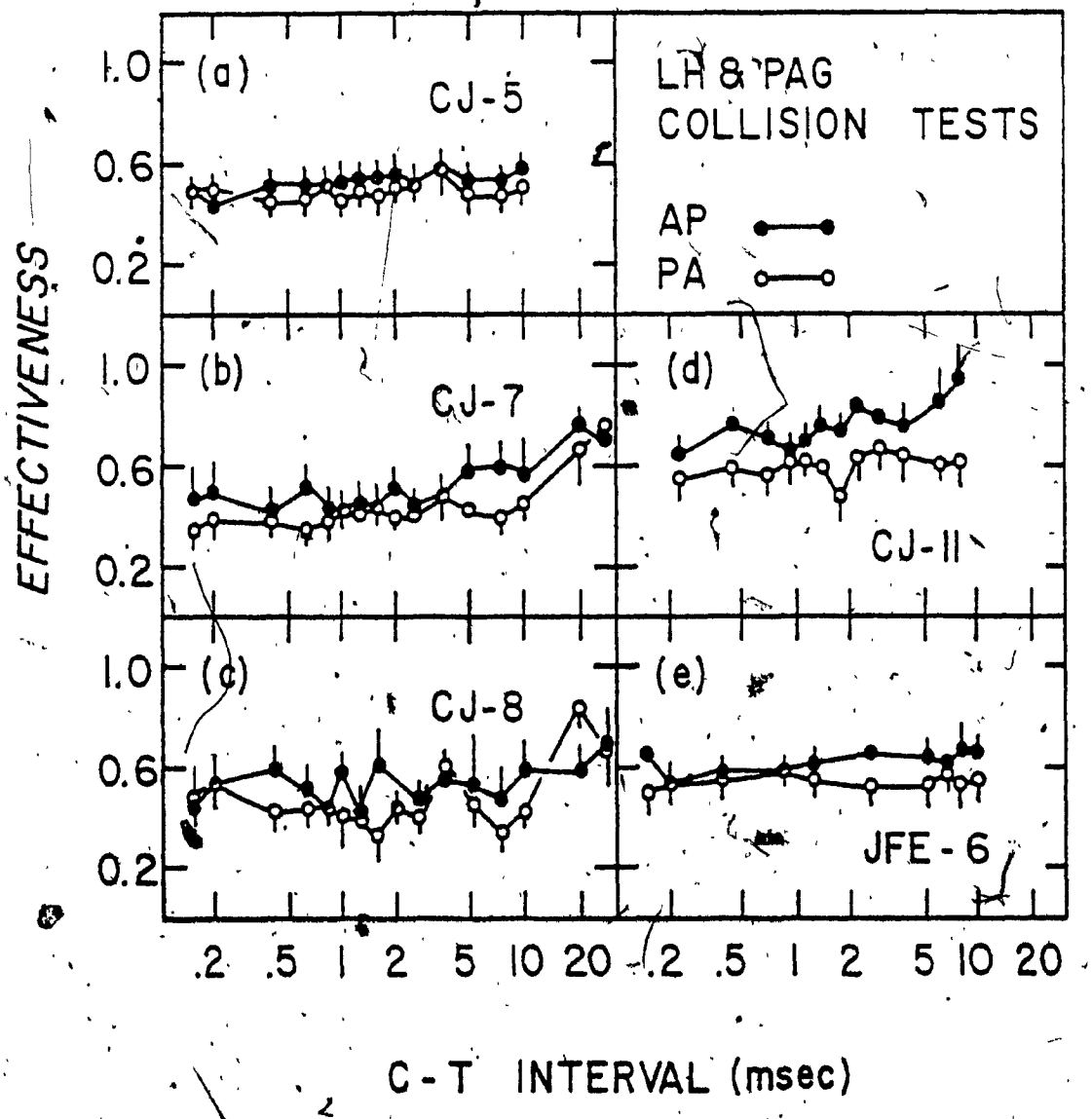
Results

Figure 8 depicts the results of the anterior--posterior (AP) and posterior-anterior (PA) tests for each subject. The data from individual subjects were analyzed using a 2-way ANOVA with repeated measures. The entire source table for each subject is presented in Appendix II.

In each subject, the main effect of the AP/PA tests was not significant. No significant interaction between C-T interval and AP/PA conditions was obtained in any subject. The main effect of C-T interval was significant in three out of five subjects (Figure 8b, c, d). Tukey post-hoc tests were performed to determine the source(s) of this main effect. All possible pairwise comparisons were made. For animal CJ-11 (Figure 8d), E values for C-T intervals of 0.2, 0.8 and 1.5 msec differed significantly from the E value for 7.5 msec. The 0.2 vs 2.5 msec comparison was also significant. E values for

FIGURE 8

The results of the collision tests from each subject. The effectiveness of paired-pulse stimulation is plotted as a function of C-T interval. In the AP condition (filled circles) the C pulse was delivered to the LH and the T pulse was delivered to the PAG; in the PA condition (open circles) the order of presentation of pulses was reversed with the C pulse applied to the PAG and the T pulse applied to the LH. The alphanumeric appearing in each box identifies the subject.


LH & PAG
COLLISION TESTS
AP
PA
(a)
CJ-5
(b)
CJ-7
(c)
CJ-8
(d)
CJ-11
(e)
JFE-6
EFFECTIVENESS
1.0
0.6
0.2
.2 .5 1 2 5 10 20
C-T INTERVAL (msec)

higher than all other C-T intervals in the case of subject CJ-8 (Figure 8c), with the following exceptions; 25 vs 0.2, 25 vs 0.4, 20 and 25 vs 3.5, and 20 vs 25. For subject CJ-7 (Figure 8b), E values for 20 and 25 msec, although not different from each other were significantly higher than all remaining C-T intervals.

In summary, two of the five sets of curves are flat. The remaining three are "bumpy" and appear to rise at C-T intervals of 20 and 25 msec.

Discussion

The major findings of this study can be described as follows: the curves from two subjects (CJ-5 and JFE-6) are clearly flat, suggesting that no collision block occurred. There was no effect of C-T interval, AP/PA tests, or interaction of these two factors. These results are consistent with the view that the two electrodes stimulate different reward-related fibers.

In the remaining three subjects, a significant main effect of C-T interval was found, with no main effect of AP/PA tests or interaction. It will be argued below that these data as well fail to provide evidence for a collision block.

2.5 and 7.5 msec) contributed to the significant main effect. The collision effects previously demonstrated (Shizgal et al., 1980) were reflected in step-like E vs C-T curves with flat regions at short and long pulse-pair intervals separated by an abrupt rise. The results from subject CJ-11 do not show this pattern. The erratic fluctuation in the E values as C-T interval is increased is likely due to error variance, since no consistent pattern was observed from session to session.

For subject CJ-8 (Figure 8c), the main effect of C-T interval was mainly due to the increase in E at the longest pulse-pair intervals tested (20 and 25 msec). With a few exceptions, all shorter C-T intervals produced significantly lower E values than 20 and 25 msec. The curves from subject CJ-7 are very similar to the flat sets of curves from CJ-5 and JFE-6 except for the points at 20 and 25 msec which were not tested in the latter two subjects.

If one examines the refractory period data from subjects CJ-7 and CJ-8 (Figure 7c, e), an increase in E is seen at C-T intervals of 20 and 25 msec. The LH refractory period curve from subject CJ-5 exhibits this phenomenon while it is seen more strongly in the PAG curve from subject CJ-8. Since this effect was also seen in the single-electrode data from these subjects,

there is no reason to attribute it to the effects of two-electrode stimulation. Perhaps, a synaptic event beyond the point of convergence is responsible for the increased output at the long C-T intervals. If so, it is not surprising that this result is obtained from double-pulse stimulation of one site, or of both sites concurrently. It is not clear, however, why the same phenomenon was not seen in subject JFE-6. Although this subject's PAG refractory period curve (Figure 7i) rises between pulse-pair intervals of 5 and 10 msec, the collision curve (Figure 8e) remained flat at the same C-T intervals. Nonetheless, in all cases where increases in E values at long C-T intervals were observed in two-electrode tests, increases in E at the same C-T intervals were observed in single-electrode data.

The results of the collision tests for each subject in Figure 8 are consistent with the notion that the substrates for self-stimulation in the LH and PAG are anatomically distinct. However, it is possible that an examination of more subjects would reveal some overlap in neurons that are directly stimulated by LH and PAG electrodes. The suggestion that the LH and PAG do not share any of the same reward fibers must be made tentatively for the following reason.

When collision-like effects from concurrent

stimulation through two electrodes are observed, the most parsimonious interpretation is that there are fibers common to both stimulation fields. The failure to observe collision-like effects does not necessarily imply the contrary - that there are no fibers common to both stimulation fields.

There are two explanations that could account for the failure to observe collision-like effects: either a) the electrodes stimulate different but converging axon bundles, or b) the electrodes stimulate different fibers within the same axon bundle. If the second explanation is correct, then one would expect that raising the current intensity would increase the probability of obtaining collision effects. Shizgal et al. (1980) have used this strategy to recruit behaviorally relevant fibers common to both LH and ventral tegmental area stimulation fields. Such efforts to maximize the size of the stimulation fields were made in each animal in this study during pilot testing, and did not produce results different from those seen here. Nonetheless, it is possible that the two stimulation fields were so misaligned in the putative bundle that even the highest currents that were used (> 1 mA) could not stimulate the same reward-related neurons at both sites.

Although clear collision effects were not obtained

from any of the subjects in this study, summation of the rewarding effects from concurrent LH and PAG stimulation did occur. Double-pulse stimulation produced E values greater than 0 for all subjects. Furthermore, the magnitude of the summation effect was similar in each case. Thus, it appears that while the rewarding signals produced by LH and PAG stimulation do not propogate through the same axon bundle, concurrent stimulation at the two placements produces a behavioral effect that is greater than stimulation at either placement alone, suggesting a functional relationship of the reward substrates coursing through the LH and PAG.

General Discussion

The simplest explanation of the results of the three experiments is that LH and PAG stimulation activate different reward-relevant neurons with converging outputs. This interpretation takes into account the differences in the single-electrode refractory period estimates, the failure to observe clear collision-like effects from two-electrode stimulation, and the consistent level of summation across subjects.

The refractory period results from Experiments 1 and 2 suggest that a) LH and PAG stimulation recruit reward-relevant neurons with overlapping ranges of absolute refractory periods and/or b) PAG stimulation recruits neurons with longer relative refractory periods. The refractory period estimates obtained from these studies cannot distinguish between these two possibilities. However, Yeomans' (1979) unequal-pulse technique can dissociate the separate contributions of absolute and relative refractory periods and should be used for this purpose in a future study.

The results also lay the groundwork for future two electrode experiments. Despite the large across-animal variance in the PAG electrode placements in the anterior/posterior plane, all subjects showed longer

refractory periods at these placements than at MFB sites. The application of the collision test to electrodes scattered longitudinally along the PAG would determine the extent of this fiber tract. Conduction velocities could then be generated and compared to those obtained from MFB placements.

The experiments described in this thesis used a psychophysical approach in order to infer from behavior, neurophysiological properties of the PAG reward substrate. The MFB has been well studied in this manner and recently this approach has been extended to the examination of reward neurons in the frontal cortex (Schenk et al., 1980). Together with electrophysiological and autoradiographic mapping data, the results of these behavioral studies may well enable us to identify, describe, and understand the circuit(s) subserving brain-stimulation reward.

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Appendix I

Source Tables of ANOVAs on Regression Analyses

a) Refractory period results from subject CJ-5

LH (Transformed E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	4.094	1
Error	1.245	29
Total	5.339	30

PAG (Transformed E values for C-T intervals from 0.6 to 2.0 msec)

Source of Variation	SS	df
Regression	2.791	1
Error	5.063	35
Total	7.854	36

LH and PAG (Pooled transformed data)

Source of Variation	SS	df
Regression	3.761	1
Error	9.779	65
Total	13.540	66

Appendix I (Cont'd)

b) Refractory period results from subject CJ-7

LH (Transformed E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	3.570	1
Error	1.386	29
Total	4.956	30

PAG (Transformed E values for C-T intervals from 0.4 to 2.0 msec)

Source of Variation	SS	df
Regression	3.518	1
Error	1.570	41
Total	5.088	42

LH and PAG (Pooled transformed data)

Source of Variation	SS	df
Regression	5.534	1
Error	4.520	71
Total	10.054	72

Appendix I (Cont'd)

c) Refractory period results from subject CJ-7

LH (Transformed E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	3.570	1
Error	1.386	29
Total	4.956	30

PAG (Transformed E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	2.237	1
Error	0.750	29
Total	2.987	30

LH and PAG (Pooled transformed data based on LH E values for C-T intervals from 0.4 to 1.2 msec and PAG E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	5.776	1
Error	2.484	59
Total	8.260	60

Appendix I (Cont'd)

d) Refractory period results from subject CJ-8

LH (Transformed E values for C-T intervals from 0.4 to 1.5 msec)

Source of Variation	SS	df
Regression	1.810	1
Error	0.883	23
Total	2.693	24

PAG (Transformed E values for C-T intervals from 0.6 to 2.0 msec)

Source of Variation	SS	df
Regression	2.157	1
Error	1.223	28
Total	3.380	29

LH and PAG (Pooled trasnformed data)

Source of Variation	SS	df
Regression	3.415	1
Error	2.674	52
Total	6.089	53

Appendix I (Cont'd)

e) Refractory period results from subject CJ-11

LH (Transformed E values for C-T intervals from 0.4 to 2.5 msec)

Source of Variation	SS	df
Regression	3.811	1
Error	2.873	46
Total	6.684	47

PAG (Transformed E values for C-T intervals from 0.8 to 2.5 msec)

Source of Variation	SS	df
Regression	3.493	1
Error	4.043	40
Total	7.536	41

LH and PAG (pooled transformed data)

Source of Variation	SS	df
Regression	6.808	1
Error	7.530	87
Total	14.338	88

Appendix I (Cont'd)

f) Refractory period results from subject CJ-11

LH (Transformed E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	2.295	1
Error	1.157	28
Total	3.452	29

PAG (Transformed E values for C-T intervals from 0.8 to 2.5 msec)

Source of Variation	SS	df
Regression	3.493	1
Error	4.043	40
Total	7.536	41

LH and PAG (Pooled transformed data based on LH E values for C-T intervals from 0.4 to 1.2 msec and PAG PAG E values for C-T intervals from 0.8 to 2.5 msec)

Source of Variation	SS	df
Regression	4.749	1
Error	6.574	69
Total	11.323	70

Appendix I (Cont'd)

g) Refractory period results from subject JFE-6

LH (Transformed E values for C-T intervals from 0.4 to 1.2 msec)

Source of Variation	SS	df
Regression	1.772	1
Error	5.035	29
Total	6.807	30

PAG (Trasnformed E values for C-T intervals from 0.6 to 5.0 msec)

Source of Variation	SS	df
Regression	3.435	1
Error	3.518	43
Total	6.953	44

LH and PAG (Pooled transformed data)

Source of Variation	SS	df
Regression	3.612	1
Error	10.106	73
Total	13.768	74

Appendix II

Source Tables of ANOVA's

a) ANOVA on results of collision tests from subject CJ-5

Source	df	MS	F	p
C-T Interval (C-T)	13	50.28	1.379	.19
Error (C-T x Repeated Measures (RM))	65	36.47		
Order of Presentation of Pulses (AP/PA)	1	179.18	0.158	.71
Error (AP/PA x RM)	5	1135.01		
C-T x AP/PA	13	34.53	0.948	.51
Error (C-T x AP/PA x RM)	65	36.45		

*significant ($p \leq .05$)

b) ANOVA on results of collision tests from subject CJ-7

Source	df	MS	F	p
C-T Interval (C-T)	15	1129.84	6.812	5.3×10^{-9}*
Error (C-T x Repeated Measures (RM))	75	165.86		
Order of Presentation of Pulses (AP/PA)	1	2610.75	0.628	.46
Error (AP/PA x RM)	5	4159.95		
C-T x AP/PA	15	107.91	0.726	.75
Error (C-T x AP/PA x RM)	75	148.60		

*significant ($p \leq .05$)

c) ANOVA on results of collision tests from subject CJ-8

Source	df	MS	F	p
C-T Interval (C-T)	15	802.39	4.267	2.6×10^{-5} *
Error (C-T x Repeated Measures (RM))	60	188.07		
Order of Presentation of Pulses (AP/PA)	1	305.26	0.171	.70
Error (AP/PA x RM)	4	1784.49		
C-T x AP/PA	15	382.38	1.295	.23
Error (C-T x AP/PA x RM)	60	295.31		

*significant ($p \leq .05$)

d) ANOVA on results of collision tests from subject CJ-11

Source	df	MS	F	p
C-T Interval (C-T)	11	277.46	2.618	.02*
Error (C-T x Repeated Measures (RM))	33	105.98		
Order of Presentation of Pulses (AP/PA)	1	7385.04	4.605	.12
Error (AP/PA x RM)	3	1603.71		
C-T x AP/PA	11	120.13	0.980	.48
Error (C-T x AP/PA x RM)	33	122.62		

*significant ($p \leq .05$)