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DOUBLE-PULSE STIMULATION USED TO PROBE THE ANATOMY OF THE SELF-STIMULATION SYSTEM OF THE RAT

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

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BY

DWIGHT CHARLES GERMAN

Oklahoma City, Oklahoma

1972

DOUBLE-PULSE STIMULATION USED TO PROBE THE

ANATOMY OF THE SELF-STIMULATION

SYSTEM OF THE RAT

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DOUBLE-PULSE STIMULATION USED TO PROBE THE ANATOMY OF THE SELF-STIMULATION SYSTEM OF THE RAT

CHAPTER I

INTRODUCTION

The double-pulse stimulation technique has been used for some time as a neurophysiological probe to explore the poststimulation excitatory cycle of axons and synapses in the peripheral nervous system (Helmholtz, 1854; Sherrington, 1906). This technique utilizes a pair of liminal pulses, one being the conditioning or C pulse whose onset is followed at a parametrically varied interval by another, the test or T pulse. The double-pulse or C-T technique has been used to demonstrate such axonal phenomena as neural refractoriness (Erlanger and Gasser, 1937; Grundfest, 1940; Hursh, 1939) and latent addition (Lucas, 1910; Erlanger and Gasser, 1937). At the synaptic level, this technique has been used to demonstrate such things as inhibitory effects (Eccles, 1964), and homosynaptic (Eccles, 1946; Li and Chou, 1962; Hubbard and Schmidt, 1963) and heterosynaptic temporal summation (Lloyd, 1946;

Albe-Fessard and Chagas, 1954; Fadiga and Brookhart, 1962).

The above axonal and synaptic phenomena have generally been demonstrated using electrophysiological response measures on acute preparations of single neurons or fiber systems. For example, if one were measuring synaptic inhibition, the C pulse could be delivered to an inhibitory presynaptic neuron, and at a parametrically varied interval the T pulse would be delivered to an excitatory presynaptic neuron. The response measured as a function of the C-T interval is usually the electrophysiological behavior of the postsynaptic neurons converged upon by the inhibitory and excitatory fibers. The stimulation-response function thus determined indicates that the shorter the C-T interval, the less likely it is that the T pulse will fire the postsynaptic neuron since the inhibitory effect will have had less time to decay. Alternatively, if axonal conduction phenomena are being analyzed, electrical responsiveness is recorded from the same neuron or fiber system that is being electrically stimulated.

Overt behavior can also be used, in place of electrophysiological measures, to analyze neurophysiological properties. Various operant behaviors, such as instrumental escape (Kestenbaum et al., 1970), self-stimulation (Deutsch, 1964; Rolls, 1971a; Smith and Coons 1970; Terry, 1971; Ungerleider and Coons, 1970) and runway performance (Gallistel et al., 1969), have been shown to vary systematically

when animals were stimulated intracranially with trains of parametrically varied C-T pulse pairs. Figure 1 illustrates the characteristics of the C-T pulse train. Such stimulation parameters as pulse duration and intensity, C-C interval, and train duration are typically held constant and the overt behavioral change is recorded as a function of changes in the C-T interval. Such overt behaviors have proven to be reliable measures of underlying central neural processes and they have demonstrated all of the same properties observed classically by means of direct electrophysiological measures (i.e., latent addition, refractory periods, temporal summation, and inhibitory effects).

Numerous species have been shown to learn various responses which are positively reinforced by electrical stimulation of diencephalic structures (fish, Boyd and Gardner, 1962; chick, Andrews, 1967; rabbit, Bruner, 1967; cat, Wilkenson and Peele, 1963; dog, Stark and Boyd, 1963; goat, Persson, 1962; monkey, Bursten and Delgado, 1958; dolphin, Lilly and Miller, 1962; rat, Olds and Milner, 1954; human, Bishop et al., 1963). The medial forebrain bundle (MFB) and the structures it interconnects most frequently produce the strongest positive reinforcing effects (Olds and Olds, 1962, 1963; Battig, 1969). Self-stimulation of MFB-related structures is the behavior to be learned in the present experiment, and it involves an animal learning to press a lever to electrically stimulate his own

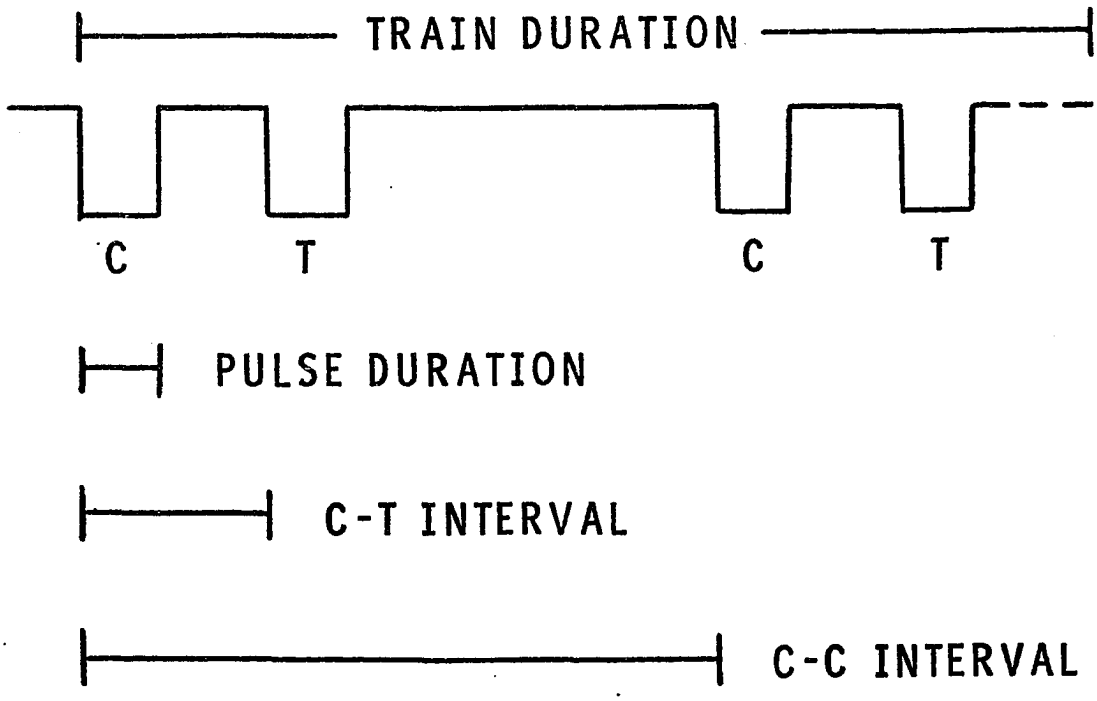


Figure 1. Schematic representation of a train of negative-going C-T pulse pairs. The pulse duration, C-T interval, and C-C interval characteristics of the train are illustrated.

brain. While various electrode placements result in different rates of self-stimulation behavior (Olds and Olds, 1962, 1963; Battig, 1969), at any particular site the effectiveness is related to such stimulation parameters as pulse frequency, train duration, and stimulus intensity (Kessey, 1962; McIntire and Wright, 1965; Su et al., 1966; Deutsch, 1964; Wetzel, 1971). Since self-stimulation rate is a function of the stimulation parameters, it is possible to use this behavior for studying the neurophysiological properties of self-stimulation areas of the brain and their possible relation to other neuronal structures which may mediate this behavior.

Heterosynaptic temporal summation has been demonstrated in the MFB self-stimulation system by administering C pulses to the MFB on one side of the brain and T pulses to a homologous site on the opposite side of the brain (Ungerleider and Coons, 1970). As the C-T interval was decreased, self-stimulation performance increased in a linear fashion. Such data indicated that MFB impulses from opposite sides of the brain converge upon a common neuronal pool, since heterosynaptic temporal summation effects could not be demonstrated unless neuronal convergence existed. Since the MFB is known to converge at sites upstream as well as downstream from the stimulating electrodes, however, it was not possible to differentiate between the possible convergence sites. In the present experiment rats were bilaterally implanted with MFB self-stimulation elec-

trodes. However, rather than placing the electrodes in homologous MFB sites on opposite sides of the brain, the electrodes were implanted in MFB-related anterior and contralateral posterior self-stimulation areas. With each lever press delivering a train of pulse pairs through a single electrode (stimulation parameters as in Figure 1), it was postulated that the rate of self-stimulation, as a function of changes in the C-T interval, would reveal the time constants characteristic of such properties as latent addition, neural refractoriness, and temporal summation for both anterior and posterior self-stimulation areas. And secondly, it was postulated that by administering C pulses through the anterior electrode and parametrically delayed T pulses through the contralateral posterior electrode (and vice versa), predictably different stimulation-response functions would result depending upon whether self-stimulation impulses ascend or descend within the complex MFB system.

The remainder of the Introduction is divided into four parts. Section A contains a discussion of prior studies in which the C-T technique was used to reveal stimulation-response relationships between the electrical stimulation of neurons and the electrical responses recorded (a) from the axons of the same neurons and (b) from the postsynaptic neurons onto which the stimulated neurons project. Section B discusses the ways in which the same stimulation-response functions have been revealed using the C-T technique with

overt behavior as the dependent variable instead of the electrical responses of the neural tissue. Section C is concerned with self-stimulation behavior and the anatomical interconnections within the MFB system. Finally, Section D describes the general strategy of the present experiment and makes predictions concerning the different stimulation-response functions that might be found depending upon whether the ascending or descending MFB fibers are functional in self-stimulation behavior.

A. Double-Pulse Stimulation with Electrical Response Measures

Helmholtz (1854), and later Sherrington (1906), were among the first to use the double-pulse technique (C-T technique) to study the changes in neural excitability which follow the generation of an action potential. In the classical Sherringtonian application of the technique, an electrical measure of neural excitation is taken as a function of the C-T stimulation interval. Any number of response measures may be taken, such as: (a) the level of current required to maintain a predetermined rate or probability of firing or magnitude of response; (b) the rate of firing or probability of firing, in the case of single neurons; (c) the magnitude of the gross evoked response in the case of multifibered nerve; and (d) the intracellular recorded magnitude of the change in membrane potential, in studies of certain synaptic processes. Since Helmholtz first introduced this technique, it has been used extensively to explore the various aspects

of both axonal conduction and synaptic transmission.

The study of axonal conduction is accomplished by applying both the C and T pulses to the same neuron or nerve fiber from which the excitability changes are measured. The axonal phenomenon of neural refractoriness has been demonstrated in this manner (Erlanger and Gasser, 1937; Grundfest, 1940; Hursh, 1939). The absolute refractory period represents an inexcitability of a nerve fiber during and for a short period of time after the action potential. Absolute refractory period values range from 0.4 msec. to 2.0 msec. for mammalian fibers depending primarily upon fiber diameter (0.4 to 1.0 msec. for A type fibers; 1.2 msec. for B type fibers; and 2.0 msec. for C type fibers; Grundfest, 1940). Thus, at C-T intervals of this size or smaller, the T pulse, no matter how intense, is unable to induce firing. During the succeeding relative refractory period (i.e., at slightly longer C-T intervals) the probability that the T pulse will induce firing increases as the excitation threshold returns to its resting level.

The C-T technique has also been used to investigate the axonal phenomenon of subliminal summation, or as it was sometimes called, latent addition (Lucas, 1910; Erlanger and Gasser, 1937). This property was believed to be the result of the neuron's ability to store and summate charge capacitively on its membrane for up to 0.2 msec. With the advent of intracellular recording techniques,

however, the term 'latent addition' was abandoned since it tended to oversimplify the various excitability changes which result from neural stimulation. Today this property is subsumed under the heading of "cable properties" which takes into account not only membrane capacitance, but also membrane and plasma resistance as well (Ruch et al., 1961). Neurophysiologically, this effect manifests itself in the combined ability of subliminal C and T pulses to fire the neuron at C-T intervals shorter than 0.2 msec. The term latent addition continues to be used in the psychological literature only as a descriptive label for the behavioral changes at C-T intervals less than 0.2 msec.

The stimulation-response relationships described above hold for both the single fiber and the multifiber nerve preparation. In the latter case, the C-T technique can be used to differentiate the various fiber groups which constitute the nerve. Because larger diameter fibers recover from refractory periods sooner, as the C-T interval is gradually increased, sudden increases in firing level will mark the end of refractory periods of different fiber groups. Erlanger and Gasser (1937) used this technique to separate alpha and beta subgroups of mammalian A fibers. In this situation, however, it is imperative that the current levels of the C and T pulses be at maximal strength, since at less than maximal strength the smaller sized fibers in the mixed nerve will not be fired due to their higher

spike threshold.

Excitability changes have been observed mainly in isolated peripheral nerve preparations, as mentioned above. However, numerous studies have demonstrated various excitability dynamics of intact human nerve preparations. For example, Gilliatt and Willison (1962) have demonstrated excitability changes in the intact human median nerve by applying C-T pulse pairs at the wrist and recording the resulting action potentials farther along the nerve at the elbow. This nerve was shown to have an absolute refractory period of 0.6 msec.

A slight alteration in the C-T technique allows an examination of synaptic stimulation-response functions. Homosynaptic temporal summation can be demonstrated when both C and T pulses are delivered to a single presynaptic neuron, and the electrical response is recorded from the postsynaptic neuron. This phenomenon is evidenced by the fact that the smaller the C-T interval, the more effective is the T pulse in generating a response, except for those C-T intervals within the refractory period range of the stimulated presynaptic fibers (Eccles, 1946; Li and Chou, 1962; Hubbard and Schmidt, 1963). This effect is due to the interaction of the neurotransmitter with the postsynaptic membrane such that the duration of the postsynaptic potential is a function of the transmitter inactivation rate and the amount of neurotransmitter released per pulse. When

C and T pulses are applied to different presynaptic neurons, and the response is recorded from the postsynaptic neurons onto which they converge, evidence for heterosynaptic temporal summation is obtained (Lloyd, 1946; Albe-Fessard and Chagas, 1954; Fadiga and Brookhart, 1962). This phenomenon differs from homosynaptic temporal summation mainly because the rise in response level with increasingly shorter C-T intervals is not limited by the refractory period of the stimulated presynaptic neuron. The duration of heterosynaptic temporal summation is also a function of the duration of the postsynaptic potential, as described above. Lloyd (1946) observed a 14.0 msec. temporal summation decay time in spinal reflexes, while Eccles (1946) found this interval to be 10.0 msec. for spinal motoneurons. Much longer temporal summation values were found for cortical pyramidal cells (Li and Chou, 1962) and thalamic neurons (Phillips, 1961) where excitatory postsynaptic potentials lasted for up to 80.0 msec.

Synaptic inhibition has also been studied with the C-T technique. In this case, the C pulse is delivered to an inhibitory neuron and the T pulse is delivered either (a) to the postsynaptic neuron onto which the inhibitory neuron projects, or (b) to an excitatory presynaptic neuron projecting to the same neuron as does the inhibitory presynaptic neuron. The time course of the inhibitory effect is traced by taking the electrical response of the postsynaptic neu-

ron as a function of the C-T interval. The more closely the T pulse follows the C pulse, the less effective it is in generating a response, evidently because more of the inhibitory neurotransmitter is still active at the synapse (Eccles, 1964). This type of inhibition is known as postsynaptic inhibition, and the duration of its effect includes 8.0 msec. for spinal motoneurons (Araki et al., 1960), 100.0 msec. for thalamic neurons (Purpura and Cohen, 1962), 100.0 to 200.0 msec. for cortical cells (Phillips, 1956), and over 200.0 msec. for hippocampal neurons (Kandel and Spencer, 1961).

The type of inhibition just described differs from the phenomenon known as presynaptic inhibition. This inhibitory effect is also characterized by decreased effectiveness of the T pulse at shorter C-T intervals, however, the time course is frequently longer than for postsynaptic inhibition. The depression of excitability, as measured in the spinal motoneuron, does not reach a maximum until about 20.0 msec. and lasts for over 200.0 msec., as opposed to 8.0 msec. for postsynaptic inhibition (Eccles et al., 1961). Presynaptic inhibition is thought to act on the axon terminals of presynaptic excitatory neurons in a way which decreases their ability to stimulate the postsynaptic neuron (Eccles, 1964).

To summarize, the following neural properties have been demonstrated using the C-T stimulation technique with electrical response measures: (a) latent addition; (b) refractory periods; (c) ho-

mosynaptic and heterosynaptic temporal summation; and (d) postsynaptic and presynaptic inhibition.

B. Double-Pulse Stimulation with Behavioral Response Measures

Many of the early experiments employing the C-T technique used behavioral measures for the study of stimulation-response relationships in axons and synapses. Since much of this work focused on the study of neuromuscular phenomena and spinal reflexes, however, the "behavior" often consisted of nothing more than muscle fiber contractions (Helmholtz, 1854; Sherrington, 1906). For example, Lloyd (1946) used the spinal reflex to study the time course of heterosynaptic temporal summation. A subliminal stimulus (C pulse) delivered to one branch of a biceps nerve was followed at a varied interval by a second stimulus (T pulse) delivered to the other branch of this nerve. The magnitude of the resulting reflex showed an exponential decay of temporal summation as the C-T interval was lengthened up to 14.0 msec.

More recently, higher order behaviors have been used to measure underlying neural processes. Because single pulse pairs will not elicit behavior from central motivational systems, however, the C-T technique required modification for use in these instances. Instead of a single pair of pulses, trains of equally spaced pulse pairs (i.e., C-C interval held constant) are employed with the stimulation parameters as represented in Figure 1. Such a modification of

the C-T technique has resulted in the self-stimulation behavioral demonstration of latent addition, neural refractoriness, homosynaptic and heterosynaptic temporal summation, and synaptic inhibition.

Deutsch (1964) was the first to use the C-T technique to behaviorally measure refractory periods of "self-stimulation fibers". Rats were trained to press a lever for positively reinforcing electrical stimulation of the brain consisting of trains of parametrically varied C-T pulse pairs delivered via chronically implanted electrodes. With a 10.0 msec. C-C interval, and depending upon the behavioral effect being measured (drive or reward), the rate of lever pressing was found to undergo a sudden increase after C-T intervals of 0.5 to 0.6 msec. and 0.8 to 1.1 msec. This was attributed to the recovery from refractoriness of two separate fiber groups having different axon diameters. In other words, at very short C-T intervals, the T pulses arrive during the refractory period of the C-stimulated fibers and thus do not influence behavior. As the C-T interval is lengthened, the T pulses eventually start arriving outside the refractory period and become effective in fiber excitation. This point is reflected at the behavioral level by the sudden increase in lever pressing. The duration of the initial period of low responding is thus a measure of the refractory period of the fibers activated by the stimulating electrode. Similar refractory periods (0.5 to 0.6 msec. for "reward" fibers, and 0.9 to 1.1 msec. for "drive" fibers) have

subsequently been found using a runway behavioral measure and C-T manipulated hypothalamic stimulation (Gallistel et al., 1969).

An analogous experiment in which self-stimulation was taken as the measure of poststimulation excitatory changes yielded very similar results and extended the above findings somewhat (Smith and Coons, 1970). The dependent variable in this study was the minimum current intensity required to maintain a fixed number of lever presses per minute, and the C-C interval was 400.0 msec. Evidence of latent addition was found at the 0.1 msec. C-T interval since at this interval the current intensity required to elicit lever pressing was less than that for either the control condition (where only C pulses were given in the train) or for C-T intervals within the refractory period range of the stimulated fibers. Absolute refractoriness occurred at C-T intervals up to 1.2 msec., and relative refractoriness from 1.2 to 5.0 msec. Finally, as the C-T interval was lengthened from 5.0 to 200.0 msec., a gradual increase in current was required to maintain criterion lever pressing. The greater current intensity required to elicit behavior at these longer C-T intervals was thought to reflect synaptic rather than axonal events, since recovery from refractoriness should be complete and both pulses should generate action potentials which are conducted to the synapse. Thus, the increase in self-stimulation threshold is probably due to the decreasing ability of the C and T pulses to summate temporally at the syn-

apse as they become separated by increasingly greater temporal intervals.

A methodological point seems to be in order here. The data of Deutsch (1964), Gallistel et al., (1969), and Smith and Coons (1970) all show evidence of latent addition which Rolls (1971a) believes casts some doubt on the validity of their refractory period findings. The higher the current level (within limits), it is argued, the greater is the probability that the C pulses will fire all of the fibers in the electrode vicinity and thus rule out the possibility that any subliminally depolarized fringe of axons will be able to briefly store this charge (Lucas, 1910) and be fired by the closely succeeding T pulses. It is believed that if the current is twice the self-stimulation threshold then latent addition should not appear at less than a 0.2 msec. C-T interval. It is further suggested that the presence of latent addition indicates that what appears to be the absolute refractory period is only the relative refractory period since a twice threshold stimulating current would have resulted in a shorter refractory period value. This argument is based on the assumed contribution of just-threshold fibers activated at the electrode fringe by a less than twice threshold stimulating current. Such a submaximal stimulus would result in fewer fibers firing at shorter C-T intervals than would be fired by the twice threshold stimulus since the relative refractory state of the peripheral fibers would be overcome by the supramaxi-

mal stimulus and thus add to the number of fibers activated to elicit the self-stimulation behavior. Therefore, at a given relative refractory period the submaximal stimulus should indicate absolute refractoriness, whereas the supramaximal stimulus should result in a higher level of self-stimulation performance and thus indicate the actual relative refractory state of all of the stimulated fibers. Equally susceptible to this criticism is the following C-T escape study.

The instrumental escape behavior of rats in response to C-T stimulation of the mesencephalic "pain" system has also been shown to reflect underlying neural processes (Kestenbaum et al., 1970). Rats were taught to press a lever to obtain short rest periods from an ongoing train of pulse pairs administered to the medial lemniscus or to the mesencephalic portion of the spinothalamic tract. With a C-C interval of 100.0 msec., a plot of the rate of lever pressing as a function of the C-T interval showed evidence of latent addition at 0.2 msec., refractory periods at 0.4 to 0.9 msec., 0.9 to 1.0 msec., and 1.0 to 2.5 msec., and temporal summation declining in effect from 2.5 to 50.0 msec. The refractory period data suggested the recovery from refractoriness of three different fiber groups, and the values correspond well with the known refractory periods of mammalian fibers 10 to 20 microns in diameter, 1 to 10 microns in diameter, and poorly myelinated fibers, respectively (Grundfest, 1940). The temporal summation findings were also found to closely reflect

the decay of temporal summation measured electrically in peripheral preparations (Hursh, 1939; Grundfest, 1940; Lloyd, 1946).

In each of the previous experiments, the effects of temporal summation were obscured at the shorter C-T intervals by the refractory periods of the neurons stimulated. Since both C and T pulses were delivered via the same electrode, all the impulses arriving at the synapse were conducted along the same set of fibers. Thus, a T pulse following a C pulse by an interval of time smaller than the fiber's refractory period would fail to generate an action potential. The response strength measured in these prior studies, therefore, declined sharply when the C-T interval was shortened beyond a certain point, rather than continuing to show the performance increase characteristic of temporal summation. When excitatory impulses converge upon the postsynaptic neuron from separate presynaptic fibers, however, the entire time course of temporal summation is apparent. Temporal summation has been investigated under these conditions by having the C and T pulses delivered via separate electrodes (i.e., C pulses through one electrode, and T pulses through another) implanted bilaterally in the lateral hypothalamic arms of the MFB which are believed to converge upon a common neuronal pool in the midbrain ventral tegmental area (Ungerleider and Coons, 1970). When the strength of self-stimulation behavior, measured as the latency to the first lever press, was plotted as a function of the C-T interval,

a curve was obtained which, as in the electrical studies of heterosynaptic temporal summation, showed an uninterrupted increase in response strength for progressively shorter C-T intervals down to 0.1 msec.

Self-stimulation behavior also lends itself to the study of inhibitory processes since it is subject to inhibition deriving from the activation of the ventromedial hypothalamic nucleus (VMN)(Olds and Olds, 1962; Oomura at al., 1964; Hoebel, 1968; Morgane, 1969). With C pulses delivered to the VMN, and T pulses delivered to the ipsilateral lateral hypothalamic MFB, the self-stimulation response rate as a function of the C-T interval revealed a bimodal inhibitory interaction between these two brain areas (Terry, 1971). One possible explanation given for the bimodal relationship was that the inhibitory synapses could be differentially related to the two types of neurons in the self-stimulation system - the so-called reward and drive neurons (Deutsch, 1963; Gallistel et al., 1969).

C. Self-Stimulation Behavior and Anatomical Connections within the MFB

The phenomenon of self-stimulation refers to the fact that when electrodes are chronically implanted in certain subcortical sites in the brain, animals will repeatedly perform a response to electrically stimulate these areas. Self-stimulation behavior has been found for variously placed stimulating electrodes in a variety of animals, however, it has been most extensively studied in the rat. A com-

prehensive review of the anatomical data lists the following as some of the sites for self-stimulation: gyrus dentatus, area amygdaloidae anterior, nucleus caudatus, nucleus anteromedialis thalami, nucleus ventralis thalami, nucleus paraventricularis thalami, nucleus reticularis thalami, area supramammillaris, nucleus mammillaris lateralis, nucleus ventralis tegmenti (Tsai), substantia grisea periventricularis, and chiasma opticum, with the most effective and reliable sites being in the septal region and in the lateral to posterior hypothalamic course of the MFB. Topographic maps of self-stimulation sites in the rat brain place most of the structures within the trajectory of the MFB (Olds and Olds, 1963; Battig, 1969).

The MFB is a phylogenetically old, heterogeneous fiber system which follows a parasagittal path ventrally through the brain, connecting most of the telencephalon and diencephalon with a complex of brain stem nuclei (Nauta, 1958, 1960). As the MFB passes between these regions, the lateral zone of the hypothalamus and preoptic regions are traversed. The ascending MFB elements, comprising cholinergic (Shute and Lewis, 1966), and noradrenergic, dopaminergic and serotonergic axons (Ungerstedt, 1971), inter alia, seem to serve generalized arousal and sleep functions. The descending MFB elements serve as part of the control mechanisms for the brain stem nuclei. Thus, the MFB consists of several parallel intermingling pathways, which behavioral analysis has shown to be functionally discrete, and

which degeneration studies and electrophysiological analyses have shown to be anatomically dissociable with respect to origin, trajectory, number of synapses interposed, and fields or influence (Morgane, 1969).

Axonal degeneration studies have been performed to trace the course of the MFB fibers in the brain. Such studies, however, have proven difficult for two reasons: (1) the MFB courses through the lateral hypothalamic area (LHA) which is a "bed nucleus" (Gurdjian, 1927) for many diencephalic fiber tracts. Thus, lesions placed in the LHA may nondifferentially affect many fibers which are not related to the MFB system; and (2) the fibers of the MFB are poorly myelinated, and of small diameter which causes them to stain poorly and makes degeneration studies difficult to perform and ambiguous to interpret. This latter problem has been partially solved by the use of the Nauta silver staining techniques and their derivatives (Nauta and Gygax, 1951, 1954; Fink and Heimer, 1967).

Studies of fiber degeneration following lesions of the MFB have been done by Guillery (1957) and Wolf and Sutin (1966) in the rat, and by Nauta (1958) and McClure and Clark (1968) in the cat. Their results can be described in terms of ascending and descending degeneration from the LHA. Other tissue staining techniques have shown the course of ascending cholinergic (Shute and Lewis, 1966) and monoaminergic (Ungerstedt, 1971) pathways in the MFB.

Ascending Projections

From the LHA, fiber degeneration has been traced to the lateral preoptic area, the diagonal band of Broca, and the medial, and to a lesser extent, the lateral septal nucleus. Some fibers pass through the medial septal nucleus and enter the medial part of the cingulate cortex. A dorsomedial MFB pathway can be traced into numerous thalamic nuclear groups (i. e., nucleus reticularis, ventralis anterior, parataenialis, anterodorsalis, and dorsomedialis), with residual fibers reaching the lateral habenular nucleus via the stria medullaris. The degenerating fibers which join the stria terminalis follow the bundle to the amygdaloid complex, as well as degenerating fibers which exit from the preoptic area and enter the amygdaloid complex medially. Guillery's (1957) data suggest that the ascending fibers which terminate in the medial septal nucleus have a midbrain origin, whereas those ending in the lateral septal nucleus arise from cells in the LHA. Degeneration has been followed contralaterally in both the medial and lateral septal nuclei, with crossover occurring in the supraoptic commissures and perhaps also in the septum.

The histochemical studies demonstrate that cholinergic and monoaminergic fibers also ascend in the MFB. The noradrenergic fibers arise from various brain stem nuclei and terminate in limbic forebrain regions. The locus coeruleus is one of the noradrenergic brain stem nuclei, and it has been importantly linked to self-stim-

ulation behavior (Ritter and Stein, 1972). Dopaminergic fibers arise from the substantia nigra and terminate in the caudate-putamen complex. Serotonergic neurons, originating in the raphe nuclei, terminate in numerous telencephalic and diencephalic regions as do the cholinergic fibers which originate in nucleus cuneiformis reticularis and in areas near the substantia nigra.

Descending Projections

The majority of the descending MFB fibers pass through the LHA and reach the midbrain ventral tegmental area of Tsai. Prior to reaching the area of Tsai, some fibers leave the bundle medially and some enter both medial mammillary body nuclei and some enter the lateral mammillary nucleus ipsilaterally. Residual fibers pass through the supramammillary decussation and extend caudally to the contralateral area of Tsai. Of the two offshoots from the MFB at the level of the caudal mammillary body, one group terminates in the central gray and the other terminates in the rostral tip of the interpeduncular nucleus. A few fibers can be traced from the area of Tsai into the raphe nuclei, the central gray, and the nucleus mesencephalicus profundus pars lateralis. For the most part, however, descending MFB fibers seem to terminate in the midbrain ventral tegmental area.

To summarize, the MFB is a diffuse and complex two-way

fiber system that (a) is represented bilaterally in the brain, (b) can be traced from the forebrain to the brain stem, and (c) converges in the ventral tegmental area of the brain stem and also in the septal nuclei and mammillary body.

Self-Stimulation and MFB Anatomy

Since the anatomical evidence points to both upstream and downstream sites of MFB convergence, self-stimulation behavioral studies have been performed to determine which direction positively reinforcing impulses follow within the MFB. In general, the technique here is to lesion either anterior or posterior from the electrode which elicits self-stimulation. A loss or decline in self-stimulation resulting from the lesion suggests that the outflow of the system has been interrupted and presumably indicates at least whether convergence of the MFB is upstream in the septal area, for example, or downstream perhaps in the ventral tegmental area. Massive lesions of the septal nuclei (Ward, 1960) or of the amygdaloid nuclei (Ward, 1961) had little effect on the rate of tegmental self-stimulation in rats. Similarly, Miller (1963) reported that bilateral septal lesions had no effect on self-stimulation of the hypothalamus. However, bilateral MFB lesions at the level of the hypothalamus abolished self-stimulation of the septal area. Further evidence from Olds and Olds (1964) showed that lesions anterior to MFB electrodes produced only slight effects with rapid recovery to prior self-stim-

ulation rates, whereas posterior lesions produced a complete and irreversible loss of self-stimulation. Thus, these studies suggest that the outflow of the MFB self-stimulation system is in a downstream direction toward the brain stem. To localize where in the brain stem these fibers converge, Schiff (1964) studied the effects of both ventral and dorsal tegmental lesions on septal self-stimulation rates in rats. Self-stimulation rates were decreased after ventral tegmental lesions, but not affected after dorsal tegmental lesions.

Although the downstream site of convergence of MFB "self-stimulation fibers" has received some degree of behavioral experimental support, other behavioral evidence is not compatible with such a notion. For example, Valenstein (1966), Boyd and Gardner (1967), and Lorens (1966) found that extensive lesions of major MFB areas (preoptic area, mammillothalamic tract, ventral tegmental area, anterior-posterior hypothalamus, etc.) had little effect on the rate of septal or lateral hypothalamic self-stimulation so long as sufficient post-operative recovery was allowed (sometimes as long as 2 months). Such results led these authors to conclude that the "self-stimulation system" was characterized by "redundancy" and "plasticity". On the other hand, Morgane (1964) has concluded that ablations anywhere along the MFB trajectory, either anterior or posterior to the stimulating electrode, causes marked self-stimulation suppression. Thus

the data are by no means consistent with respect to the effects of MFB lesions on self-stimulation behavior, since data exists to suggest: (a) that the descending MFB fibers are critical for self-stimulation; (b) that the MFB fibers are not critical for self-stimulation; and (c) that ascending and descending MFB fibers are important for self-stimulation.

Stein (1968) stresses the importance of the ascending noradrenergic fibers found in the MFB for self-stimulation behavior. He attempts to resolve the discrepancies in the electrolytic lesion data by suggesting that when time is allowed to pass between lesion and testing, degeneration can occur. Thus, posterior lesions might be most debilitating since the degeneration would ascend beneath the stimulating electrode making self-stimulation rates low at best. Furthermore, since the MFB is compact posteriorly and fans out anteriorly, posterior lesions would result in a greater amount of MFB damage simply due to the fiber geometry. Other changes, such as denervation supersensitivity, could also occur during lesion recovery which could facilitate, as well as inhibit, self-stimulation behavior. In order to avoid such problems, Stein (1968) used "reversible chemical lesions", either anterior or posterior to the self-stimulation electrode, and found that both ascending and descending MFB fibers were involved in self-stimulation. This conclusion is certainly consistent with the anatomical data, and it also explains the bidirectional deficits re-

ported in some of the previously mentioned experiments. Thus perhaps both ascending and descending MFB fibers are functional in self-stimulation behavior, and the directionality of reinforcing impulses merely depends upon the electrode location within the MFB.

D. Experimental Strategy and Predictions

The present experiment will address itself to two main issues. First, since most C-T studies of the neurophysiological properties of "self-stimulation fibers" have been done in posterior hypothalamic MFB areas (Deutsch, 1964; Gallistel et al., 1969; Rolls, 197la, 197lb; Smith and Coons, 1970; Ungerleider and Coons, 1970), the present experiment will attempt to study some of the neurophysiological properties of anterior MFB area "self-stimulation fibers". Since self-stimulation response rates are generally lower as electrodes are placed more anterior in the MFB (Olds and Olds, 1963; Battig, 1969), perhaps the neurophysiological properties of the "self-stimulation fibers" in anterior MFB areas will be different from those in posterior MFB areas. Second, since there is considerable controversy as to the directionality of positively reinforcing impulses within the MFB system, as mentioned above, the present experiment will attempt to differentiate between the possibilities of ascending vs. descending sites of MFB self-stimulation convergence.

The C-T technique of electrical stimulation was used to

study both axonal and synaptic stimulation-response relationships in the MFB self-stimulation system of the rat. The stimulation consisted of trains of parametrically varied C-T pulse pairs delivered at a constant interval separating the onsets of the C pulses (C-C interval). Thus, the frequency of stimulation and therefore the amount of current applied to the tissue remained constant, with only the pattern of stimulation being manipulated. The response measure was the number of lever presses per minute-trial as a function of the C-T interval.

There were two stimulation conditions employed: (1) unilateral stimulation - both C and T pulses applied to the MFB on the same side of the brain, once to the preoptic area MFB (POA-MFB), and once to the posterior hypothalamic MFB (PH-MFB); and (2) bilateral stimulation - the C pulses applied to the POA-MFB, and the T pulses applied to the contralateral PH-MFB (condition Ca-Tp), and vice versa (Cp-Ta). It was hypothesized that the following stimulation-response relationships reflecting axonal and synaptic processes would result. For unilateral stimulation, (a) if the C pulses are too weak to fire all of the fibers stimulated, then, at C-T intervals shorter than 0.2 msec., latent addition should occur. At these brief intervals, an increase in response rate should result compared to the case in which the T pulses are omitted from the stimulation train (i.e., C-C condition), and the case where the C-T interval is

longer than 0.2 msec. but still within the refractory period range of the stimulated fibers. (b) Given C pulses that are sufficiently intense to fire all of the stimulated fibers, then, at C-T intervals shorter than the absolute refractory period, the behavior elicited should not differ from the C-C condition. As the C-T interval is increased beyond the absolute refractory period, the self-stimulation rate to double-pulse stimulation should become progressively greater than that for C-C stimulation. Such increases can be attributed to either the relative refractory period, or the recovery from the absolute refractory period of a smaller diameter second group of fibers. (c) Homosynaptic temporal summation should be observed at C-T intervals greater than the refractory period of the stimulated fibers. Thus, as the C-T interval is lengthened beyond the relative refractory period, the self-stimulation rate should rapidly increase.

For bilateral stimulation, (a) administering C pulses through one electrode and T pulses contralaterally through the other electrode should demonstrate heterosynaptic temporal summation if there is some form of convergence of these fibers onto common postsynaptic neurons. Since separately stimulated pathways can only summate temporally if there is spatial summation as well, the presence of heterosynaptic temporal summation in such a situation is a physiological test for whether or not fiber systems converge in the brain. Such temporal summation would be manifest by the fact that self-

stimulation rates do not decline in the refractory period range of C-T intervals as they do in the unilateral stimulation conditions. (b) The self-stimulation rate should vary predictably when C pulses are given through the anterior electrode and parametrically delayed T pulses are given through the posterior electrode (and vice versa) depending upon whether the site of self-stimulation convergence is upstream or downstream in the MFB. If reinforcing impulses descend from the bilateral stimulating electrodes and influence the activity of a common neuronal pool in the brain stem, for example, then the self-stimulation rate should be highest: (1) at an intermediate C-T interval and drop off at longer and shorter C-T intervals when condition Ca-Tp is administered; and (2) at the shortest C-T interval and drop off as the C-T interval is lengthened when the opposite condition is administered, Cp-Ta. Likewise, in this latter condition, the self-stimulation rate at the shortest C-T interval should approach that of the shortest C-T interval under condition Ca-Tp. The opposite predictions would be made if the site of self-stimulation convergence is upstream, for example, in the septal area.

The above predictions on the rate of self-stimulation behavior and thus the magnitude of heterosynaptic temporal summation are based on the fact that when stimulating electrodes activate two separate fiber systems which are equidistant from the postsynaptic convergence neurons, the shorter the C-T interval, the greater the a-

mount of temporal summation (Ungerleider and Coons, 1970). In the present experiment, where electrodes activate fibers at non-equidistant points from the convergence neurons, maximal temporal summation would be expected at the C-T interval which results in the closest simultaneity of action potentials from the two activated systems. Thus, if the "self-stimulation convergence neurons" are located downstream in the MFB and condition Ca-Tp is administered, then the C-T interval which allows for the closest temporal contiguity of action potentials at the convergence site will result in the greatest self-stimulation rate. This interval should not be at the shortest C-T interval since the C-pulse-action-potentials should presumably arrive at the convergence site after the T-pulse-action-potentials, whereas at slightly longer C-T intervals contiguity should occur. When condition Cp-Ta is administered, since the posterior electrode is closer to the downstream convergence site, self-stimulation rates should progressively increase as the C-T interval is decreased since greater temporal contiguity of impulses at the convergence site will be approached.

CHAPTER II

METHODS AND PROCEDURES

Subjects

Eleven adult male albino rats, weighing between 270 and 340 grams at the time of surgery, were each chronically implanted with two indwelling monopolar electrodes. Six rats had effective self-stimulation electrodes in the POA-MFB and in the contralateral PH-MFB, another three rats had effective self-stimulation electrodes only in the POA-MFB, and two rats had effective self-stimulation electrodes only in the PH-MFB.

Surgical Procedures

Each animal was anesthetized with Nembutal and its head was secured in a Kopf stereotaxic instrument adjusted so that the incisor bar was raised 5.0 mm above the interaural line. The following DeGroot (1959) coordinates were employed for electrode implantation: POA-MFB -- AP = 7.2 to 7.8, LAT = +2.3 to +2.5, VENT = -2.0 to -2.2; PH-MFB -- AP = 3.8, LAT = -1.2, VENT = -3.0. The electrodes were size 00 stainless steel insect pins insulated to with-

in 0.5 mm of the tip. An electrode pin was attached to the more anterior of two jewelers screws secured in the animal's skull, and served as the indifferent electrode for stimulation. The electrodes and indifferent pin were cemented to the skull with dental acrylic.

Histology

Following the completion of behavioral testing, animals were perfused intracardially with isotonic saline followed by 10% formalin. Their brains were removed and frozen sections were taken at 50 micron thicknesses. Verification of electrode placements was made with reference to the histological sections found in the Pellegrino and Cushman (1967) rat brain atlas.

Brain Stimulation and Test Apparatus

Brain stimulation pulses were produced by a system comprised of Digibit logic modules (BRS Electronics), a Grass PS-2 Photo Stimulator, and two Grass SD-9 Stimulators. The pairs of negative-going square-wave pulses were administered through a 1 microfarad capacitor in series with a 68 kilohm resistor (Deutsch, 1966) to minimize electrode polarization. The voltage amplitude of the pulses was controlled by a 50 kilohm variable resistor placed prior to the capacitor and the series resistor and connected to ground. Leads from this coupling circuit connected the rat's electrodes to the stimulators. The connectors attaching the leads to the electrodes were

tie-pin protectors.

Each time the experimenter operated a switch, or the rat pressed the lever in the operant conditioning box, the stimulators delivered pulse pairs in a train whose duration was controlled by a Digibit variable one-shot. The first pulse of each pair in the train was called the conditioning (C) pulse, and the second pulse was called the test (T) pulse. One SD-9 Stimulator provided the C pulses, and the other SD-9 Stimulator provided the T pulses. The interval between the onsets of the two pulses within a pair was called the C-T interval, and the interval between the onsets of the first pulses of two consecutive pairs was called the C-C interval. The duration of each pulse was 0.1 msec., and this variable was set on each SD-9 Stimulator. The duration of the C-T interval was controlled by the Delay dial on the T-pulse-SD-9-Stimulator, and the duration of the C-C interval was controlled by the Frequency dial on the PS-2 Photo Stimulator which simultaneously drove the two SD-9 Stimulators. The C and T pulses within each pair could either be delivered through the same electrode to a single site in the brain (unilateral stimulation), or segregated so that C pulses went to one electrode and T pulses went to the other electrode (bilateral stimulation).

The dependent variable consisted of the number of lever presses within a one-minute trial during which the rat had access to the lever. This variable was recorded on a Digibit counter.

Brain stimulation parameters were continuously monitered on a Heath EU-70A dual beam oscilloscope as the rat pressed the lever. The test apparatus was a 12 x 10 x 9 in. sound attenuated Lehigh Valley operant conditioning box. An insulated retractable lever, when pressed, allowed the rat to initiate trains of brain stimulation.

Selection Criteria and Training

Animals were screened for self-stimulation behavior approximately one week after surgery. Each lever press during screening delivered a half-second train of C pulses at a C-C interval of 5.0 msec., with each pulse having an intensity not greater than 200 microamperes. To be included in the experiment, an animal had to self-stimulate at a rate of at least 30 lever presses per minute. This was to insure the effectiveness of the stimulation as a positive reinforcer.

The 11 qualified self-stimulators, of which 6 animals were bilateral self-stimulators, were then trained over 2 one-hour sessions to adapt them to some of the experimental procedures which would prevail during the succeeding test phase. Such procedures included the priming procedure which preceded each trial, the method of initiating and terminating trials by inserting and withdrawing the lever, and certain changes in the duration of both the pulse train and the C-C interval.

Testing

Unilateral Stimulation

Unilateral stimulation involves administering both C and T pulses through the same electrode. Nine rats had POA-MFB electrodes for which they would self-stimulate, and eight rats had effective PH-MFB self-stimulation electrodes. In six of these rats, both electrodes were effective in the same animal, and in this case the PH-MFB electrode was always tested before the POA-MFB electrode. Each electrode was tested for 4 sessions administered over consecutive days. The session consisted of 35 one-minute trials, each trial being separated by a one-minute rest with the lever absent. During a session, the train duration per press was 0.6 sec. (0.5 sec. for rats DG-41 and DG-42), and the C-C interval was held constant; both electrodes were tested with either a 20.0, 25.0, 30.0, 40.0, or 50.0 msec. C-C interval. Such different C-C intervals were used because a number of animals had been tested at shorter C-C intervals before it was decided that a longer C-C interval would be necessary for testing the bilateral stimulation conditions. Six C-T intervals were tested, plus a single pulse control (C-C condition) consisting of trains of C pulses with the T pulses omitted. The C-C condition was a control to provide a baseline from which the effectiveness of the addition of the T pulses at various C-T intervals could be assessed. These intervals were varied from trial to trial according to a 7 x 7

latin square design. The size of the C-T interval varied somewhat per C-C interval employed. All animals were tested at C-T intervals of 0.1, 0.5, 0.8, and 1.5 msec. However, the remaining two C-T intervals were either 4.0 or 6.0 msec. when the C-C interval was 20.0 msec., 5.0 and 10.0 msec. when the C-C interval was either 25.0, 30.0, or 40.0 msec., or 5.0 and 25.0 msec. when the C-C interval was 50.0 msec.

Bilateral Stimulation

Bilateral stimulation involves administering C and T pulses to separate electrodes on opposite sides of the brain either in condition Ca-Tp or condition Cp-Ta. Four consecutive days of testing were administered for each bilateral condition. For 3 rats, each daily session consisted of 5 trials at each of 7 pulse-pair intervals, and 3 rats were given 5 trials per day at each of 6 pulse-pair intervals. The train duration was 0.6 sec. per lever press (0.5 sec. for DG-41 and DG-42), and the C-C interval was held constant; five rats were tested with 40.0 msec. C-C intervals, and one rat was tested with a 50.0 msec. C-C interval. The rats which were tested at 20.0, 25.0 and 30.0 msec. unilateral C-C intervals were subsequently tested with 40.0 msec. C-C intervals and higher current intensities before the start of bilateral testing, however, since minimal data were collected they will not be reported. Three rats were tested first under condition Ca-Tp, and then condition Cp-Ta, and three

rats were tested with the opposite sequence. Half of the rats were tested with C-T intervals of 0.1, 0.5, 0.8, 1.5, 5.0 and 20.0 msec. plus the C-C condition, and half were tested with 0.1, 1.0, 3.0, 6.0, and 20.0 or 25.0 msec. C-T intervals plus the C-C condition. The first sequence of C-T intervals was employed in order to demonstrate that performance was much higher for bilateral stimulation at the same C-T intervals which resulted in very low self-stimulation rates for unilateral stimulation. The second sequence of C-T intervals was employed in order to assess the effects of a different set of C-T intervals on bilateral stimulation performance. The C-T intervals were varied from trial to trial according to either a 7 x 7, or a 6 x 6 latin square design, depending upon the number of pulse-pair intervals tested.

Just before inserting the lever at the beginning of a trial, the rat was primed with 4 trains of pulses at the same interval (C-T or C-C) for which he could self-administer during that trial. The number of lever presses was recorded for each one-minute trial and served as the dependent variable. Finally, the current level to be employed for a given electrode was the intensity for which the animal would press approximately 10 times per minute under the C-C condition. These procedures were common to both unilateral and bilateral testing conditions.

CHAPTER III

EXPERIMENTAL RESULTS

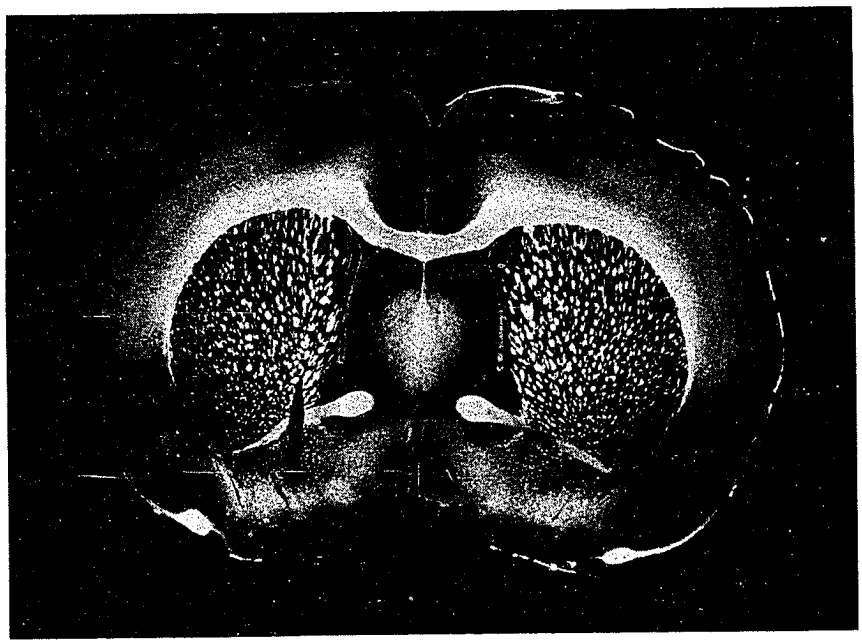
Electrode Placements

Figure 2 provides an example of the histological frontal sections of rat brain showing the approximate electrode tip position for a POA-MFB placement (Part A), and a PH-MFB placement (Part B). Figures 3 and 4 illustrate the electrode tip loci for the PH-MFB and the POA-MFB electrodes, respectively. As can be seen, the PH-MFB electrodes were located in a relatively confined region of the lateral hypothalamic area of the MFB, ranging from AP = 4.4 to 3.8. The POA-MFB electrodes, however, were somewhat more widely distributed along anterior portions of the MFB, ranging from AP = 8.0 to 6.8. The average AP offset between the anterior and posterior MFB electrodes was 3.26 mm.

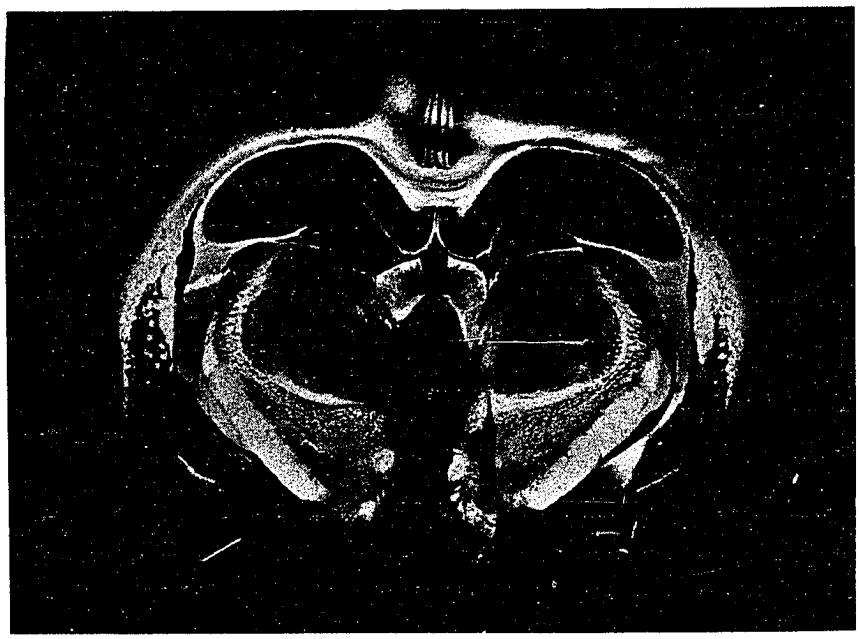
Data Analysis

The unilateral and bilateral data presented in the text are group curves averaged over all rats within the respective experimental conditions. The means and standard deviations for the number of

Figure 2. Histological frontal sections of rat brain revealing the electrode tip for a POA-MFB placement (Part A) and a PH-MFB placement (Part B). The POA-MFB placement is from rat DG-23, and the PH-MFB placement is from rat DG-42.



A. EXAMPLE OF A POA-MFB PLACEMENT



B. EXAMPLE OF A PH-MFB PLACEMENT

Figure 2.

Figure 3. Electrode tip loci for the PH-MFB placements. Frontal sections from AP plane 4.4 to plane 3.8 in the Pellegrino and Cushman (1967) rat brain atlas are represented. The AP plane represents the distance in millimeters from the interaural line. The abscissa scale represents the millimeter distance from the midline of the brain, and the ordinate specifies the horizontal distance from the surface of the brain. A black dot indicates the 0.5 mm uninsulated electrode tip for each rat. (Brain areas are abbreviated as follows: MFB = medial forebrain bundle; LHA = lateral hypothalamic area; ZI = zona inserta; PC = cerebral peduncle; SUM = supramammillary area; MM = medial mammillary nucleus; MT = mammillothalamic tract; ML = lateral mammillary nucleus).

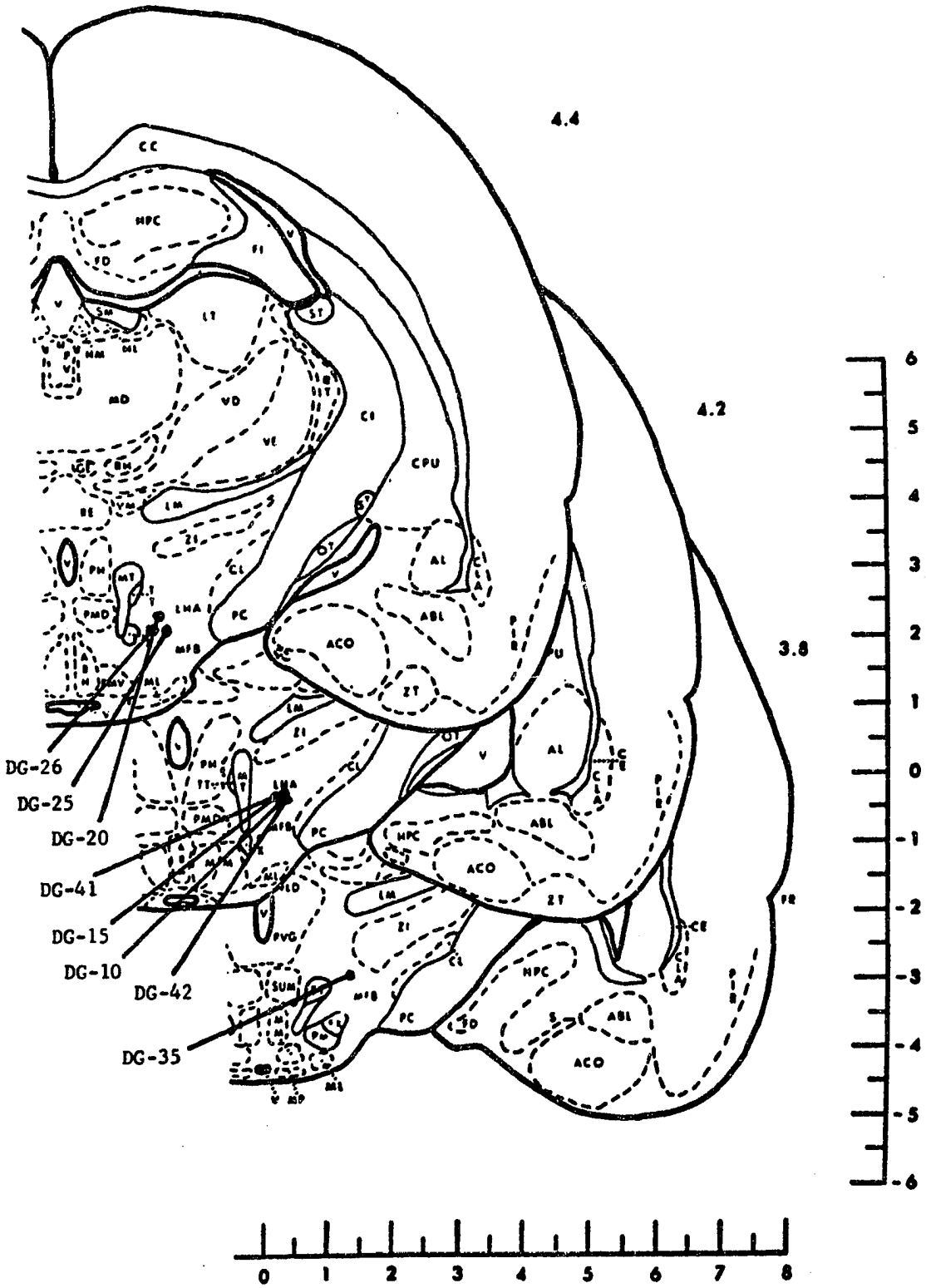


Figure 3. Electrode tip loci for the PH-MFB placements.

Figure 4. Electrode tip loci for the POA-MFB placements. Frontal sections from AP plane 8.0 to plane 6.8 in the Pellegrino and Cushman (1967) rat brain atlas are represented. The AP plane, ordinate and abscissa scales, and black dot have the same definition as in Figure 3. (Brain areas are abbreviated as follows: MFB = medial forebrain bundle; CA = anterior commissure; POA = lateral preoptic area; CO = optic chiasma; LHA = lateral hypothalamic area; SM= stria medullaris thalami; NOT = nucleus of olfactory tract).

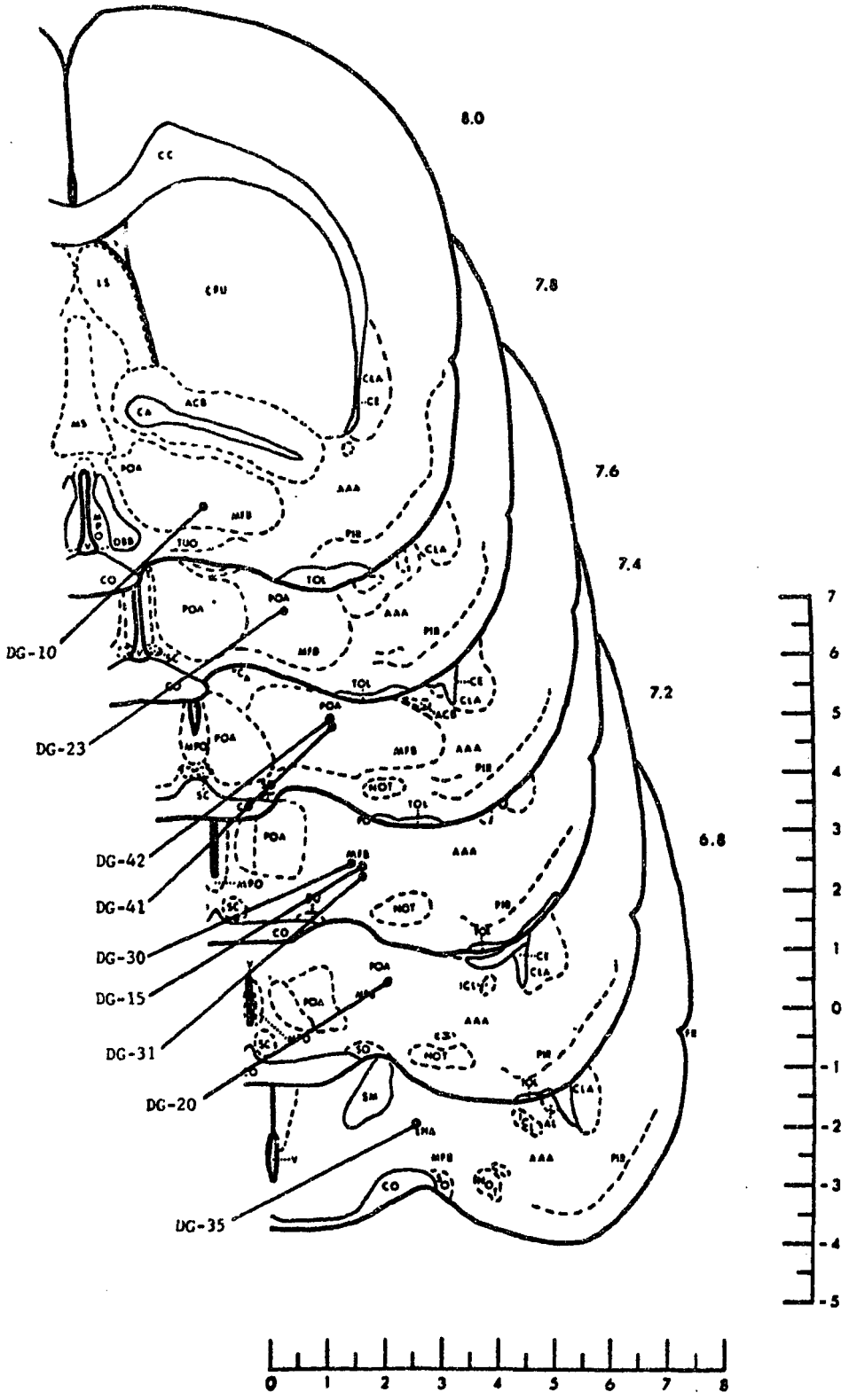


Figure 4. Electrode tip loci for the POA-MFB placements.

lever presses per minute as a function of the C-T interval are presented for individual rats in Appendix A (Tables 6 and 7 for unilateral stimulation data, and Tables 10 and 11 for bilateral stimulation data). All statistical analyses performed on individual rats under both unilateral and bilateral stimulation conditions are also presented in Appendix A (Tables 8, 9, and 12). It will be seen that the effects present in the group curves are also largely reflected in most of the individual animals' data.

Both one- and two-tailed tests were used in the analyses of the unilateral and bilateral stimulation data. One-tailed tests were used for unilateral C-T interval comparisons since self-stimulation performance only increases above the C-C interval level as T pulses are administered at progressively longer C-T intervals. Although maximal self-stimulation performance has been found for lateral hypothalamic MFB stimulation at the 5.0 msec. C-T interval (Smith and Coons, 1970; Ungerleider and Coons, 1970), it was not known whether self-stimulation performance at a similar site or in the POA-MFB would increase, stay the same or decrease when the C-T interval was lengthened to 6.0, 10.0, or 25.0 msec. Thus two-tailed tests were used for unilateral C-T interval comparisons beyond 5.0 msec. For bilateral stimulation, one-tailed tests were used in making C-T interval comparisons between the longest and a relatively short C-T interval since self-stimulation behavior increases as the C-T interval

is decreased within this range. Two-tailed tests, however, were required for testing relatively short C-T intervals with the shortest C-T interval since self-stimulation performance could be higher or lower at the shortest C-T interval than at the relatively short interval depending upon which direction reinforcing impulses take for MFB convergence.

Unilateral Stimulation

The results for unilateral stimulation of POA-MFB and PH-MFB areas averaged over rats which had either one or two effective self-stimulation electrodes are presented in Figures 5 and 6. These figures show that performance under both unilateral stimulation conditions did not seem to be related to whether the same or different rats were used in determining the C-T self-stimulation relationships. Grouping rats into PH-MFB and POA-MFB catagories seemed justified since an F test of the coefficient of concordance showed a highly significant relationship among the several C-T intervals in the pattern of self-stimulation performance for rats within each group ($F = 113.68$, $df = 5, 29$, $p < .001$ for PH-MFB; $F = 117.10$, $df = 5, 29$, $p < .001$ for POA-MFB). These results also suggested that the duration of the C-C interval was not importantly related to the pattern of self-stimulation performance at either electrode site.

T tests for correlated means were used to compare performance at various C-T intervals over all rats within each unilateral

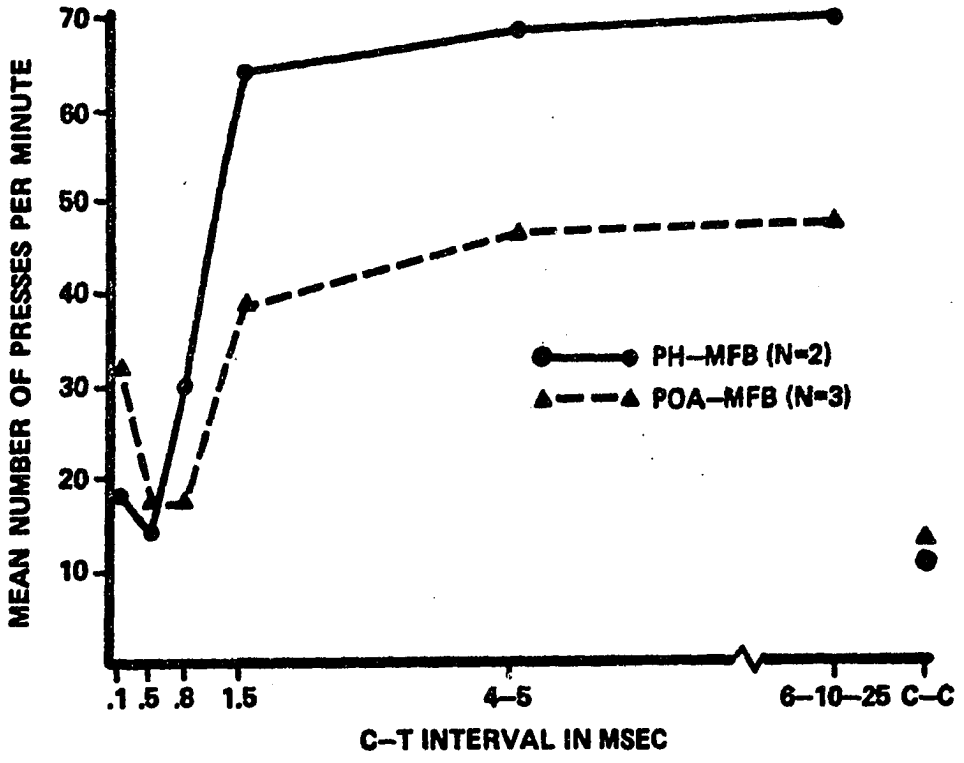


Figure 5. Mean number of lever presses per minute for unilateral stimulation as a function of the C-T interval for rats with only one effective self-stimulation electrode. The C-C interval varied from 20.0, 25.0, or 50.0 msec. between rats.

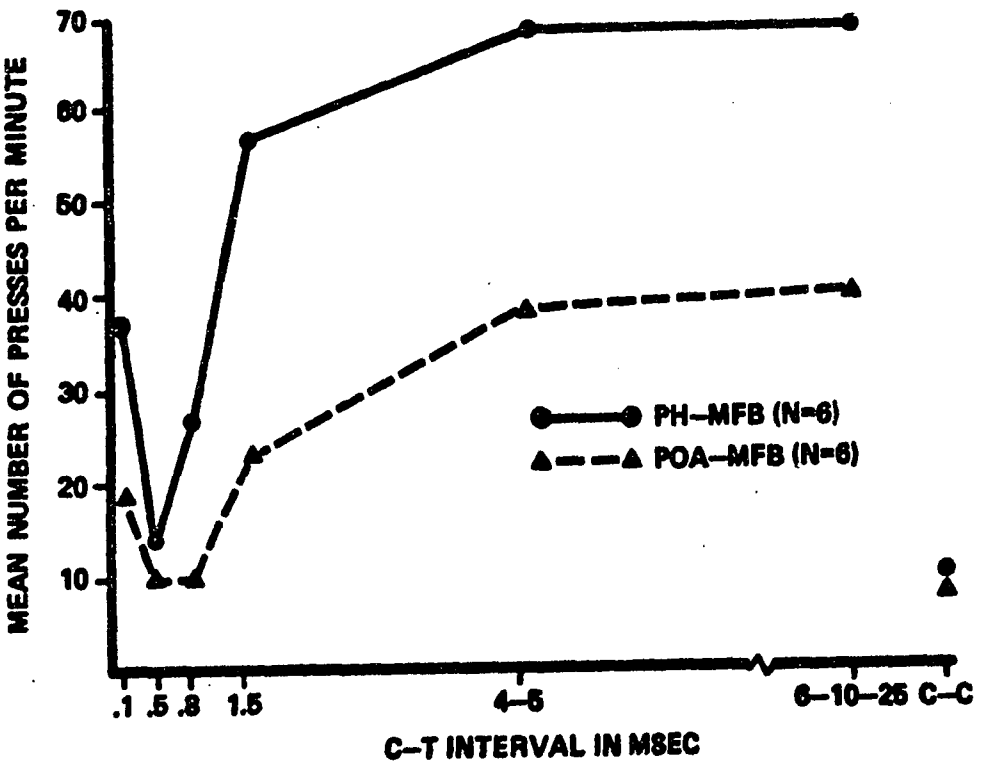


Figure 6. Mean number of lever presses per minute for unilateral stimulation as a function of the C-T interval for rats with two effective self-stimulation electrodes. The C-C interval varied from 20.0, 25.0, 30.0, 40.0, or 50.0 msec. between rats.

stimulation condition. These data are presented in Tables 1 and 2, and they revealed the following stimulation-response relationships.

Latent Addition

The lever pressing rates were significantly higher at the 0.1 msec. C-T interval than at either the 0.5 msec. C-T interval or the C-C condition for both POA-MFB and PH-MFB stimulation. Such an increase in behavior at C-T intervals less than 0.2 msec. as compared with the C-C condition or the refractory period interval is indicative of the phenomenon of latent addition. Closer inspection of the data for individual rats in Tables 8 and 9 in Appendix A, however, revealed that although most animals showed latent addition, not all rats showed the effect. This finding will be discussed later in terms of Rolls' (1971a) hypothesis that twice threshold current is required in order to eliminate latent addition phenomenon, and thus obtain a valid estimate of the actual absolute refractory period of the stimulated neurons.

Absolute Refractory Period

The lever pressing rates at the 0.5 msec. C-T interval were significantly higher than under the C-C condition for both POA-MFB and PH-MFB stimulation groups. However, for the PH-MFB group, lever pressing was significantly higher at 0.8 msec. than at 0.5 msec., whereas for the POA-MFB group such a C-T interval com-

Table 1

CORRELATED MEAN T TESTS ON THE NUMBER OF LEVER PRESSES PER MINUTE - SIGNIFICANT DIFFERENCES BETWEEN C-T INTERVALS FOR RATS TESTED UNDER THE UNILATERAL PH-MFB STIMULATION CONDITION

C-T Comparisons	t Value	Significance	Direction
Latent Addition			
0.1 vs. C-C	3.53	.005	0.1>C-C
0.1 vs. 0.5	3.34	.01	0.1>0.5
Refractory Period			
0.5 vs. C-C	2.07	.05	0.5>C-C
0.8 vs. C-C	4.66	.005	0.8>C-C
0.8 vs. 0.5	4.89	.005	0.8>0.5
Recovery from Refractoriness			
0.5 vs. 0.8	4.89	.005	0.8>0.5
0.8 vs. 1.5	6.31	.001	1.5>0.8
1.5 vs. 4,5	4.38	.005	4,5>1.5
Temporal Summation			
4,5 vs. 6,10, or 25	1.95	N.S.[a]	4,5=6,10,25

[a] Two-tailed test.

Table 2

CORRELATED MEAN T TESTS ON THE NUMBER OF LEVER PRESSES PER MINUTE - SIGNIFICANT DIFFERENCES BETWEEN C-T INTERVALS FOR RATS TESTED UNDER THE UNILATERAL POA-MFB STIMULATION CONDITION

C-T Comparisons	t Value	Significance	Direction
Latent Addition			
0.1 vs. C-C	4.01	.005	0.1> C-C
0.1 vs. 0.5	3.71	.005	0.1> 0.5
Refractory Period			
0.5 vs. C-C	3.12	.01	0.5> C-C
0.8 vs. C-C	3.45	.005	0.8> C-C
0.8 vs. 0.5	1.05	N.S.	0.8= 0.5
Recovery from Refractoriness			
0.8 vs. 1.5	6.50	.001	1.5 >0.8
1.5 vs. 4,5	5.02	.001	4,5>1.5
Temporal Summation			
4,5 vs. 6,10, or 25	2.99	.02[a]	6,10,25>4,5

[a] Two-tailed test.

parison was not significant. These comparisons were thought to reflect the absolute refractory period of the neurons being stimulated, and they suggested an absolute refractory period of 0.8 msec. for the stimulated POA-MFB fibers, and 0.5 msec. for the stimulated PH-MFB fibers. That these intervals are representative of the absolute refractory period of the respective groups is further clarified by inspection of Tables 8 and 9 in Appendix A. Table 8 shows that for PH-MFB stimulation, only two rats performed significantly higher at the 0.5 msec. C-T interval than at the C-C interval, while all other rats in this group did not differ significantly in this C-T interval comparison. Furthermore, only two of the eight rats failed to perform significantly higher at the 0.8 msec. C-T interval than at the 0.5 msec. interval. Table 9 reveals that for POA-MFB stimulation, only one rat performed significantly higher at the 0.5 msec. C-T interval than at the C-C interval, and none of these rats differed significantly in their performance between C-T intervals of 0.8 and 0.5 msec. Furthermore, a correlated mean t test showed that there was no significant difference in self-stimulation rate at the 0.5 msec. C-T interval between the POA-MFB and the PH-MFB electrodes in the six rats which had two effective self-stimulation placements ($t = 1.09$, $df = 5$, N.S.). Likewise, an independent t test showed nonsignificance in the same comparison between rats with only one effective self-stimulation placement ($t = 0.45$, $df = 5$, N.S.). However,

at the 0.8 msec. C-T interval, self-stimulation rates were higher for PH-MFB stimulation than for POA-MFB stimulation (correlated $t = 2.70$, $df = 5$, $p < .05$; independent $t = 1.91$, $df = 3$, N.S.). Thus, 0.5 and 0.8 msec. do seem representative of the group absolute refractory periods for the stimulated PH-MFB and the POA-MFB areas respectively, since performance at these C-T intervals was no different than when the T pulses were omitted from the stimulation train altogether.

Relative Refractory Period

The lever pressing rate rose significantly from 0.5 to 0.8 msec., from 0.8 to 1.5 msec., and from 1.5 to 4.0 or 5.0 msec. for PH-MFB stimulation. A comparable increase in lever pressing performance was seen for POA-MFB stimulation beginning at 0.8 msec. and increasing through 6.0, 10.0, or 25.0 msec. This increase in self-stimulation performance was thought to reflect the recovery from refractoriness for the stimulated MFB fibers. This increase in lever pressing suggests that at least some of the fibers stimulated, probably those closest to the electrode tip where current is most intense, are capable of being fired by the T pulse.

Temporal Summation

The shortest C-T interval which results in the highest level of self-stimulation behavior is generally thought to mark the point

where recovery from refractoriness is complete and where peak temporal summation occurs. Tables 1 and 2 show that lever pressing rates continued to increase from 4.0 or 5.0 msec. out to 6.0, 10.0, or 25.0 msec. for POA-MFB stimulation ($p<.02$), but for PH-MFB stimulation there was no significant difference in performance between these intervals. For PH-MFB stimulation, such results suggest that by 5.0 msec. the C pulse stimulated neurons have fully recovered from their refractoriness and are producing maximal temporal synaptic summation. For POA-MFB stimulation, however, it appears that by 5.0 msec. not all of the stimulated neurons have fully recovered from refractoriness since self-stimulation rates continued to increase beyond this C-T interval. Thus, maximal temporal summation occurred later for the stimulated POA-MFB fibers than for the stimulated PH-MFB fibers.

POA-MFB vs. PH-MFB Performance

Table 3 presents the current levels (in microamperes) used for individual rats in both unilateral stimulation conditions. This table also presents the mean lever pressing rate for each rat at the 5.0 msec. C-T interval. As can be seen, self-stimulation rates were consistently higher for PH-MFB stimulation than for POA-MFB stimulation ($t = 3.14$, $df = 15$, $p<.01$), although significantly lower current levels were needed for PH-MFB self-stimulation than for POA-MFB self-stimulation ($t = 2.96$, $df = 15$, $p<.01$). Since there was no

Table 3

MEAN NUMBER OF LEVER PRESSES PER MINUTE (MLP) AT THE 5.0 MSEC. C-T INTERVAL AND CURRENT LEVELS USED FOR RATS TESTED UNDER THE UNILATERAL STIMULATION CONDITIONS

	PH-MFB			POA-MFB	
Rat	MLP	Current	Rat	MLP	Current
DG-35	61.06	250	DG-35	44.40	280
DG-20	49.05	160	DG-20	33.65	270
DG-15	47.50[a]	150	DG-15	27.63[a]	240
DG-25	86.30	250	DG-23	49.60	280
DG-10	56.60[a]	185	DG-10	39.25[a]	200
DG-26	51.35	240	DG-31	56.90	250
DG-41	97.75	190	DG-41	49.25	190
DG-42	106.40	190	DG-42	34.30	250
			DG-30	31.60	280
Mean	69.50	201.87		40.73	248.89

[a] C-T interval = 4.0 msec.

significant difference between the durations of the C-C intervals employed in testing both electrode sites, the current-level and response-rate differences did not seem to be biased by the C-C interval.

Bilateral Stimulation

Table 4 presents the current intensities (in microamperes) and the mean number of lever presses per minute at the C-C interval for each of the six rats tested under the bilateral stimulation conditions. Although the current intensities were changed somewhat from those used under the unilateral stimulation conditions, overall, a higher current was needed to meet the 10 presses per minute criterion rate for C-C stimulation at POA-MFB placements than at PH-MFB placements. Also it can be seen that the average response rate for both self-stimulation electrodes was 10 presses per minute for C-C stimulation.

Three rats were tested under the bilateral stimulation conditions with the same set of C-T intervals as was used in testing the unilateral stimulation conditions, while three rats were tested with different C-T intervals. Since self-stimulation performance was quite similar under the two sets of C-T intervals, all rats were combined for group analysis with the following C-T intervals - 0.1, 1-1.5, 5-6, 20-25 msec., and the C-C condition (40.0 or 50.0 msec.).

It was predicted that if heterosynaptic temporal summation

Table 4

MEAN NUMBER OF LEVER PRESSES PER MINUTE (MLP) AT THE C-C INTERVAL AND CURRENT LEVELS USED FOR RATS TESTED UNDER THE BILATERAL STIMULATION CONDITIONS

	POA-MFB		PH-MFB	
Rat	MLP	Current	MLP	Current
DG-35	6.50	280	2.65	300
DG-41	21.81	190	10.30	190
DG-42	8.80	250	3.80	190
DG-10	4.40	270	12.25	240
DG-15	12.70	230	16.15	240
DG-20	10.40	250	15.00	150
Mean	10.77	245.00	10.02	218.33

were accomplished by a downstream midbrain convergence of MFB impulses, for example, under condition Ca-Tp performance should peak at some C-T interval greater than the shortest C-T interval employed. However, under the opposite condition, Cp-Ta, lever pressing rates should progressively increase from the longest to the shortest C-T interval. The opposite outcome for conditions Ca-Tp and Cp-Ta would be suggestive of an upstream MFB self-stimulation convergence site. With such predictions, the critical C-T interval comparisons were 20-25 vs. 0.1 msec., 20-25 vs. 1-1.5 msec., and 1-1.5 vs. 0.1 msec.

Since previous double-pulse self-stimulation studies have shown that both homosynaptic (Kestenbaum et al., 1970) and heterosynaptic (Ungerleider and Coons, 1970) temporal summation produce power function curves, it was thought that a similar kind of curve might be found in the present experiment. Therefore, the bilateral stimulation data were plotted on a log x log scale because such a scale transforms power curves into straight lines, thereby offering a visual check on these expectations.

Figure 7 presents the bilateral stimulation group curves as a function of the C-T interval, and Table 5 presents the correlated mean t test results for the critical C-T interval comparisons for these data. As can be seen, giving C pulses anterior and T pulses posterior (Ca-Tp), lever pressing rates increased from 20-25 msec.

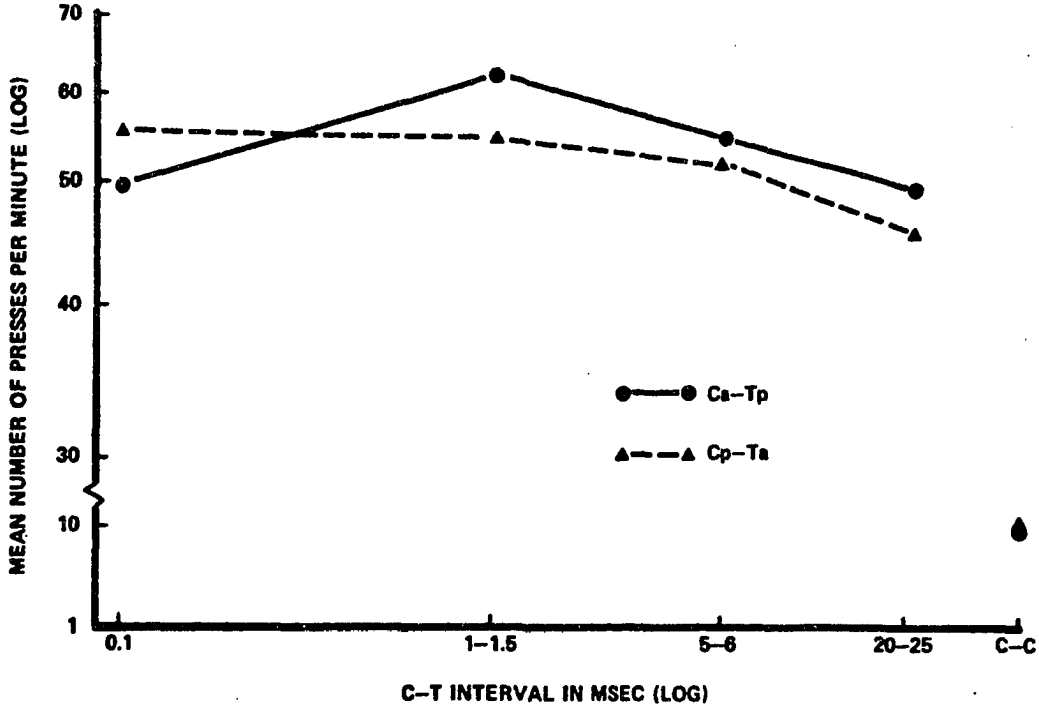


Figure 7. Mean number of lever presses per minute for bilateral stimulation as a function of the C-T interval. Five rats were tested with a C-C interval of 40.0 msec. and one rat was tested with a 50.0 msec. C-C interval.

Table 5

CORRELATED MEAN T TESTS ON THE NUMBER OF LEVER PRESSES PER MINUTE - SIGNIFICANT DIFFERENCES BETWEEN C-T INTERVALS FOR RATS TESTED UNDER THE BILATERAL STIMULATION CONDITIONS

C-T Comparisons	Ca-Tp	Direction	Cp-Ta	Direction
0.1 vs. 20-25	-0.12	0.1=20-25	4.66[c]	0.1>20-25
0.1 vs. 1-1.5	-2.55[a]	1-1.5>0.1	0.46	0.1=1-1.5
1-1.5 vs. 20-25	2.81[c]	1-1.5>20-25	2.57[b]	1-1.5>20-25

[a] Significance, $p<.06$, two-tailed test.

[b] Significance, $p<.05$, one-tailed test.

[c] Significance, $p<.005$, one-tailed test.

to 1-1.5 msec. ($p<.05$), but then decreased from 1-1.5 to 0.1 msec. ($p<.06$). However, when C pulses were given through the posterior electrode and T pulses given through the anterior electrode, Cp-Ta, lever pressing rates increased from 20-25 msec. to 0.1 msec. in a linear fashion ($p<.005$), with no significant increase between 1-1.5 and 0.1 msec. Furthermore, self-stimulation performance was not found to differ significantly at C-T intervals of 0.1 msec., 5-6 msec., or 20-25 msec. between conditions Ca-Tp and Cp-Ta. However, performance was significantly higher at the 1-1.5 msec. C-T interval under condition Ca-Tp than under condition Cp-Ta ($t = 2.57$, $df = 5$, $p<.05$). These results strongly support the downstream convergence hypothesis.

T tests for correlated means were used to test the slope differences within and between the two bilateral stimulation conditions. No significant difference was found in the rate of decline in self-stimulation performance as the C-T interval was lengthened from 1-1.5 to 20-25 msec. under the two bilateral stimulation conditions. Although the rate of decline from 1-1.5 msec. to 0.1 msec. was steeper under condition Ca-Tp than the rate of increase between these C-T intervals under condition Cp-Ta, the difference was not significant. Finally, no significant difference was found between the slopes under condition Ca-Tp comparing self-stimulation performance changes from 1-1.5 to 0.1 msec. vs. 1-1.5 to 5-6 msec.

Tables 10 and 11 in Appendix A present individual means and standard deviations for each rat tested under both bilateral stimulation conditions, and Table 12 in Appendix A presents independent t test results for the above mentioned critical C-T interval comparisons. There seemed to be a fairly high degree of similarity among rats' performance under the bilateral stimulation conditions suggesting, as did the group data, that the convergence of MFB self-stimulation impulses is downstream. In order to test the degree to which individual rats' performance coincided with the observed group ranking in C-T interval self-stimulation performance, Lubin-Lyerly (1961) tests were performed. As would be predicted if convergence were downstream, the group curve for condition Cp-Ta showed a monotonic increase in self-stimulation performance from the longest to the shortest C-T interval, and the Lubin-Lyerly test indicated a highly significant ($p < .01$) degree of agreement among rats' performance under this condition. Testing rats' performance under condition Ca-Tp for a similar monotonic relationship resulted in nonsignificance. Testing performance under condition Ca-Tp, however, for the degree of agreement among rats with the observed inverted-U curve resulted in a significant correlation ($p < .03$). Rats' performance under condition Cp-Ta were not significantly related to the inverted-U curve. Such results indicate that the group of rats performed quite consistently within the two respective bilateral stimulation conditions.

If one animal were an exception to the downstream convergence hypothesis, it would be DG-35. Under condition Cp-Ta, self-stimulation performance increased from the 25.0 msec. C-T interval to the 3.0 msec. interval ($t = 4.30$, $df = 38$, $p < .001$), and then decreased to the 0.1 msec. C-T interval ($t = 1.84$, $df = 38$, $p < .10$). Under condition Ca-Tp, however, self-stimulation performance increased from the 25.0 msec. C-T interval to the 0.1 msec. interval ($t = 1.53$, $df = 38$, $p < .10$). Comparing self-stimulation performance at each C-T interval between the two bilateral stimulation conditions, however, resulted in no significant differences in performance at any of the C-T intervals. If an ascending MFB convergence of self-stimulation impulses were demonstrated, the general shape of both bilateral stimulation curves would be similar to those found for DG-35, however, one would also expect the overall curve for condition Cp-Ta to be somewhat higher than that for condition Ca-Tp, and the point on the Cp-Ta curve where maximal performance occurs should be significantly higher than performance at the same C-T interval under condition Ca-Tp. Although some of DG-35's data suggest an ascending MFB self-stimulation convergence, other aspects of the data suggest that DG-35 merely represents a variant example of the downstream MFB convergence exemplified in the group bilateral stimulation results. From the present data, it was not possible to differentiate between these two possibilities. For a discussion of possible

methodological problems in using bilateral C-T stimulation to assess the directionality of reinforcing MFB impulses, see Appendix B.

CHAPTER IV

DISCUSSION

Unilateral Stimulation

The unilateral stimulation portion of the experiment has replicated a number of previous findings, and has also extended our knowledge of the neurophysiological properties of MFB self-stimulation fibers. Evidence of latent addition, absolute and relative refractoriness, and temporal summation have been found, and their significance will be discussed below.

The phenomenon of latent addition was suggested by the fact that lever pressing rates were significantly higher at the 0.1 msec. C-T interval than at the 0.5 msec. C-T interval or at the C-C interval for stimulation at most PH-MFB and POA-MFB sites. Prior behavioral studies using the C-T technique have also found latent addition effects at 0.1 msec. in the self-stimulation system (Deutsch, 1964; Gallistel et al., 1969; Smith and Coons, 1970; Ungerleider and Coons, 1970), and at intervals less than 0.3 msec. in the so-called pain system (Kestenbaum et al., 1970). The behavioral increase at these short C-T intervals is thought to reflect the existence of a

subliminally depolarized fringe of neurons located some distance from the electrode tip where neurons are stimulated with suprathreshold current. This subliminal fringe of neurons is thought to store the C-pulse-produced charge and subsequently be fired by the T-pulse-produced depolarization which follows within 0.2 msec. Since more fibers are excited, the magnitude of self-stimulation behavior is increased at these shorter C-T intervals than when either C-C or refractory period C-T stimulation is tested.

For those PH-MFB and POA-MFB placements that did not show latent addition effects, it is presumed that the stimulating electrode fired all of the neurons in its vicinity with the C pulses. Now at the 0.1 msec. C-T interval, all of the stimulated fibers are absolutely refractory to being refired by the T pulses. Thus the rate of self-stimulation behavior at the 0.1 msec. C-T interval in this case will be no different than at the C-C interval or at the 0.5 msec. C-T interval.

Rolls (1971a) maintains that twice threshold stimulation is required when behaviorally measuring refractory periods in order to eliminate latent addition effects and thus get at valid absolute refractory period estimates. This hypothesis states that the presence of latent addition results in longer absolute refractory period values than when latent addition is absent. The present latent addition findings for individual rats, however, do not agree with this hypothesis. Some

rats within both unilateral stimulation conditions showed latent addition effects and some did not, but the absolute refractory period values were unaffected by the presence or absence of latent addition. Although it is reasonable to assume the existence of a just-threshold and an adjacent subliminally depolarized fringe of neurons surrounding the central core of supraliminally excited neurons, the present results suggest that the just-threshold fringe contributes insignificantly to the absolute refractory period determination, and perhaps is not importantly related to the number of subliminally depolarized neurons which influence the magnitude of the latent addition effect.

The behaviorally determined absolute refractory period values of 0.5 and 0.8 msec. for MFB self-stimulation fibers are similar to the refractory period of MFB fibers measured electrophysiologically. Electrophysiological studies have found 0.5 to 0.6 and 0.8 to 1.1 msec. absolute refractory periods for MFB fibers which were activated by lateral hypothalamic area self-stimulation electrodes (Gallistel et al., 1969; Rolls, 1971b). Furthermore, the noradrenergic MFB fibers which are thought to be important for self-stimulation behavior (Stein, 1968) have been found to have axon diameters from 1 to 4 microns (Fuxe, 1965). Such MFB fibers would thus seem to represent a subgroup which corresponds to mammalian A-type fibers which have absolute refractory periods from 0.4 to 1.1 msec. (Grundfest, 1940; Erlanger and Gasser, 1937; Hursh, 1939). Although the spacing of pulses be-

tween 0.5 and 1.5 msec. C-T intervals was not refined enough in the present experiment to precisely determine the point where absolute refractoriness ended and relative refractoriness began, it seems certain that the absolute refractory period is generally longer for POA-MFB stimulation than for PH-MFB stimulation.

The fact that two PH-MFB placements showed 0.8 msec. absolute refractory period values, as opposed to 0.5 msec., merely indicates that the smaller sized fibers which have been predominantly demonstrated at POA-MFB placements can also be found at PH-MFB sites. Finding 0.5 and 0.8 msec. refractory periods for PH-MFB stimulation is in agreement with previous studies demonstrating that both "drive" and "reward" fibers seem to exist in an area excited by a single PH-MFB electrode (Deutsch, 1964; Gallistel et al., 1969). For example, absolute refractory period values of 0.4 to 0.5 msec. have been found for PH-MFB stimulation in a number of experiments, some of which were specifically aimed at measuring the refractory period of the so-called reward fibers (Deutsch, 1964; Gallistel et al., 1969; Smith and Coons, 1970; Ungerleider and Coons, 1970). When the experimental arrangement was set to measure the so-called drive fibers, however, PH-MFB stimulation resulted in absolute refractory period values between 0.8 and 1.1 msec. (Deutsch, 1964; Gallistel et al., 1969; Rolls, 1971a). The refractory periods of drive system fibers have been separated from those of reward system fibers on op-

erational grounds by varying the C-T interval of the priming stimulus (drive manipulation) and holding the C-T interval constant for the stimulation that the animal can self-administer by pressing the lever (reward manipulation). By varying one C-T stimulation interval and holding the other constant, it is possible to determine the neurophysiological properties of each subsystem separately. In the present experiment, since the priming C-T interval was always the same as the animal could self-administer during that trial, it was not possible to relegate the refractory period differences between anterior and posterior MFB areas to predominantly drive vs. reward effects. What is suggested, however, is that the stimulated POA-MFB fibers which elicited the behavioral changes at C-T intervals were consistently of a smaller axon diameter than most of the stimulated PH-MFB fibers.

The relative refractory period group data tend to corroborate the absolute refractory period group data, since on neurophysiological grounds, the longer the absolute refractory period the longer the relative refractory period. The stimulated PH-MFB neurons had an absolute refractory period of 0.5 msec. and recovered from relative refractoriness by 5.0 msec., whereas the stimulated POA-MFB neurons had an absolute refractory period of 0.8 msec. and recovered from relative refractoriness after 5.0 msec. The fact that self-stimulation response rates continue to increase as the C-T interval

is lengthened within the above mentioned limits suggests that the relative refractory state of the stimulated neurons is declining allowing more fibers to fire to the T pulse. Thus, not until 5.0 msec. (for PH-MFB fibers) or after 5.0 msec. (for POA-MFB fibers) is the full effect of the T pulse made on fiber excitation. The data of both Smith and Coons (1970) and Ungerleider and Coons (1970) support these findings and suggest that recovery from refractoriness is complete by 5.0 msec. for PH-MFB stimulation.

An alternative explanation for the increase in self-stimulation performance between 1.5 and 5.0 msec. C-T intervals is the recovery from absolute refractoriness of a second group of smaller diameter fibers characterized by a longer absolute refractory period. The presence of small diameter poorly myelinated fibers exhibiting absolute refractory periods of 2.0 msec. (Grundfest, 1940) would be consistent with these data. From the present experiment, however, it was not possible to differentiate between these two alternatives.

Maximal self-stimulation performance was reached after 5.0 msec. for POA-MFB stimulation and at 5.0 msec. for PH-MFB stimulation. The point where maximal self-stimulation performance occurs suggests that both C and T pulses are fully effective in fiber excitation since at these longer C-T intervals the refractoriness of the stimulated fibers is exceeded. Temporal summation best explains these data, and its magnitude is a function of the rate at which im-

pulses arrive at the synapse. One mechanism for this phenomenon is that closely spaced impulses, each releasing the same number of neurotransmitter packets, may summate on the postsynaptic membrane by adding successive depolarizations. A second possible mechanism is a presynaptic effect in which closely spaced impulses arriving at the same synapse cause the release of more neurotransmitter packets (presynaptic facilitation), again causing summation of postsynaptic potentials (Eccles, 1964). Since previous studies have found temporal summation effects to be strong even at C-T intervals as long as 60.0 msec. for lateral hypothalamic MFB stimulation (Ungerleider and Coons, 1970; Smith and Coons, 1970), it was not too surprising that the present PH-MFB and POA-MFB temporal summation effects were strong at the 6.0, 10.0 or 25.0 msec. C-T interval. Such temporal summation effects are thought to reflect long lasting excitatory postsynaptic potentials (EPSPs) produced by MFB stimulation (Ungerleider and Coons, 1970). Although temporal summation has frequently been found to decay rather rapidly (Eccles, 1946; Lloyd, 1946), EPSPs may last for up to 80.0 msec. in cortical pyramidal cells and thalamic neurons (Li and Chou, 1962; Phillips, 1961). Since long lasting temporal summation effects could also result from recurrent excitatory loops and other neuronal interactions, the present results only allow speculation as to the temporal summation mechanism.

The finding that unilateral PH-MFB self-stimulation rates were higher than POA-MFB self-stimulation rates is consistent with previous findings from self-stimulation mapping studies of the rat diencephalon. Olds and Olds (1962, 1963) and Battig (1969) have found that self-stimulation rates tend to decrease as electrodes were placed more rostral in the MFB. Such results are believed to be related to the fiber geometry of the MFB. Nauta (1958, 1960), Morgane (1969) and others have pointed out that the MFB fibers are more compact in the posterior and lateral hypothalamic areas, and fan out as they ascend toward limbic forebrain structures. Since the magnitude of self-stimulation behavior is believed to be related to the overall number of "self-stimulation fibers" excited, it would be expected that more fibers could be excited, and with less current, by stimulating PH-MFB sites as opposed to POA-MFB sites where the fibers are less compactly arranged.

Bilateral Stimulation

The bilateral stimulation data not only confirmed that heterosynaptic temporal summation could be demonstrated in the MFB self-stimulation system, but also suggested that convergence of reinforcing impulses from different MFB loci on opposite sides of the brain occurs downstream from the stimulating electrodes.

The existence of heterosynaptic temporal summation in the bilateral stimulation data was based on two major findings. First,

although the animals would press approximately 10 times per minute for POA-MFB or PH-MFB stimulation alone (i.e., C-C stimulation), response rates were many times higher when both areas were stimulated together (i.e., C-T stimulation). Second, as the C-T interval was decreased, the self-stimulation rate increased. This was seen under condition Cp-Ta most clearly when self-stimulation rates increased as the C-T interval was decreased from 20-25 msec. to 0.1 msec., and also under condition Ca-Tp when the C-T interval was decreased from 20-25 msec. to 1-1.5 msec.

Although reinforcing effects can result from stimulating different sites along the MFB trajectory, it may not necessarily be the case that these effects are achieved via neurons commonly activated from these different sites. For example, self-stimulation in POA-MFB might activate a completely different and independent group of so-called self-stimulation neurons than are activated when an animal self-stimulates in PH-MFB. However, if there were not some form of convergence of impulses, then it would be expected that changing the C-T interval would have no effect on the self-stimulation rate since the behavior would merely be a function of the C-C pulse activation of one set of self-stimulation neurons plus the T-T pulse activation of another set of self-stimulation neurons. Furthermore, if there were no convergence of impulses, it would be expected that the magnitude of the bilateral C-T self-stimulation behavior would be

close to the sum of the combined magnitudes of the unilateral C-C stimulation rates. Since the animals would press about 10 times per minute at each electrode for C-C stimulation, if no convergence existed, the bilateral C-T self-stimulation rate should be around 20 presses per minute. Since both of these results did not occur, and self-stimulation rates increased as the C-T interval was decreased, the heterosynaptic temporal summation hypothesis seemed to be a parsimonious explanation of these results.

Temporal summation is a function of all of the postsynaptic potentials active on the postsynaptic neuron at any given time (Eccles, 1964). Since increases in repetitive firing of the same presynaptic ending can lead to an increased presynaptic mobilization and release of transmitter (presynaptic facilitation), and to an increased transmitter concentration in the synaptic cleft that cannot occur in the heterosynaptic situation (Liley and North, 1953; Bullock and Horridge, 1965), the summation of EPSPs in the heterosynaptic situation could well exhibit different temporal characteristics than are exhibited in the homosynaptic situation. Ungerleider and Coons (1970), however, suggested that the same mechanism was operative in their homosynaptic and heterosynaptic temporal summation data since the temporal summation decay curves were very similar under the unilateral and bilateral stimulation conditions. The presynaptic facilitation mechanism was ruled out as playing an important role in

the unilateral stimulation condition since this phenomenon cannot occur if separate presynaptic pathways are stimulated, as in the bilateral stimulation conditions.

In the present experiment, the C-C intervals employed in testing the unilateral stimulation conditions were too short to determine at which C-T interval temporal summation began to decay. This being the case, no comparison could be made with the decay of heterosynaptic temporal summation in the bilateral stimulation conditions. However, just as the temporal summation effects were long lasting for unilateral MFB stimulation, they were also long lasting for bilateral MFB stimulation. This was reflected in the high rate of bilateral self-stimulation at the 20-25 msec. C-T interval. Such results agree with the considerable heterosynaptic temporal summation found even at the 60.0 msec. C-T interval by Ungerleider and Coons (1970). It is interesting that such summation is quite long when compared with that observed in classical neurophysiological experiments. Eccles (1946) did not observe heterosynaptic temporal summation in motoneurons beyond C-T intervals of 8.0 msec. The duration of temporal summation is importantly related to the lifetime of the neurotransmitter at the synapse, and if there is prolonged transmitter action at the synapse, then the time over which EPSPs can summate is prolonged. Thus, assuming that the neurotransmitter released by MFB stimulation has such a long lifetime, the observed

temporal summation at the 20-25 msec. C-T interval can be explained. As was mentioned previously, however, other more complex neuronal interactions might explain these long lasting temporal summation results, and they cannot necessarily be ruled out by the present data.

Since heterosynaptic temporal summation had been shown to occur when homologous MFB sites were bilaterally stimulated (Ungerleider and Coons, 1970), the present experiment was aimed at differentiating between the possibilities of upstream vs. downstream convergence sites. For this purpose, MFB electrodes were bilaterally implanted at different anterior-posterior coordinates. Self-stimulation performance was found to increase from the longest to the shortest C-T interval when C pulses were given to the PH-MFB and T pulses were given to the POA-MFB, while performance under the opposite condition increased from the longest C-T interval to a relatively short C-T interval, and then decreased to the shortest interval. These results fit nicely with the hypothesis of a downstream convergence of MFB self-stimulation impulses. Such an hypothesis would predict that performance should increase from the longest to the shortest C-T interval under condition Cp-Ta since the C and T pulses are converging upon postsynaptic neurons more closely in time as the C-T interval is decreased because the C pulses are administered through an electrode which is neuroanatomically closer to the postsynaptic convergence neurons

than is the T-pulse-electrode. Under condition Ca-Tp, however, at very short C-T intervals the T pulses will activate the postsynaptic convergence neurons before the C pulses. As the C-T interval is lengthened, the C and T pulses begin to excite the postsynaptic convergence neurons simultaneously resulting in maximal heterosynaptic temporal summation and thus maximal self-stimulation performance. As the C-T interval is lengthened even further, the C pulses begin to excite the convergence neurons before the T pulses and performance declines as the C-T interval is lengthened thereafter.

Further evidence favoring the downstream MFB convergence model also exists. First, self-stimulation performance should not differ at the 0.1 msec. C-T interval between conditions Cp-Ta and Ca-Tp since the disparate arrival times of the C- and T-produced impulses to the convergence neurons is essentially the same. Second, the overall C-T interval curve should be lower under condition Cp-Ta than under condition Ca-Tp since the overall disparity in C-T impulse convergence times is greater in the former condition. Finally, similar declining self-stimulation slopes should be found on either side of the C-T interval where maximal self-stimulation occurs since the disparity in convergence times should increase symmetrically as the C-T interval is increased or decreased from the point of simultaneity in convergence. The results of the overall group of rats confirmed all of these predictions.

Although most of the individual rats tested under the bilateral stimulation conditions performed in a manner consistent with the downstream MFB convergence model, the possibility of ascending MFB self-stimulation convergence cannot necessarily be ruled out. A possible case in point is rat DG-35. Not only could this rat's performance be taken as suggestive of an ascending MFB convergence model, but other features were also unique to this rat. For example, the PH-MFB and POA-MFB electrodes of DG-35 were the most caudally located of the respective electrode groups. Furthermore, both electrodes were stimulating fibers with 0.8 msec. absolute refractory periods. And finally, the reinforcement value, defined in terms of the rate of self-stimulation at the C-C interval divided by the amount of current needed to elicit that rate, for the combined electrode sites in DG-35 was the lowest of the six bilateral stimulation animals. These differences suggest the possibility that both ascending and descending fibers, converging in anterior and posterior MFB sites respectively, may influence self-stimulation behavior, and the directionality of convergence results might simply depend upon the location of the stimulating electrodes in the MFB.

Finally, some speculation as to the possible forebrain and/or brain stem convergence sites seems warrented. Stein (1968) has presented a large amount of evidence to suggest the critical involvement of the ascending noradrenergic MFB fibers for self-stimulation

behavior. Many of these fibers have their cell bodies located in the pontine reticular formation (locus coeruleus), an area recently shown to sustain very high rates of self-stimulation (Ritter and Stein, 1972). These fibers ascend in a ventral bundle in the MFB and make synaptic connections with limbic forebrain structures (Ungerstedt, 1971). If one assumes that the forebrain noradrenergic synapses, in areas like the amygdala and septal nuclei, are important for self-stimulation, then: (a) descending MFB fibers which converge in the ventral tegmental area might in some way influence the locus coeruleus to indirectly effect self-stimulation behavior; or (b) ascending noradrenergic MFB fibers could be directly activated and converge in the medial septal nuclei or in the amygdaloid areas (via communication in the anterior commissure). Now, depending upon the loci of the stimulating electrodes, both ascending and descending MFB fibers could be shown to have a convergence effect upon self-stimulation behavior.

To summarize, the bilateral stimulation data suggested that heterosynaptic temporal summation was operative in the self-stimulation system when non-homologous MFB sites were stimulated, and that the directionality in convergence of MFB self-stimulation impulses was downstream from the stimulating electrodes. Furthermore, these results, (a) replicate the finding that the C-T technique can be used in a bilateral stimulation paradigm to circumvent the

refractory period limitations of the unilateral stimulation paradigm; (b) suggest that stimulation at different sites along the MFB trajectory influences common neuronal pools important for self-stimulation behavior; and (c) further validate the interpretation of the unilateral stimulation findings that axonal and synaptic events are reflected at short and long C-T intervals respectively.

CHAPTER V

SUMMARY

The double pulse stimulation technique has been used for some time as a neurophysiological probe to explore the poststimulation cycle of axons and synapses in the peripheral nervous system. This technique utilizes a pair of liminal pulses, one being the conditioning or C pulse whose onset is followed at a parametrically varied interval by another, the test or T pulse. The double pulse or C-T stimulation technique has been used to demonstrate such axonal phenomena as latent addition and neural refractoriness. At the synaptic level, this technique has been used to study such things as homosynaptic and heterosynaptic temporal summation, and synaptic inhibition.

The above axonal and synaptic properties have generally been demonstrated using electrophysiological response measures on acute preparations of single neurons or fiber systems. For example, in studying axonal properties, the electrical responsiveness is recorded from the same neuron or fiber system that is electrically stimulated. In studying synaptic phenomena, however, presynaptic fibers are stim-

ulated and the electrical responsiveness is recorded from the post-synaptic neurons.

Overt behavior can also be used, in place of electrophysiological measures, to analyze neurophysiological properties. Such operant responses as instrumental escape behavior, self-stimulation behavior, and runway performance have been shown to vary systematically when rats were stimulated intracranially with trains of parametrically varied C-T pulse pairs. The interval separating the onsets of the C pulses (i.e., C-C interval) is held constant, and behavioral changes are recorded as a function of changes in the C-T interval. Such operant behaviors have been capable of revealing the same neural properties as typically demonstrated with electrophysiological behaviors (i.e., latent addition, neural refractoriness, temporal summation, and synaptic inhibition).

The operant behavior most frequently used to study the neurophysiological properties of central motivational fiber systems is self-stimulation behavior. Such behavior involves an animal learning to electrically stimulate his own brain. Animals readily learn to press a lever, for example, when the medial forebrain bundle (MFB) and the structures interconnected thereby are electrically stimulated. Thus, if the rate of lever pressing is measured as a function of changes in the C-T stimulation interval, such properties as latent addition, neural refractoriness, and homosynaptic temporal

summation are revealed for the neurons activated by the pulse-pair train.

Since the MFB is known to be represented bilaterally in the brain, and converges in forebrain and midbrain areas, it seemed ideally suited for the behavioral study of heterosynaptic temporal summation. By administering C pulses to the MFB on one side of the brain, and T pulses to the MFB on the other side of the brain, if the MFB fibers influence a common area in eliciting self-stimulation behavior, then the shorter the C-T interval the greater the magnitude of self-stimulation. Heterosynaptic temporal summation was demonstrated, and it was considered as evidence that the anatomical convergence of MFB fibers was indeed functional in self-stimulation behavior. However, since the stimulation electrodes were placed in homologous MFB areas, it was not possible to determine whether the ascending or the descending impulses in the MFB were responsible for the self-stimulation summation effects.

The present experiment was addressed to two main issues. First, since the neurophysiological properties of "self-stimulation fibers" have been exclusively studied in posterior MFB areas, the present experiment sought to study such properties in anterior MFB areas. And secondly, by placing self-stimulation electrodes in both anterior and contralateral posterior MFB areas, it was predicted that the directionality of MFB self-stimulation convergence could be

analyzed.

Rats were bilaterally implanted with MFB self-stimulation electrodes, one implanted in the anterior preoptic MFB area (POA-MFB) and the other implanted in the contralateral posterior hypothalamic MFB area (PH-MFB). Self-stimulation during testing consisted of trains of C and T pulses delivered at a constant C-C interval. The C-T interval was varied from trial to trial. Just before each trial, the animal was primed with 4 trains of pulses at the same C-T interval for which he could self-administer during that trial. The number of lever presses per one-minute trial served as the dependent variable. There were two experimental conditions: (1) unilateral stimulation - C and T pulses delivered through a single MFB electrode, and (2) bilateral stimulation - C and T pulses delivered through separate electrodes to opposite sides of the brain.

The unilateral stimulation data revealed the time course of latent addition, neural refractoriness and homosynaptic temporal summation for the 9 anterior and 8 posterior MFB areas tested. Latent addition was suggested by the fact that the POA-MFB and PH-MFB self-stimulation rates were frequently higher at the 0.1 msec. C-T interval than at either the 0.5 msec. C-T interval or at the C-C interval. Absolute refractory periods of 0.8 msec. were found for all 9 POA-MFB placements tested, and 0.5 msec. for 6 of the PH-MFB placements. Two of the PH-MFB placements showed 0.8 msec.

absolute refractory periods. At these C-T intervals the self-stimulation rates were no different than when the T pulses were omitted from the stimulation train (i.e., C-C condition). Such refractory period differences between anterior and posterior areas of the MFB suggest that the fibers mediating changes in self-stimulation performance are generally of a smaller axon diameter in anterior MFB areas than in posterior areas. The refractory period values were not found to be related to the presence or absence of latent addition, and thus did not support Rolls' (1971a) hypothesis. As the C-T interval was lengthened from refractory period intervals to about 5.0 msec., self-stimulation rates increased suggesting not only the end of the absolute refractory period but the course of recovery from the relative refractory period. Maximal homosynaptic temporal summation was found at the 5.0 msec. C-T interval for PH-MFB stimulation, and after 5.0 msec. for POA-MFB stimulation. Such results suggested that by around 5.0 msec. the MFB fibers were fully recovered from refractoriness and were conducting both C and T impulses to the synapses. Finally, the self-stimulation rates were higher for PH-MFB stimulation than for POA-MFB stimulation, while the current levels were lower for PH-MFB self-stimulation than for POA-MFB self-stimulation. Such data replicated previous findings and suggest that self-stimulation rate is related to the number of "self-stimulation fibers" excited since more fibers can be excited

and with less current in posterior areas of the MFB than in anterior areas due to the geometry of the fiber system.

In the bilateral stimulation conditions, evidence of heterosynaptic temporal summation was obtained as well as evidence for a downstream brain stem convergence of MFB self-stimulation impulses. First, heterosynaptic temporal summation was suggested since the self-stimulation rates for bilateral C-T stimulation were many times higher than the combined rates under the two unilateral C-C conditions, irrespective of the C-T interval. In other words, at C-T intervals which behaviorally indicated neural refractoriness in the unilateral stimulation conditions, under the bilateral stimulation conditions such intervals sustained very high rates of self-stimulation. Secondly, when C pulses were given to the posterior electrode and T pulses to the anterior (Cp-Ta), self-stimulation rates increased from the longest to the shortest C-T interval in a linear fashion. When condition Ca-Tp was tested, self-stimulation increased from the longest to an intermediate C-T interval and then decreased to the shortest C-T interval. A downstream MFB convergence model most parsimoniously explains these results. Such a model assumes that the posterior MFB electrode is closer to the downstream convergence neurons (DCN) than the anterior MFB electrode, and thus under condition Cp-Ta the C impulses will always influence the DCN before the T impulses. Now, the magnitude of heterosynaptic temporal summation

and thus the rate of self-stimulation will increase as the C-T interval is decreased. Under condition Ca-Tp, however, if the C pulse is given a few milliseconds before the T pulse, then the two impulses should arrive at the DCN simultaneously and elicit maximal self-stimulation behavior. As the C-T interval is increased or decreased from this maximal summation point, the rate of self-stimulation should drop off. Although the downstream convergence model was strongly suggested in the present experiment, the possibility of upstream convergence from different MFB electrode placements cannot, of course, be ruled out.

The present experiment demonstrated that the C-T stimulation technique could, (a) be used to determine basic neurophysiological properties of anterior MFB areas as well as posterior MFB areas; (b) serve as a behavioral tool for determining whether separately stimulated pathways converge in the brain; and (c) make use of the phenomenon of heterosynaptic temporal summation to analyze the directionality of reinforcing impulses within the MFB self-stimulation system of the rat.

APPENDIX A

Table 6

NUMBER OF LEVER PRESSES PER MINUTE FOR UNILATERAL PH-MFB STIMULATION: INDIVIDUAL MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) AS A FUNCTION OF THE C-T INTERVAL

	DG-25 C-C=25.0		DG-26 C-C=20.0			DG-15 C-C=25.0		DG-10 C-C=20.0	
C-T	$\bar{X}$	S.D.	$\bar{X}$	S.D.	C-T	$\bar{X}$	S.D.	$\bar{X}$	S.D.
C-C	8.30	9.93	14.40	14.75	C-C	15.65	19.20	4.45	5.36
0.1	12.15	15.26	23.35	17.76	0.1	32.05	19.99	44.20	27.62
0.5	11.35	18.67	17.40	16.96	0.5	13.65	17.05	3.10	4.90
0.8	31.35	33.68	29.35	19.61	0.8	25.25	18.99	9.60	13.64
1.5	80.75	21.43	48.90	10.00	1.5	41.30	19.75	37.60	19.74
5.0	86.30	8.56	51.35	9.22	4.0	47.50	11.96	56.60	11.31
10.0	91.30	14.43	51.60	11.67	6.0	44.95	11.67	57.60	8.83

Table 6 -- continued

NUMBER OF LEVER PRESSES PER MINUTE FOR UNILATERAL PH-MFB STIMULATION: INDIVIDUAL MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) AS A FUNCTION OF THE C-T INTERVAL

	DG-35 C-C=50.0		DG-20 C-C=50.0			DG-41 C-C=30.0		DG-42 C-C=30.0	
C-T	$\bar{X}$	S.D.	$\bar{X}$	S.D.	C-T	$\bar{X}$	S.D.	$\bar{X}$	S.D.
C-C	8.75	13.98	4.35	6.65	C-C	18.90	13.19	8.45	8.79
0.1	61.05	17.11	10.15	12.46	0.1	35.25	11.32	40.95	29.88
0.5	21.75	23.27	4.75	5.65	0.5	24.10	11.82	17.15	11.83
0.8	28.95	21.01	7.65	8.03	0.8	47.00	19.71	38.55	19.61
1.5	54.04	16.94	27.75	15.49	1.5	88.65	17.64	90.05	21.87
5.0	61.05	9.30	49.05	10.74	5.0	97.75	17.17	106.40	22.82
25.0	62.05	11.09	49.85	11.06	10.0	104.45	15.92	110.90	26.82

Table 7

NUMBER OF LEVER PRESSES PER MINUTE FOR UNILATERAL POA-MFB STIMULATION: INDIVIDUAL MEANS ($\overline{X}$) AND STANDARD DEVIATIONS (S.D.) AS A FUNCTION OF THE C-T INTERVAL

	DG-35 C-C=50.0		DG-20 C-C=50.0		DG-23 C-C=50.0			DG-15 C-C=25.0	
C-T	$\overline{X}$	S.D.	$\overline{X}$	S.D.	$\overline{X}$	S.D.	C-T	$\overline{X}$	S.D.
C-C	4.55	5.14	5.30	8.86	7.40	9.99	C-C	11.10	8.72
0.1	12.95	12.40	8.60	9.77	9.85	10.12	0.1	23.63	8.02
0.5	9.00	11.60	4.65	6.59	9.00	10.11	0.5	12.79	10.95
0.8	7.10	10.31	3.75	4.34	8.80	7.10	0.8	13.47	6.96
1.5	26.20	16.41	13.90	10.13	40.10	16.20	1.5	24.00	6.59
5.0	44.40	7.80	33.65	8.64	49.60	8.13	4.0	27.63	6.62
25.0	49.15	9.68	37.15	6.11	49.95	8.31	6.0	30.26	8.72

Table 7 -- continued

NUMBER OF LEVER PRESSES PER MINUTE FOR UNILATERAL POA-MFB STIMULATION: INDIVIDUAL MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) AS A FUNCTION OF THE C-T INTERVAL

	DG-30 C-C=20.0		DG-31 C-C=25.0		DG-42 C-C=30.0		DG-41 C-C=40.0			DG-10 C-C=20.0	
C-T	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	C-T	$\bar{X}$	S.D.
C-C	9.00	8.84	23.05	13.62	4.15	4.68	14.15	15.70	C-C	12.75	7.71
0.1	32.30	7.27	54.40	12.81	8.98	11.21	34.60	19.76	0.1	25.95	9.74
0.5	15.80	8.46	25.26	10.70	5.30	6.21	17.20	19.87	0.5	12.85	6.04
0.8	14.85	10.22	30.30	13.52	8.95	9.72	18.10	16.76	0.8	13.90	7.93
1.5	30.45	6.71	47.80	10.26	24.50	14.51	27.35	16.26	1.5	22.95	6.79
5.0	31.60	7.21	56.90	12.65	34.30	7.60	49.25	11.59	4.0	39.25	6.15
10.0	30.90	4.93	62.00	10.24	36.55	9.20	50.20	8.91	6.0	39.20	6.62

Table 8

INDEPENDENT T TESTS ON THE NUMBER OF LEVER PRESSES PER MINUTE - SIGNIFICANT DIFFERENCES BETWEEN C-T INTERVALS FOR INDIVIDUAL RATS TESTED UNDER THE UNILATERAL PH-MFB CONDITION

C-T Comparisons	Rats DG-35	DG-20	DG-15	DG-25	DG-10	DG-26	DG-41	DG-42
Latent Addition								
0.1 vs. C-C	10.32[c]	1.79[a]	2.58[b]	0.94	6.16[c]	1.69[a]	4.10[c]	4.55[c]
0.1 vs. 0.5	5.93[c]	1.72[a]	3.06[b]	0.14	6.39[c]	1.06	2.97[b]	3.23[b]
Refractory Period								
0.5 vs. C-C	2.09[a]	0.20	0.34	0.64	0.81	0.58	1.28	2.57[b]
0.8 vs. C-C	3.49[b]	1.38	1.55	2.88[b]	1.53	2.66[b]	5.17[c]	6.11[c]
0.8 vs. 0.5	1.00	1.29	1.98[a]	2.26[a]	1.96[a]	2.01[a]	4.35[c]	4.08[c]
Recovery from Refractoriness								
1.5 vs. 0.8	4.06[c]	5.03[c]	2.56[b]	5.40[c]	5.09[c]	3.87[c]	6.87[c]	7.65[c]
4,5 vs. 1.5	1.58	4.93[c]	1.17	1.05	3.64[c]	0.79	1.61	2.26[a]
Temporal Summation								
6,10,25 vs. 4,5	0.30	0.22	0.66	1.30	0.30	0.07	1.25	0.56

[a] Significance, $p < .05$, one-tailed test.

[b] Significance, $p < .01$, one-tailed test.

[c] Significance, $p < .001$, one-tailed test.

Table 9

INDEPENDENT T TESTS ON THE NUMBER OF LEVER PRESSES PER MINUTE - SIGNIFICANT DIFFERENCES BETWEEN C-T INTERVALS FOR INDIVIDUAL RATS TESTED UNDER THE UNILATERAL POA-MFB CONDITION

C-T Comparisons	Rats DG-35	DG-20	DG-23	DG-15	DG-31	DG-10	DG-30	DG-41	DG-42
Latent Addition									
0.1 vs. C-C	2.73[b]	1.09	0.75	4.61[c]	7.30[c]	4.64[c]	8.88[c]	3.54[c]	1.72[a]
0.1 vs. 0.5	1.01	1.46	0.26	3.48[b]	7.35[c]	4.99[c]	6.45[c]	2.71[b]	1.24
Refractory Period									
0.5 vs. C-C	1.53	0.26	0.49	0.53	0.81	0.04	2.42[a]	0.53	0.64
0.8 vs. C-C	0.97	0.68	0.50	0.93	1.65	0.45	1.89[a]	0.75	1.94[a]
0.8 vs. 0.5	0.53	0.50	0.07	0.23	1.02	0.46	0.31	0.15	1.38
Recovery from Refractoriness									
1.5 vs. 0.8	4.30[c]	4.02[c]	7.72[c]	4.79[c]	4.50[c]	3.78[c]	5.57[c]	1.73[a]	3.88[c]
4,5 vs. 1.5	4.37[c]	6.47[c]	2.29[a]	1.70[a]	2.44[b]	7.76[c]	0.51	4.78[c]	2.61[b]
Temporal Summation									
6,10,25 vs. 4,5	1.67	1.44	0.13	1.41	1.37	0.02	0.35	0.23	0.82

[a] Significance, $p < .05$, one-tailed test.

[b] Significance, $p < .01$, one-tailed test.

[c] Significance, $p < .001$, one-tailed test.

Table 10

NUMBER OF LEVER PRESSES PER MINUTE FOR BILATERAL Ca-Tp STIMULATION: INDIVIDUAL MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) AS A FUNCTION OF THE C-T INTERVAL

		C-T Intervals					
Rat		20.0	6.0	3.0	1.0	0.1	C-C
DG-35	$\bar{X}$	33.95[a]	32.50	43.65	43.70	43.90	6.50
	S.D.	22.47	19.19	19.13	20.78	17.20	5.96
DG-41	$\bar{X}$	53.95	71.60	84.45	83.80	78.15	21.85
	S.D.	19.22	24.59	23.84	23.22	26.98	13.26
DG-42	$\bar{X}$	64.10	59.40	71.85	76.05	61.00	8.80
	S.D.	23.02	25.69	19.99	17.44	32.05	7.45

		C-T Intervals						
		20.0	5.0	1.5	0.8	0.5	0.1	C-C
DG-20	$\bar{X}$	56.05	52.20	55.35	58.40	49.50	42.25	10.40
	S.D.	14.42	15.46	11.93	8.83	17.78	19.95	13.73
DG-10	$\bar{X}$	46.25	66.05	68.10	65.55	59.50	30.20	4.40
	S.D.	33.26	17.87	21.76	26.36	27.11	27.67	9.53
DG-15	$\bar{X}$	51.35	51.51	56.00	56.70	54.95	45.60	12.70
	S.D.	14.10	17.65	17.52	14.76	15.64	18.87	14.04

[a] C-T interval = 25.0 msec.

Table 11

NUMBER OF LEVER PRESSES PER MINUTE FOR BILATERAL Cp-Ta STIMULATION: INDIVIDUAL MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) AS A FUNCTION OF THE C-T INTERVAL

Rat		C-T Intervals 20.0	6.0	3.0	1.0	0.1	C-C
DG-35	$\bar{X}$	24.40[a]	37.65	44.05	37.60	34.95	2.65
	S.D.	11.85	18.44	16.05	15.45	14.38	2.66
DG-41	$\bar{X}$	54.30	72.80	68.00	73.25	67.75	10.30
	S.D.	23.61	22.99	15.02	18.19	25.52	8.78
DG-42	$\bar{X}$	39.40	49.05	62.55	58.20	58.10	3.80
	S.D.	34.16	37.60	22.93	23.36	23.82	4.37

		C-T Intervals 20.0	5.0	1.5	0.8	0.5	0.1	C-C
DG-20	$\bar{X}$	61.05	57.75	59.30	55.35	63.80	65.95	15.00
	S.D.	15.77	19.31	15.60	15.86	17.83	12.81	18.27
DG-10	$\bar{X}$	51.35	57.40	54.10	57.40	54.30	56.15	12.25
	S.D.	29.04	28.58	27.77	29.12	33.66	18.18	18.92
DG-15	$\bar{X}$	47.75	48.40	51.70	57.05	52.60	56.35	16.15
	S.D.	22.68	18.33	20.15	16.93	18.04	16.05	14.08

[a] C-T interval = 25.0 msec.

Table 12

INDEPENDENT T TESTS ON THE NUMBER OF LEVER PRESSES PER MINUTE - SIGNIFICANT DIFFERENCES BETWEEN C-T INTERVALS FOR INDIVIDUAL RATS TESTED UNDER THE BILATERAL STIMULATION CONDITIONS

	Ca-Tp			Cp-Ta		
Rat	20-25 vs. 1-1.5	20-25 vs. 0.1	1-1.5 vs. 0.1	20-25 vs. 1-1.5	20-25 vs. 0.1	1-1.5 vs. 0.1
DG-35	-1.39[a]	-1.53[a]	-0.30	-2.96[c]	-2.47[c]	0.54
DG-41	-4.32[c]	-3.18[c]	0.69	-2.77[c]	-1.69[b]	0.76
DG-42	-1.80[b]	0.34	1.80[d]	-1.98[b]	-1.96[b]	0.01
DG-10	-2.40[b]	1.62[a]	4.70[e]	-0.30	-0.61	-0.27
DG-15	-0.90	1.06	1.76[d]	-0.56	-1.35[a]	-0.78
DG-20	0.16	2.44[c]	2.46[e]	0.34	-1.05	-1.44

[a] Significance, $p < .10$, one-tailed test.

[b] Significance, $p < .05$, one-tailed test.

[c] Significance, $p < .01$, one-tailed test.

[d] Significance, $p < .10$, two-tailed test.

[e] Significance, $p < .05$, two-tailed test.

APPENDIX B

Taking advantage of the convergence of MFB fibers from opposite sides of the brain, the present experiment demonstrated that the magnitude of heterosynaptic temporal summation could be used to study the directionality of reinforcing impulses within the MFB. In analyzing the bilateral stimulation data, however, it became apparent that certain differences existed between the six rats in this phase of the experiment which might be important in the determination of directionality of reinforcing MFB impulses.

Although the current level was adjusted such that the rat would press about 10 times per minute-trial under the C-C stimulation condition, lever pressing rates sometimes changed after a current level had been selected and tested for some time. Specifically, rats DG-35, DG-41 and DG-42 pressed significantly higher when unilateral C-C pulses were given to the POA-MFB electrode than when C-C pulses were given to the PH-MFB electrode (independent t tests with $df = 38$ were 2.57, $p < .02$; 3.16, $p < .01$; 2.52, $p < .02$, respectively). These three rats were said to be "anterior dominant" (AD) since the anterior electrode produced a higher self-stimulation rate than

the posterior electrode. Rats DG-10, DG-15 and DG-20, however, self-stimulated only slightly higher for PH-MFB stimulation than for POA-MFB stimulation at the C-C interval (nonsignificant differences). These three rats were said to be "non-dominant" (ND).

When the bilateral stimulation data were analyzed in terms of electrode dominance, some interesting correlations resulted. For example, when comparing the independent t values for individual rats (Table 12 in Appendix A) which express the degree of decline in self-stimulation rate from the 1-1.5 msec. C-T interval to the 0.1 msec. interval under condition Ca-Tp, it was found that the ND group accounted for most of the overall variance in the decline in self-stimulation between these intervals (ND - zero mu t = 3.35, df = 2, $p<.10$: AD - nonsignificant t). A similar finding occurred when analyzing these same independent t values comparing the slope differences between conditions Ca-Tp vs. Cp-Ta from the 1-1.5 to the 0.1 msec. C-T interval. The ND group showed a greater difference between these C-T intervals under the two bilateral stimulation conditions than the AD group (ND - correlated mean t = 5.42, df = 2, $p<.05$: AD - nonsignificant t). Such results indicate that for the ND group, the change in self-stimulation rate between the 1-1.5 and 0.1 msec. C-T intervals is highly related to which bilateral stimulation condition is tested (i.e., Ca-Tp or Cp-Ta). Such a statement, however, cannot be made for the AD group. Further analyses revealed that, in

general, the ND group demonstrated a more clear cut downstream convergence of MFB self-stimulation impulses than did the AD group. Since the current levels were not varied to create AD and ND conditions within the same animal, however, it is not possible to draw causal relations demonstrating that the electrode dominance condition effected the outcome of the directionality findings. These data do suggest that more clear cut findings on directionality will result if the current levels are adjusted to minimize the effects of electrode dominance.

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