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Comparisons of the Medial Forebrain Bundle Substrates  
Subserving Stimulation-Induced Feeding and Brain Stimulation Reward

Alain Gratton

A Thesis  
in  
The Department  
of  
Psychology

Presented in Partial Fulfillment of the Requirements  
for the Degree of Doctor of Philosophy at  
Concordia University  
Montréal, Québec, Canada

November 1985

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## ABSTRACT

Comparisons of the Medial Forebrain Bundle Substrates  
Subserving Stimulation-Induced Feeding and Brain Stimulation Reward

Alain Gratton, Ph.D.  
Concordia University, 1985

Psychophysical techniques were used to explore the possibility that stimulation-induced feeding (SIF) and brain stimulation reward (BSR) are subserved by a common or by separate populations of medial forebrain bundle (MFB) fibers. Mapping of the anterior-posterior extent of the MFB for SIF with moveable electrodes showed that feeding can be elicited with electrode placements extending from the anterior region of lateral hypothalamic area to the ventral tegmental area. The dorsal-ventral distribution of MFB sites that support SIF and BSR appear to be co-extensive and the relative sensitivities of each site to the rewarding and motivating effects of the stimulation were well correlated. Behaviorally inferred estimates of refractory periods of the fibers underlying SIF and BSR were similar, suggesting that SIF and BSR are subserved by the same population of MFB fibers or by different populations of fibers with very similar refractory period characteristics. Finally, the refractory period functions for SIF and BSR each exhibited a near-zero slope between C-T intervals of 0.6 and 0.7 msec. This feature of the refractory period functions was interpreted as suggesting that the MFB substrates for SIF and BSR each comprise two sub-populations of fibers with non-overlapping distributions of refractory periods. To test this hypothesis, the effects of

central muscarinic receptor blockade on the refractory period distribution for BSR were assessed. Because atropine blocks salivary secretion and disrupts feeding, a similar test could not be made in the SIF paradigm. Atropine sulfate eliminated the early component of the refractory period functions for BSR; initial increases in T-pulse effectiveness values occurred at a longer C-T interval (0.8 msec) under atropine sulfate treatment than seen under control conditions (approx. 0.5 msec). The dopamine receptor blocker, pimozide, had no effect on the distribution of refractory period estimates, suggesting that dopaminergic systems do not contribute to the rewarding effect of the stimulation at the first stage system. Based on current knowledge of the anatomy of cholinergic pathways, the data suggest that the reward circuitry contains a cholinergic component which receives inputs from first stage reward fibers that have refractory periods in the 0.5 to 0.7 msec range. Other interpretations of the data are presented and discussed.

Anatomical linkage between lateral hypothalamic and ventral tegmental area SIF sites was established by applying a behavioral variation of the antidromic collision test. The results of the collision study suggest that SIF-relevant fibers extend along the longitudinal axis of the MFB, at least between the lateral hypothalamic area and the ventral tegmental area. Furthermore, in animals where collision effects for SIF were obtained, similar collision effects for BSR were also obtained. Estimates of conduction velocity for SIF-relevant fibers (2.3 to 4.3 m/sec)

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were within the range of previously reported conduction velocity estimates for BSR-relevant fibers. As a whole the data greatly reduce the credibility of the hypothesis that SIF and BSR are subserved by independent MFB first stage systems.

## ACKNOWLEDGEMENTS

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## GENERAL INTRODUCTION

## A) The substrate for medial forebrain bundle self-stimulation.

Rats will learn a variety of responses, including pressing a lever, to administer trains (bursts) of electrical pulses to their own brain. Some of the most sensitive sites and those that yield some of the most vigorous self-stimulation behavior are found along the 6mm length of the medial forebrain bundle (MFB) extending between the ventral tegmental area (VTA) and the hypothalamic preoptic area.

The MFB comprises approximately 50 discrete fiber systems, some of which course through the MFB in a rostral-caudal direction, whereas other systems project their fibers in the opposite direction (Nieuwenhuys et al., 1982). It is unlikely that all these fiber systems are involved in BSR. A few or possibly only one fiber system is relevant to the rewarding effect of MFB electrical stimulation. The BSR specialist is faced with the difficult task of determining which of these fiber systems carries the reward signal triggered by the electrical stimulation. The problem is compounded by the fact that electrical stimulation is not selective in the neuronal excitation it produces; any number of fiber systems within the field of effective stimulation may be activated.

A number of different strategies have been employed since

the discovery of BSR to uncover the identity of the substrate for MFB reward. One strategy employs techniques borrowed from psychophysics. Over the past decade "trade-off" experiments have been used to establish a number of properties of the reward-relevant substrate of the MFB. In essence, trade-off experiments involve determining the value of a particular stimulation parameter required to offset the effect of varying another stimulation parameter in order to maintain a constant level of behavior. In addition to providing some of the electrophysiological and anatomical characteristics of the MFB substrate directly activated by rewarding stimulation, trade-off experiments have generated a useful model of the reward circuitry and therefore testable assumptions about the functions of each of its basic components.

1) The minimal model.

Gallistel (1978) has developed a minimal model of BSR to incorporate the basic functional components the reward circuitry should contain to account for the electrophysiological characteristics of the BSR substrate which have emerged from trade-off experiments thus far. It is a minimal model in the sense that it makes few assumptions about either the anatomical locus from which the rewarding experience arises or the precise mechanism through which it is generated.

a) The "cable" or "first stage" system comprises those MFB

reward-relevant fibers which lie directly under the electrode tip and which are depolarized by a pulse of electrical current. Unless stated otherwise, these are the fibers of concern in the present investigation.

b) The "integrator" or "second stage" system represents the neural network through which the neurophysiological signal must pass in order to eventually generate a rewarding effect. The model makes no other assumption about the second stage system except that it must sum both spatially and temporally the output from the first stage system.

c) The "conversion process" is assumed if the animal is to keep an enduring record of the rewarding effects of the stimulation. Such a postulation is made necessary by the fact that BSR, like conventional rewards, improves an animal's performance across successive trials (Gallistel et al., 1974). For this to happen the animal must remember, from previous trials the magnitude of the rewarding effect. The conversion process is where the magnitude of the rewarding effect is measured and converted into a permanent memory.

## 2) Electrophysiological & anatomical characteristics of the first stage system.

Considerable information has been gained about the manner in which the first stage system reacts to different combinations



of stimulation parameters. From this information, several electrophysiological and anatomical characteristics of the first stage system have emerged. The neurophysiological properties of the first stage system have been derived from four types of trade-off experiments.

a) Post-stimulation excitability cycles.

An action potential will leave in its wake a portion of the axon in a state of refractoriness to any further stimulation. The amount of time an axon remains in this state is the refractory period. When conventional unit recording techniques are used, the refractory period of a neuron can be measured by estimating the minimum interval that must be allowed to pass before a second pulse will elicit a second action potential. Hence, by systematically shortening the interval between the first (C-pulse) and second (T-pulse) pulse of a pair of pulses, a point will be reached where the two pulses will elicit only one action potential; this indicates that the T-pulse falls within the refractory period of the action potential elicited by the C-pulse. This approach has been used to estimate the refractory periods of the first stage system of the reward circuit, except that in this case the ability of the T-pulse in eliciting a second action potential was inferred behaviorally by determining the number of pulse pairs required for criterial behavior at various intra-pair (C-T) intervals. Because a population of relevant fibers with varying refractory periods is activated by

the stimulation, the function relating the C-T interval to the effectiveness of the T-pulse in producing a second action potential rises gradually. In the MFB, the reward relevant fibers have refractory periods ranging from 0.4 to 2.0 msec (Bielajew et al., 1981; Rompre & Miliaressis, 1980; Yeomans, 1975). It appears that these values reflect in large part the contribution of absolute refractory periods of the reward relevant fibers (Yeomans, 1979), although a contribution of relative refractory periods has been demonstrated (Bielajew et al., 1982). These behaviorally inferred estimates of neural refractory periods fall within the range of refractory periods of myelinated axons recorded in the CNS by conventional electrophysiological techniques (Waxman & Swadlow, 1977) and suggest that the first stage system is made up of fine myelinated fibers in the 0.2 to 3.0 micrometer diameter range (Szabo et al., 1974; Yeomans, 1975).

That the behaviorally inferred refractory periods of the first stage system truly reflect the post-stimulation excitability properties of reward relevant fibers is suggested by several features of the refractory period curve. For instance, following the refractory period some axons may become unusually sensitive to further stimulation (Swadlow & Waxman, 1979). This period of hyperexcitability (or supernormal period) is observed when unequal amplitude pulse pairs (C>T) are used and manifests itself by an unusually high T-pulse effectiveness at C-T intervals in the 2.0 to 10.0 msec range (Rompre, 1984; Yeomans, 1979). However, at

very short C-T intervals (0.15 to 0.4 msec) the effectiveness of the T-pulse decreases as a function of increasing the C-T interval. This portion of the refractory period curve reflects summation of local non-propagated potentials at the fringes of the field of effective stimulation. Axons which are not brought to firing threshold by the C-pulse define the fringe of the field of effective stimulation. These axons, will, however be partially depolarized by the C-pulse. If the T-pulse arrives fast enough it will be able to build upon the depolarization left behind by the C-pulse and bring some of the axons to firing threshold. At longer C-T intervals however, the depolarization elicited by the C-pulse will have dissipated before the arrival of the T-pulse; in this case no local potential summation will occur. Using current threshold measures Yeomans et al. (1979) concluded that local potentials in reward relevant fibers decay exponentially (at C-T intervals from 0.05 to 0.35 msec) with a time constant of approximately 0.1 msec, although there was evidence for some fibers with longer time constants. The exponential rate of decay of local potentials in reward fibers found by Yeomans et al. (1979) compared favorably with their own data obtained from unit recordings, indicating that presumed local potential summation effects observed with behavioral measures can be reasonably ascribed to a true neurophysiological property of reward fibers.

The contention that refractory periods inferred from behavioral measures can map the post-stimulation excitability

cycles of reward relevant fibers is also supported by several studies that show different rates of recovery from refractoriness at other BSR sites. Prefrontal cortex (Schenk et al., 1980), periaqueductal grey (Bielajew et al., 1981), midline hindbrain (Rompre, 1984) and thalamic (Rompre, 1984) BSR sites all appear to be subserved by fibers with slower rates of recovery from refractoriness. The technique is also capable of discriminating between the substrates for different electrically elicited behaviors. The rate of recovery from refractoriness for fibers in the hindbrain rotation substrate (Miliaressis & Rompre, 1980) and fibers in the lemniscal aversion substrate (Dennis et al., 1976) is faster than what is seen for MFB self-stimulation.

b) Anatomical linkage

By applying a pair of pulses through the same electrode and varying the C-T interval, an estimate of the range of refractory periods of the first stage system is obtained. If the C and T pulses are applied through two different electrodes and the C-T interval is varied in the same manner, it is possible to determine if the two BSR sites are connected by a common set of reward-relevant fibers. The double electrode technique used to determine anatomical linkage takes advantage of the fact that a suprathreshold pulse of cathodal current will trigger two action potentials. One action potential will propagate antidromically towards the soma, while the orthodromic action potential will come to bear upon synaptic transmission. If electrode A and

electrode B are stimulating different portions of the same axon and assuming that electrode A lies between electrode B and the soma, one of two things will happen depending on the interval between the C and T pulses and the conduction velocity properties of the axon. At short C-T intervals, the orthodromic impulse triggered by the C-pulse in electrode A will not have time to propagate past electrode B before the antidromic impulse is triggered by the T-pulse. In this case collision between the two impulses will ensue and only the orthodromic impulse from electrode B (T-pulse) will reach the synapse. However, if the C-T interval is sufficiently long, the orthodromic impulse (and its refractory period) originating from electrode A will clear electrode B before any impulse is triggered by the T-pulse. In this case the orthodromic action potentials triggered at both electrode sites will reach the synapse and presumably double the net post-synaptic level of excitation. The interval required by the impulse from electrode A to propagate past electrode B is the collision interval which is the sum of the time of conduction of the impulse between the two electrode sites and its refractory period.

Anatomical linkage of ventral tegmental and lateral hypothalamic BSR sites has been demonstrated by adapting the collision test to behavioral trade-off experiments, where again the number of pulse pairs is traded-off against the C-T interval (Shizgal et al., 1980). By applying a train of C-pulses to the lateral hypothalamus (LH) and a train of T-pulses to the ventral

tegmental area (VTA), Shizgal et al. (1980) showed that within a restricted range of C-T intervals (as short as 0.5 msec) the effectiveness of the T-pulse rises abruptly. This unique feature of the function relating T-pulse effectiveness to C-T interval in the double electrode experiment reflects the collision interval of reward relevant fibers coursing between, and common to LH and VTA BSR sites. The fact that T-pulse effectiveness increases abruptly at the same critical C-T interval when the T-pulse is applied to either the LH or the VTA strengthens the conclusion that these two BSR sites are linked by a common set of fibers and suggests that conduction of the reward signal between the two sites is not interrupted by a synapse.

Behavioral collision experiments have also provided the means to estimate the conduction velocity of reward relevant fibers. By dividing the inter-electrode distance by the collision time (collision interval - refractory period = collision time), it has been concluded that reward relevant fibers have conduction velocities in the 1.0-7.8 m/sec range (Bielajew & Shizgal, 1982; Shizgal et al., 1980). Again these conduction velocity estimates are compatible with those of small myelinated fibers (Waxman & Bennett, 1972) which are known to exist in the MFB (Szabo et al., 1974).

#### c) Current integrating properties

The intensity of a pulse of cathodal current necessary to

elicit a single action potential in a neuron depends to large extent on the duration of the pulse and the nature of the neural element being stimulated (i.e. soma, myelinated vs unmyelinated axon). As the pulse duration is increased the required current intensity decreases until any further increases in pulse duration entail negligible reductions in required current intensity. The required current intensity at infinitely long pulse durations is the rheobase and in essence defines the limits of the neuron's ability to integrate current over time. Expressed differently, the rheobase reflects the minimum current flux that will maintain a suprathreshold depolarization over an infinitely long period of time. A somewhat more convenient measure of the rate at which the intensity or strength-duration function approaches its rheobase is the chronaxie: the pulse duration at which the required current intensity is twice the rheobase. Several variables will affect the shape of the strength-duration function. An analysis of each is beyond the scope of this review. The process of accommodation, however, is relevant to present purposes and will be briefly explained later.

By trading-off current intensity against pulse duration, Matthews (1977) inferred the current integrating properties of MFB reward fibers. Chronaxies in the 0.7 to 3.0 msec range (mean=1.5 msec) were obtained by fitting a hyperbolic function to the resulting strength-duration data (Gallistel, 1978; Matthews, 1977). Similar chronaxie estimates for MFB reward fibers have been reported in a more recent study (Schenk & Shizgal, 1985).

Contrasted against chronaxies of 0.15 to 0.48 msec for cortico-spinal fibers subserving skeletal muscle twitches (Matthews, 1977), these data suggest that some reward fibers are capable of integrating current over exceptionally long periods. In fact, most of Matthews' (1977) strength-duration curves do not reach rheobase at pulse durations of up to 15.0 msec: thus the necessity to derive chronaxie values from a hyperbola fitted to the data. One possible explanation for the continued decrease in current intensity requirements is that some reward fibers fire several times in response to pulses of long duration. This would be equivalent to increasing the pulse frequency thus allowing the current intensity required to maintain criterial behavior to be lowered.

Also telling of the current integrating properties of the first-stage system are the strength-duration data using pulses of anodal current. Matthews (1977) showed that anodal current requirements at various pulse durations were about twice the cathodal current requirements, except at longer pulse durations where the required anodal current was at times lower than the required cathodal current. Because anodal current will hyperpolarize the membrane, the fact that anodal current is more effective at long pulse durations than cathodal current can probably be ascribed to anodal break firing. Unlike cathodal current, hyperpolarization produced by a prolonged anodal pulse will cause the firing threshold of the neuron to accommodate to a new, more negative potential. Immediately upon offset (break) of



the anodal pulse, the rapid depolarization of the membrane towards its resting potential may cause it to overshoot the new firing threshold and generate an action potential. At shorter pulse durations however, the fact that anodal current is capable of maintaining self-stimulation can probably be attributed to a depolarizing current flowing at some point removed from the electrode tip. The hyperpolarizing influx of current during an anodal pulse will be counterbalanced at a remote portion of the neuron by a depolarizing current efflux which may trigger an action potential. The effectiveness of the remote current efflux in generating an action potential will depend on its density along the membrane. If the current efflux is restricted to a small patch of membrane, as would be the case in a myelinated axon, the depolarizing current may bring the potential to firing threshold. Under these conditions the nodes of Ranvier would be susceptible to a suprathreshold depolarization. This explanation would be consistent with other evidence indicating that the first stage system comprises myelinated fibers.

d) Normal direction of conduction of the reward-relevant signal

The MFB comprises several discrete fibers systems some of which project rostro-caudally while others project in the opposite direction. Several attempts have been made to determine the normal direction of conduction of the first stage system. These studies usually involved lesioning tissue either anterior

or posterior to the BSR electrode and measuring the subsequent loss of responsiveness to the rewarding stimulation. In general posterior lesions appear to be more effective than anterior lesions in suppressing rate of responding for BSR (Keesey & Powley, 1973; Olds & Olds, 1969; Stellar & Neeley, 1982). The straightforward interpretation of these data is that the reward-relevant signal is carried in the rostral-caudal direction and that posterior lesions disconnect the BSR electrode from the reward relevant synapse. However, as discussed by Bielajew and Shizgal (note 3), these studies are subject to several criticisms which diminish the significance of their conclusions. First there is no way of knowing whether the lesion destroys fibers of the first stage system or fibers at several synapses removed from the first stage system. Second, loss of responsiveness to BSR can be ascribed to other effects associated to MFB lesions which may or may not be related to a decreased effectiveness of the rewarding stimulation; these include aphagia and adipsia (Teitelbaum & Epstein, 1962) and loss of sensory-motor integration (Marshall & Teitelbaum, 1974). Third and most importantly, anterior lesions which are assumed to disconnect the BSR electrode from the soma may also affect the conductivity of the distal part of the axon. Furthermore MFB fibers have been shown to regenerate following transection (Foerster, 1982). Thus any conclusion on the directionality of the reward signal based on lesion studies has to be considered with caution.

A more selective way of establishing directionality of the

reward signal in the first stage system would be to temporarily diminish the excitability of a portion of the reward fibers which lies between the BSR electrode and reward relevant synapses. Shizgal et al. (1980) and Bielajew & Shizgal (note 1) used a variation of the collision test to demonstrate that reward relevant fibers extending between the LH and VTA carry the reward signal primarily in the rostral-caudal direction. Using rats, which showed a collision-like effect with LH and VTA electrodes, they reasoned that if a hyperpolarizing pulse of anodal current is administered through the electrode which lies between the reward-relevant synapse and the depolarizing electrode, the orthodromically propagated reward signal would be blocked at the region of hyperpolarization and not reach the reward relevant synapse. No such effect would be expected if the anodal pulse is applied to the portion of the reward fibers which lies between the soma and the depolarizing electrode.

The hyperpolarization block experiment is in effect a cathodal strength-duration experiment using four different electrode configurations. Under the two control electrode configurations either one of the depth electrodes (LH or VTA) serves as the cathode while the anode is an indifferent electrode (a set of screws imbedded in the cranium). The experimental conditions consist using each one of the depth electrodes as the cathode while the other is used as the anode. Shizgal et al. (1980) showed that the strength-duration curve obtained with the cathode in VTA and anode in LH did not differ from the

corresponding control curve: the curve obtained with the cathode in VTA and the anode in the indifferent electrode. When the reverse configuration was used (anode in VTA and cathode in LH) the resulting strength-duration curve deviated upwards from the corresponding control curve (cathode in LH and anode in indifferent electrode) at long pulse durations. In other words, compared to the control curve, the cathodal current intensity requirements for criterial LH self-stimulation increased at long pulse durations (1.0 to 5.0 msec) when the VTA electrode served as the anode. The fact that cathodal current intensity requirements increase only at relatively long pulse durations suggests that at shorter pulse durations the hyperpolarization block had dissipated and thus did not impede the propagation of the orthodromic reward signal triggered by the LH electrode. Hence it appears that when the region of the VTA is hyperpolarized, the effectiveness of rewarding stimulation applied to the LH is reduced; no such loss in effectiveness of VTA rewarding stimulation is seen when the LH is hyperpolarized. The straightforward interpretation of these data is that the reward signal is propagated by a significant portion of the reward substrate in the caudal-rostral direction: from LH to VTA.

e) Neurochemical identity of the first stage

The neurotransmitter responsible for carrying the reward signal across the first stage synapse has not yet been identified. Whether or not it is reasonable to assume that only one

neurotransmitter is involved in this task is also open to debate. However some evidence has recently been marshalled for an involvement of ventral tegmental muscarinic receptors in BSR (Yeomans et al., 1985). The idea of a cholinergic system as the first stage reward system for BSR will be discussed later on in this work.

Although the trade-off experiments outlined above have not yet identified the pharmacological properties of the first stage system, they have provided the electrophysiological criteria to guide the search for a possible candidate. The candidate system must comprise small myelinated fibers some of which should course rostral-caudally along the longitudinal extent of the MFB from the lateral hypothalamus to the ventral tegmental area. Most of the fibers of the first stage system should have absolute refractory periods in the 0.4 to 2.0 msec range and propagate impulses at velocities of 1-8 meters per second. The local non-propagated potentials of the first stage fibers should decay exponentially with a time constant of approximately 0.1 msec. Finally the reward fibers should have chronaxies of 0.7 to 3.0 msec, be able to integrate cathodal current over periods as long as 15.0 msec and fire on the offset of anodal pulses longer than 5.0 msec.

The monoaminergic systems which course through the MFB, and in particular midbrain dopaminergic neurons which play a role in BSR, cannot constitute a significant part of the first stage

system. The behaviorally inferred electrophysiological properties of the first stage system do not fit those of dopaminergic neurons and therefore cannot account for the effects of rewarding stimulation directly under the electrode tip. Dopaminergic neurons ascend through the MFB (Lindvall & Bjorklund, 1974) whereas at least a major portion of the first stage system descends through the MFB. The first stage system is capable of propagating impulses at velocities which by far exceed those which are seen in dopaminergic neurons (Feltz & Albe-Fessard, 1972; German et al., 1980; Yim & Mogenson, 1980). The role of dopamine in BSR must therefore come into play at least one, but maybe several synapses away from the electrode tip.

### 3) Quantitative properties of the second stage system

The second stage system or "integrator" is that component of the reward circuit which receives the synaptic output of the first stage system. The second stage system must summate both in time and in space the net output of the first stage system. The manner in which the second stage subsequently treats the reward signal it receives from the first stage system has been characterized by trading-off current intensity against the number of pulses in a train of fixed duration.

#### a) Spatial-temporal integration: the counter model.

The rate at which a rat self-stimulates can be augmented by increasing either the current intensity or the number of pulses in a train of fixed duration. Although these two variables affect the rate of self-stimulation behavior in the same direction, they do so by different means. Pulse frequency is a temporal variable. Assuming that each pulse fires a reward fiber only once, then varying the pulse frequency changes the number of times each first stage fiber will fire. Thus there is a scalar relation between the number of times a fiber fires and the frequency of stimulation. Current intensity is a spatial variable; varying the current intensity changes the size of the field of effective stimulation and, by extension, the number of first stage fibers that are fired by the stimulation. The current flux of effective stimulation decreases as the square root of the radial distance from the electrode tip (Fouriezos & Wise, 1985; Ranck, 1975). However, assuming a homogeneous distribution of reward fibers around the electrode tip, the number of reward fibers within a certain field of stimulation should increase as the square of the radius of that field. From this it follows that the number of fibers recruited by the stimulation should be proportional to the current intensity. Thus it is fair to assume that the function relating the number of stimulated fibers to current intensity should be linear. If each pulse produces only one action potential in each reward fiber stimulated, it is reasonable to assume that the level of excitation the second stage system "sees" is the product of (a) the number of impulses propagated by each of the first stage

neurons and (b) the number of first stage fibers fired by the stimulation. If the role of the second stage system is simply to count the total number impulses propagated by the fibers of the first stage system, then the function relating current intensity ( $I$ ) required for criterial behavior to the reciprocal of the pulse frequency ( $1/N$ ) should be linear. A linear  $I$  vs  $1/N$  function would imply that the output of the second stage system is constant over a wide variety of pulse frequency and current intensity combinations. A non-linear  $I$  vs  $1/N$  function would imply that the first stage system did not react to the temporal or spatial variables of electrical stimulation in a linear fashion or that the second stage system integrated the reward signal it receives from the first stage system in a non-linear fashion.

Gallistel (1978) determined, for trains of fixed duration, the required current intensity for criterial running speed at various pulse frequencies. By plotting the required current against the reciprocal of the frequency (Shizgal et al., note 2) a linear relation was shown between the effects of these two parameters. The linearity of the current  $I$  vs  $1/N$  function indicated that the second stage system behaved as an impulse counter that is blind to the spatial-temporal distribution of a burst of impulses carried by the first stage system. In other words the second stage system treated the input of two first stage fibers each carrying two impulses in the same way it treated four fibers each carrying one impulse; in both

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cases the integrator "saw" four impulses. The linearity of the I vs  $1/N$  curve also suggests that, within a range of frequencies and current intensities, the reward signal does not undergo synaptic facilitation or accommodation at the second stage.

b) Neurochemical identity of the second stage system

The neurotransmitter(s) used by the second stage system has not yet been identified. Dopamine, however appears to be a potential candidate. There is now considerable evidence indicating that the midbrain dopaminergic cell groups A9 and A10 mediate the effects of various rewards including BSR. Blockade of dopaminergic post-synaptic receptors by neuroleptics (pimozide) produces, in animals working for BSR, response decrements that are consistent with what is seen when the stimulation is decreased (Fouriezos & Wise, 1976; Franklin & McCoy, 1979). Neuroleptics will also decrease the animals responsiveness to BSR; dose dependant increases in pulse frequency (Gallistel & Karras, 1984) or current intensity (Franklin, 1978) are necessary to offset their response attenuating effects. Although these data do not directly implicate dopamine as the neurotransmitter of the second stage system, they do point to a role for dopamine at some point in the reward circuitry.

B) The medial forebrain bundle substrate for stimulation-induced feeding.

In addition to acting as a powerful reward, electrical stimulation of the MFB appears to motivate or drive the animal. Given the right stimulation parameters and electrode placement, MFB stimulation will elicit various consummatory behaviors. Of all the behaviors that can be induced in the rat by electrical stimulation of the MFB, feeding is certainly the behavior that has been subjected to the most systematic analysis. Of great interest is the fact that stimulation induced feeding (SIF) is obtained at MFB sites which also support BSR (Margules & Olds, 1962).

At a time when drive reduction theory was the dominant theoretical framework for the analysis of central motivational systems, the fact a rat would feed in response to lateral hypothalamic stimulation as well as press a lever to stimulate its own lateral hypothalamus, was seen as paradoxical.

Drive reduction theory proposes that lateral hypothalamic stimulation produces a drive which in turn elicits a response presumably aimed at reducing the aversive impact of the drive state. Thus a rat was thought to eat in response to lateral hypothalamic stimulation because the stimulation makes it hungry and because food reduces the aversiveness of this hunger. The paradox resides in the fact that the same rat finds electrical

stimulation of that same MFB site highly rewarding. Drive reduction theory would predict the opposite outcome; the rat should avoid any opportunity to stimulate its lateral hypothalamus if the "drive" produced by the stimulation were aversive.

The drive-reward paradox forced many specialists in the field to assume that the motivating and rewarding effects of MFB stimulation are subserved by two independent substrates. In the following sections the literature pertaining to the anatomical specificity of the motivating and rewarding effects of MFB stimulation will be examined. Most of the literature reviewed will focus on the anatomical specificity of SIF in relation to the anatomy of the MFB substrate for BSR. The question of interest throughout the review will be: "Are SIF and BSR two behaviors that are subserved by a common or by a separate MFB first stage systems?" However, before reviewing the relevant literature several assumptions underlying the study of SIF as well as the working models of the substrate of SIF will be exposed in the following two sections.

- 1) Stimulation induced feeding: phenomenology and basic assumptions.

Probably the most striking feature of SIF is that it is temporally locked to the period of stimulation. Upon onset of the stimulation, a sated rat will begin to explore the floor of his

cage and when it encounters a piece of food will pick it up with its forepaws, assume a sitting position and chew (and swallow) the food with the avidity of a normal, hungry rat. Upon offset of the stimulation, the rat will drop the food pellet and, while chewing on the last mouthful of food, will resume a normal pattern of behavior. In this sense SIF appears to be stimulation bound. This is not to say that SIF is stereotyped. The sequence of movements which brings the animal in contact with the food as well as the consummatory response itself are not bound to the stimulation but rather to the contingencies of the environment. Feeders will not chew in response to lateral hypothalamic stimulation if no food is available but will perform an operant response to obtain food if they have been previously trained to do so (Coons et al., 1965). Hence lateral hypothalamic stimulation does not elicit an invariant motor response which operates in isolation of the animal's environment. Rather it appears that the stimulation induces feeding by operating through motivational factors.

The exact nature of the motivational variables that are called into play by SIF is unknown. Superficially, a feeder reacts to lateral hypothalamic stimulation as a food deprived rat would, when given access to food. It could therefore be concluded that lateral hypothalamic stimulation makes the animal hungry. This conclusion brings us no closer to defining the motivational state imposed by the electrical stimulation, for what is hunger? Hunger is most often defined by operation-

alization. For instance, hunger and variations in the intensity of the hunger experience are usually inferred from the number of hours an animal has been food deprived. Hence an animal deprived of food for 23 hours appears, judging by the greater amount of food it consumes, to be hungrier than when it is deprived for 1 hour. The circularity involved in defining an inferred motivational state in terms of another inferred motivational state is obvious. Whether electrical stimulation of the lateral hypothalamus elicits feeding by producing a state of hunger (whatever hunger may be) or by increasing the rewarding impact of the food, for instance, is not important for the moment. What is important is that lateral hypothalamic stimulation can reliably elicit a feeding behavior which is sensitive to most of the factors which impinge upon and influence normal feeding (Devor et al., 1970; Wise, 1974). Neither is it reasonable to assume that SIF is an exact replicate of normal feeding. It is obvious that normal feeding is under the control of several factors, both central and peripheral. It is reasonable to assume, however, that whatever the multiplicity of physiological systems involved in the normal elaboration of the feeding act, the system which lies under the electrode tip of a feeder is of great, if not central importance.

The ability to induce a feeding response electrically offers the advantage of subjecting what is presumed to be the substrate for normal feeding to quantitative analysis by using the psychophysical techniques which have been applied to

determine the quantitative properties of the MFB reward substrate. Trade-off experiments can be used to determine the electrophysiological and anatomical characteristics of the feeding-relevant fibers which lie directly under the electrode tip. Determining the quantitative properties of the "first stage system" of the substrate for SIF is important in its own right. However, the question of interest in the present investigation is whether SIF and BSR have common or separate first stage systems.

2) Anatomical models of the MFB substrate for stimulation-induced feeding.

The anatomical specificity of the rewarding and motivating effects of MFB electrical stimulation is an issue that has been subjected to experimental analysis for approximately 25 years. Evidence has been marshalled for and against the idea that BSR and SIF are subserved by a common substrate. Yet, as will be seen later, no strong conclusions can be made concerning the relationship, if any, between the substrates for BSR and SIF. Nonetheless, in light of what is known of the anatomy of the first stage system for BSR (re. section A), there are at least four basic anatomical models that can be developed to describe the anatomy of the SIF substrate. These models will provide a useful framework to guide the review of the literature relevant to the relationship between SIF and BSR.

The models for the substrate for SIF contain certain

assumptions that are parallel to those made for the substrate for BSR. First, it is assumed that the feeding-relevant fibers which lie under the electrode tip do not give rise to the feeding drive. The feeding-relevant fibers of the MFB are assumed to carry the neurophysiological signal triggered by the electrical stimulation to some other part of the brain. This component of the feeding substrate corresponds to the cable or first stage system of the BSR substrate.

The output of the first stage system for SIF is also assumed to be summed, both temporally and spatially, by an integrator or second stage system. This assumption appears to be supported by the linearity of the function relating the required pulse frequency to current intensity for SIF (Gratton and Wise, note 3). As is the case for the second stage system of BSR, no assumptions are made concerning the location or anatomical arrangement of the second stage system of SIF.

The necessity to postulate a conversion process for SIF is difficult to assess. A superficial observation of a rat feeding in response to MFB electrical stimulation suggests that the effects of the stimulation on a given trial are transient and have little bearing on the animal's behavior on subsequent trials. However other variables, such as changes in the palatability of the food, will influence the animal's responsiveness to the stimulation on subsequent trials (Smith, 1972; Tenen & Miller, 1964; Wise & Albin, 1973). For such

changes in the animal's behavior to occur, it must remember from previous trials how the food tasted. In this case a conversion process which measures the rewarding impact of the food and produces an enduring record of the magnitude of the rewarding effect must be assumed. However, for the purpose of the present investigation only the first and second stage systems for SIF are of direct concern.

a) Models of separate SIF and BSR first stage systems

The first model (Figure 1a) represents anatomically distinct first and second stage systems for SIF and BSR. The first stage system for SIF is shown coursing perpendicularly to the first stage system for BSR. This merely serves to illustrate the type of arrangement that would be anticipated if sites supporting SIF were to be localized in a restricted region of the MFB reward system. In this case electrical stimulation applied at the intersection of the SIF and BSR first stage systems would induce feeding as well as support self-stimulation behavior. Electrical stimulation of any other portion of the MFB would not induce feeding but would presumably support self-stimulation.

The second model (Figure 1b) represents the case where the first stage systems for SIF and BSR while being functionally distinct, course parallel to each other through the MFB. Such an arrangement implies that sites that are positive for SIF are not restricted to any particular region of the MFB but can be found



FIGURE 1

Anatomical models of separate BSR and SIF first stage systems.

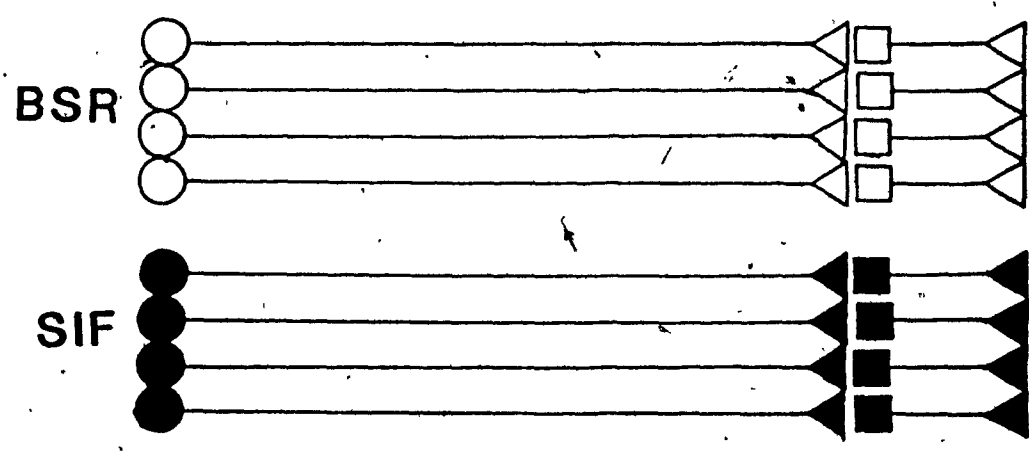
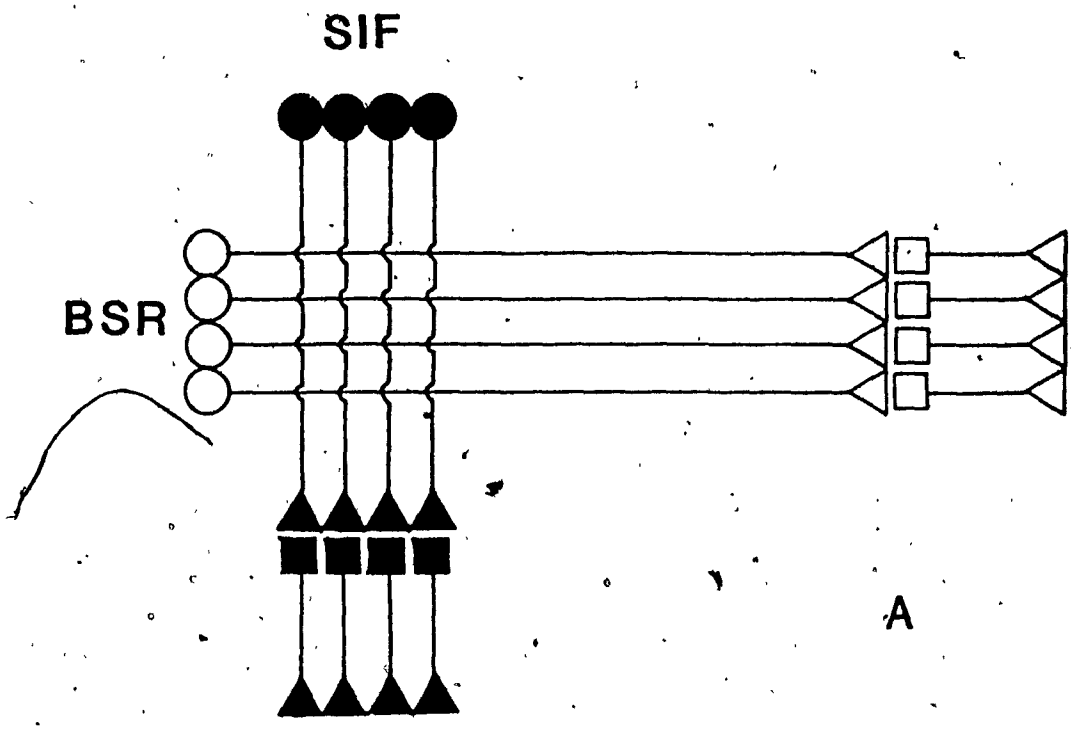
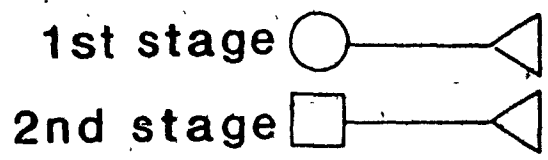


Figure 1.

all along the rostral-caudal extent of the MFB. The model, as it is shown in Figure 1b, also implies that the first stage systems for SIF and BSR, although parallel to each other, are confined to different portions of the MFB. For example, SIF relevant fibers could course through the MFB in a position lateral to the BSR relevant fibers. This may or may not be the case. The model also allows for an arrangement whereby the SIF relevant fibers and BSR relevant fibers are closely intertwined within the MFB. Hence if electrical stimulation of a particular site of the MFB were shown to support self-stimulation as well as elicit a feeding response, it could not be concluded that SIF and BSR had a common first stage system. It may be that the field of effective stimulation recruits distinct SIF and BSR relevant fibers which course through the MFB in close anatomical proximity.

b) Models of a common SIF and BSR first stage system

The model depicted in Figure 2a assumes that SIF and BSR are subserved by the same first stage system. It makes the further assumption that the feeding- and reward-relevant signals propagated by the first stage system are summed temporally and spatially by a common second stage system. This is equivalent to saying that SIF and BSR are different behavioral manifestations of the same neurophysiological event in the first and second stage systems. It is difficult to imagine a single system which can integrate the indiscriminate excitation produced by a train of electrical pulses to generate two different behavioral

outputs. At some point beyond the second stage system, the feeding- and reward-relevant signals must input into different systems. The present model makes no assumptions concerning the nature of the feeding-relevant circuitry beyond the second stage system, except that it should at some point diverge from the reward-relevant circuitry.

Figure 2b illustrates a single first stage system common to both SIF and BSR which synapses onto two different second stage systems. This model assumes that the impulses triggered by electrical stimulation are equally propagated by two branches of the first stage system and summated temporally and spatially by two independent second stage systems-- one relevant to feeding and the other to reward. The present model differs from the preceding model only in the point of divergence of the feeding- and reward-relevant signals. Hence, in order to generate two different behavioral outputs, the present model must still postulate the existence of a third stage system in the SIF circuitry which differs in its function from the conversion process for BSR.

### 3) Anatomical specificity of stimulation induced feeding

Stimulation-induced feeding in the rat has long been thought to be restricted to the anterior portion of the lateral hypothalamic MFB (i.e. Caggiula, 1969). Brain stimulation reward, however, is supported by electrical stimulation of sites which

## FIGURE 2

Anatomical models of a common BSR and SIF first stage system.

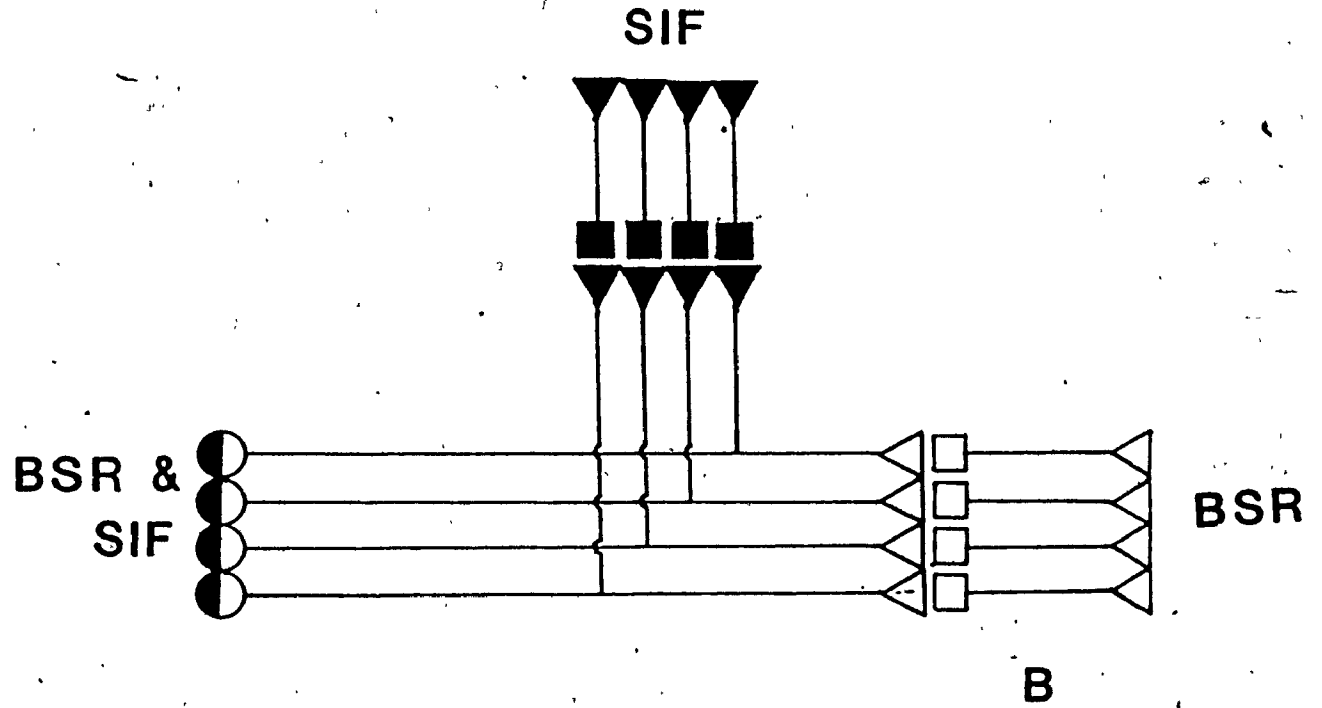
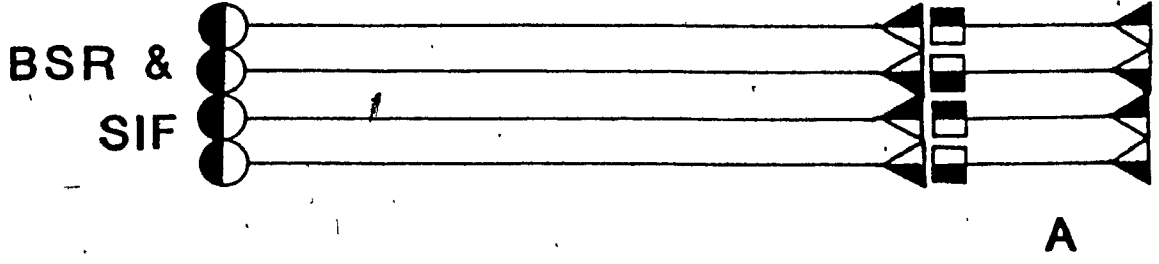
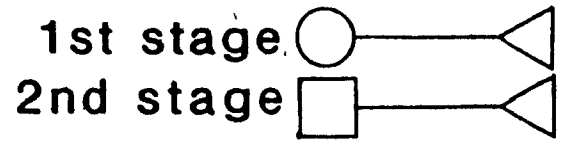


Figure 2

extend the full rostral-caudal trajectory of the MFB. The notion of distinct SIF and BSR substrates on the one hand and the more general idea of anatomically distinct motivational systems on the other rests in large part on studies showing that electrical stimulation of different regions of the hypothalamic MFB elicit different behaviors. It has been assumed in the past that if BSR is obtained all along the MFB while SIF and other elicited behaviors are obtained only from restricted areas of the MFB, then the substrates underlying BSR, SIF and the other elicited behaviors must have distinct anatomies. However, the anatomical specificity of SIF and of electrically elicited behaviors in general has been questioned because (a) the sites which support SIF do not appear to be as localized as once suggested, (b) stimulation of a single MFB site can elicit several different behaviors. These two issues will be examined in the next two sections.

a) Distribution of stimulation induced feeding sites.

Electrical stimulation of the lateral hypothalamic MFB induces feeding (Delgado & Anand, 1953; Margules & Olds, 1962; Wise, 1971) and drinking (Mendelson, 1970; Mogenson & Stevenson, 1967; Wise, 1971). Male copulatory behavior, however is reported to be elicited by electrical stimulation of a more caudal region of the lateral hypothalamic MFB (Caggiula, 1969; Caggiula & Hoebel, 1966).

The apparent localization of specific stimulation induced feeding, drinking and copulation sites along the MFB is consistent with the anatomical model in Figure 1a. This model can be expanded to depict the first stage systems for feeding and drinking as being restricted to the lateral hypothalamic MFB, whereas the first stage system for copulation would be confined to a more posterior region of the lateral hypothalamic MFB. The first stage system for BSR, however, courses through both the lateral and posterior hypothalamic regions of the MFB (Shizgal et al., 1980). The fact that feeding sites and copulation sites also support BSR (Caggiula & Hoebel, 1966; Margules & Olds, 1962) suggests, in the context of the present model, that first stage feeding and copulation fibers intersect first stage BSR fibers coursing through the MFB.

There is, however, a considerable amount of data suggesting that the anatomical model in Figure 1a is invalid. For the most part these data argue against the site specificity of electrically elicited behaviors in rat and by extension against the notion of separate first stage systems for BSR and SIF.

Consider first the case of stimulation induced feeding. Cox & Valenstein (1969) showed that feeding (and drinking) can be elicited by stimulating MFB sites extending from the anterior region of the lateral hypothalamus to the ventral tegmental area. Waldbillig (1975) later partially confirmed and extended these findings by showing that feeding can be elicited at sites



extending into the lateral tegmentum of the hindbrain. Taken together, these two studies suggest that the first stage fibers underlying SIF are not confined to any particular region of the MFB, but extend along the entire longitudinal axis of the MFB.

Consider now the issue of site specificity of stimulation induced copulation. Copulation sites appear to be associated with the posterior hypothalamus. There is, however, considerable ambiguity associated with what has been termed "posterior hypothalamic copulation center", since copulation is reportedly not elicited by stimulation of the posterior hypothalamic nucleus per se, but rather by stimulation of the posterior region of the lateral hypothalamic MFB. The posterior hypothalamic nucleus is a periventricular nucleus lying 1.0-1.5 mm medial to MFB stimulation sites which are reported to elicit copulation (Caggiula, 1969). Therefore the term "posterior hypothalamic copulation center" does not refer to any conventional anatomical delineation of the hypothalamic nuclei, but rather to a loosely defined area corresponding to the more posterior levels of the lateral hypothalamic MFB. In view of these facts, the most caudal copulation sites reported by Caggiula (1969) can be seen to be encompassed by the lateral hypothalamic area.

The anatomical separation between MFB copulation and feeding sites does not argue strongly for anatomically distinct motivational systems. Indeed, there is considerable overlap between the so-called posterior hypothalamic copulation sites and

lateral hypothalamic feeding sites. Close inspection of Caggiula's (1969) data shows that of the 15 electrode placements which are positive for copulation, at least half are clearly located within the lateral hypothalamic region which has been traditionally associated with SIF. In light of Cox & Valenstein's (1969) study showing SIF sites that extend beyond the confines of the hypothalamic MFB, the anatomical dissociation of stimulation induced feeding and copulation is not as clear as was once suggested (Caggiula, 1969; Caggiula & Hoebel, 1966; Hoebel, 1969).

It may be easier to consider the anatomical models depicted in Figures 1b, 2a and 2b as more plausible representations of the anatomy of the SIF first stage system. All three of these models represent the first stage system for SIF as a bundle of fibers coursing along the longitudinal extent of the MFB. Although models 2a and 2b make the additional postulation of a common first stage system for BSR and SIF, nothing in the literature reviewed thus far invalidates model 1b, whereby SIF and BSR are subserved by separate first stage systems. Indeed the fact remains that stimulation of the anterior region of the MFB does seem to preferentially elicit feeding, whereas stimulation of more posterior MFB sites appears to preferentially elicit copulation. It may be, however, that more careful mapping of the MFB for various elicited behaviors will uncover a medial to lateral dissociation between specific motivational systems. However, Caggiula's (1969) study also shows

five sites at which both feeding and copulation could be elicited, suggesting a considerable amount of overlap between the substrates for feeding and copulation. The fact that stimulation at a single site elicits more than one behavior has been used to argue against the anatomical specificity of electrically elicited behaviors. This issue will be examined next.

b) Modification of stimulation induced feeding

Rats that feed in response to MFB stimulation will frequently exhibit other elicited behaviors if they are stimulated long enough in the presence of the appropriate goal stimuli. Valenstein et al. (1968) showed that rats which initially exhibit SIF can be made to drink or gnaw on a piece of wood. Wise (1971) mapped the dorsal-ventral extent of the lateral hypothalamic MFB for stimulation induced feeding and drinking and showed that sites which support feeding support drinking as well. Furthermore, Caggiula (1969) as well as Gallistel (1969) found that both feeding and copulation can be elicited at the same stimulation site. The gradual emergence of a second elicited behavior first observed by Valenstein et al. (1968) occurs without manipulating the stimulation parameters and can be accelerated by replacing the initially preferred goal stimulus (i.e. food) with a second goal stimulus (water or a block of wood). Once the second behavior is elicited reliably, it will persist even after the initial goal stimulus is reintroduced in the test box. In this case, however, the rat

will, over a given number of trials, exhibit both the initial and the new elicited behaviors.

Valenstein et al. (1968) interpreted the extensive stimulation experience necessary for the new elicited behavior to emerge as additional support for the notion that MFB stimulation does not activate anatomically distinct motivational systems, but rather a general, non-specific motivational system. He argued that if the electrical stimulation excited specific MFB motivational systems, then the appearance of additional elicited behaviors should be immediate once the animals are exposed to the appropriate goal stimuli. Wise (1968) suggested an alternative interpretation of the data of Valenstein's et al. (1968). He proposed that the electrical stimulation may be activating distinct motivational systems which have different thresholds of excitation. Wise (1968) based his interpretation on the fact that repeated stimulation progressively decreases the stimulation threshold for eliciting a particular behavior. He assumed that the threshold of excitation of the underlying substrate decreased with repeated stimulation. This assumption led Wise (1968) to hypothesize that if the substrate of the second behavior is initially stimulated at intensities below its excitation threshold, then the second behavior would only be observed when its excitation threshold is sufficiently lowered. Although Wise (1968) did show that initial stimulation at high current intensities elicits more than one behavior and that prolonged stimulation experience is not necessary before a second behavior

emerges, others (Cox & Valenstein, 1969; Mogenson, 1971) have failed to observe the initial emergence of more than one behavior even when high current intensities were used.

The manifestation of multiple behaviors by stimulation of the same brain site also argues against the idea that the MFB contains anatomically distinct SIF and BSR first stage systems; although such a case cannot be argued as strongly based on the literature being reviewed presently. A lack of anatomical specificity of the rewarding and motivating effects of MFB stimulation would be expected according to the anatomical models shown in Figures 2a and 2b. Both these models postulate a common first stage system for BSR and SIF, implying that both the rewarding and motivating effects of electrical stimulation arise by initially activating a single population of MFB fibers. In this case, the behavioral expression of BSR and SIF would have to be determined at some point beyond the first stage system.

The elicitation of multiple behaviors at the same stimulation site does not preclude the possibility, however, that the stimulation activates distinct, but tightly interwoven first stage fiber systems (Figure 1b). There are a few lines of evidence that have been marshalled to argue for such an anatomical arrangement. These studies however, are merely suggestive in that they only indirectly test the hypothesis of a common first stage system for the various electrically elicited behaviors. For instance, food-related manipulations (food

deprivation, food stomach loading) selectively affect SIF, while water related manipulations selectively affect stimulation induced drinking (Devor et al., 1970). While this study does demonstrate that stimulation induced feeding and drinking are selectively influenced by the appropriate physiological variables (i.e. food vs water satiation), it does not prove that feeding and drinking are elicited by activation of distinct MFB substrates. At best the study of Devor et al. (1970) suggests either that, at some point, the substrates for drinking and feeding diverge and exhibit a certain degree of functional specificity or that they receive converging inputs from different physiological systems.

The differential effects of high and low stimulation frequencies on feeding and drinking have also been used to argue for distinct feeding and drinking first stage systems (Wise, 1974). Mogenson et al. (1971) reported that feeding is preferentially elicited at low stimulation frequencies whereas drinking predominates at higher stimulation frequencies. Wise (1974) inferred from the data of Mogenson et al. (1971) that the stimulation was activating distinct feeding and drinking fibers systems. Although it may be that the first stage systems for feeding and drinking have different frequency tracking capabilities, it may also be that feeding and drinking share a common first stage system which inputs onto two distinct second stage systems with different frequency integrating properties (i.e. Figure 2b). Thus the high and low frequency effects on

drinking and feeding do not necessarily imply that differences between the drinking and feeding substrates are found at the first stage level.

While it is difficult to find direct evidence of anatomically distinct motivational systems in the rat MFB, there are suggestions that stimulation-induced behaviors in other species (opposum, cat) are subserved by anatomically distinct MFB fiber systems (Roberts, 1969; Roberts et al., 1967). Hence it may be that in more encephalized species the degree of anatomical overlap between various fiber systems is not as great as in rat. Such a contention is supported by the fact that, in rat, small diameter electrodes through which low currents are applied have been reported to selectively elicit either feeding, drinking or brain stimulation reward (Olds et al., 1971). The increased spatial resolution afforded by small electrode tips and low current diffusion can conceivably permit the anatomical dissociation of distinct, but tightly packed fiber systems. The data of Olds et al. (1971) appear, therefore, to be consistent with the idea that the first stage systems for SIF and BSR are anatomically distinct, but course through the MFB in close anatomical proximity (Figure 1b). It is surprising, however, that mapping the lateral hypothalamic MFB for feeding and drinking with a moveable electrode has not uncovered any dramatic anatomical dissociations between sites that are positive for SIF and sites positive for stimulation induced drinking (Wise, 1971).

c) Summary and conclusions

There is at least one major conclusion to be drawn from the literature reviewed in the two previous sections (3a & 3b) which is relevant to the present investigation. Feeding is not elicited exclusively from the lateral hypothalamic MFB. Feeding can be elicited by stimulating at different anterior-posterior levels of the MFB. This finding casts doubt on the notion of lateral hypothalamic feeding center which has been widely held in the past. The distribution of MFB sites positive for SIF indicates that the anatomical model depicted in Figure 1a is not a plausible representation of the anatomy of the first stage system for SIF. The fact that SIF is elicited along the full longitudinal axis of the MFB, suggests that the first stage system for SIF is composed of fibers coursing along the MFB. Such an arrangement is consistent with either one of the anatomical models shown in Figures 1b, 2a or 2b.

Whether or not BSR and SIF share a common first stage system cannot be confidently determined from the literature reviewed thus far, although there are suggestions that this may be the case. Most of the literature reviewed dealt with the anatomical specificity of MFB substrates underlying stimulation induced feeding, drinking and copulation. The basic conclusion is that, at least in the rat, there is no direct evidence that anatomically distinct MFB fiber systems subserve these behaviors. In fact the demonstration that several behaviors can be elicited



at the same stimulation site suggests that a general, non-specific motivational system underlies stimulation induced feeding, drinking and copulation. Such a system may also be responsible for the rewarding effects of MFB electrical stimulation.

4) Effects of motivational variables on brain stimulation reward.

If one assumes that electrical stimulation of the MFB produces its rewarding effect by activating the substrate which carries the reward-relevant signal produced by conventional reinforcers, then an animal's responsiveness to BSR should be selectively influenced by factors which normally influence its responsiveness to conventional reinforcers. If one further assumes that the MFB contains anatomically specific motivational systems, then the rewarding effect produced by activating a particular MFB motivational system should be specifically influenced by the appropriate motivational variables. This line of reasoning underlies several studies aimed at uncovering the presence of anatomically specific motivational systems in the MFB.

For the most part, the studies that will be reviewed next, examined the effects of food-, water- and sex-related factors on electrical self-stimulation of the MFB. These studies are of interest to the present investigation because they assume that the rewarding and motivating effects of MFB stimulation are produced

by activating a common MFB fiber system. Although it is never explicitly stated, it appears that the authors of these reports held the view that MFB contains fiber systems which when stimulated could both reward an operant response as well as elicit a consummatory response. Hence the problem addressed by these studies is not to determine if the motivating and rewarding effects of MFB stimulation are subserved by a common fiber system. Rather these studies sought to determine if food, water and sex rewards activate distinct or common MFB reward fiber systems.

Hence two issues are of prime importance when interpreting the results of such studies: the selectivity and the anatomical specificity of drive manipulations on BSR. In the context of the present review, selectivity refers to the direction of the effects produced by drive manipulations. For example, if an animal presses a lever to stimulate its food reward substrate, then food deprivation and food stomach loading should selectively influence its responsiveness to the rewarding effect of the stimulation. Since food deprivation increases food intake, then food deprivation would be expected to increase a rat's responsiveness to the rewarding stimulation. However food stomach loading, which decreases food intake, should cause a decrease in responsiveness to rewarding MFB stimulation.

The anatomical specificity of drive manipulations on BSR refers to their ability to anatomically dissociate motivational

systems. For instance, if MFB electrical stimulation specifically activates the substrate for food reward, then food deprivation should specifically increase the animal's responsiveness to the rewarding stimulation, whereas water deprivation should have no such effect.

a) Selectivity of drive manipulations on BSR.

i) Hunger, thirst and supersatiation effects. Brady et al. (1957) first reported, in rat and cat, that food and water deprivation enhances rates of lever pressing for caudate and septal electrical stimulation. Furthermore the magnitude of the rate enhancing effect of food deprivation reported by Brady et al. (1957) was positively related to the number of hours of deprivation; 48 hour food deprivation produced a greater enhancement than 4 hour food deprivation. There have since been several independent reports of the rate enhancing effects of food and water deprivation on BSR (Becker-Rose et al., 1972; Gallistel & Beagley, 1971; Goodall & Carey, 1975; Hodos & Valenstein, 1960; Margules & Olds, 1962; Olds, 1958; Stellar & Gallistel, 1975; Wilkinson & Peele, 1962).

While food deprivation appears to increase responsiveness for rewarding electrical stimulation, supersatiation by intragastric intubation of a liquid diet has been claimed to decrease lever pressing rates (Hoebel, 1968; Hoebel & Teitelbaum, 1962) or increase the stimulation intensity threshold for lateral

hypothalamic BSR (Hoebel, 1968; Mount & Hoebel, 1967). Gastric distention produced by inflating an intragastric balloon apparently also decreases a rat's responsiveness to rewarding lateral hypothalamic stimulation (Hoebel, 1968).

ii) Food related hormone effects. Administration of insulin in the normal rat causes hypoglycemia which results in hyperphagia. Glucagon injections have the opposite effect; they produce hyperglycemia and, consequently, hypophagia. Insulin and glucagon have been claimed to have opposite effects on rewarding lateral hypothalamic stimulation; insulin causes an increase in response rate for BSR, glucagon causes a decrease in response rate (Balagura & Hoebel, 1967). Similar inhibitory effects on BSR observed with glucagon administration is produced by intravenous infusions of hypertonic glucose solutions (Hoebel, 1968).

iii) Castration and sex hormone effects. Olds (1958) claimed that castration and subsequent testosterone administration could influence the rate of lever pressing for rewarding electrical stimulation. Generally, castration appeared to decrease response rates, whereas testosterone replacement generally brought the response rates back to pre-castration levels. However, the castration and testosterone effects in Olds' (1958) study do not appear to be reliable. In some instances castration had very little effect, while in other instances where castration did produce response decrements, testosterone did not appear to reinstate pre-castration response

rates. However Caggiula (1969,1970) also reported, in three rats, moderate castration induced response decrements for BSR which were reversed by testosterone administration, whereas testosterone administered to intact rats produced moderate response increments for BSR.

Prolonged estrus induced by chronic estrogen administration is also claimed to increase, at times, response rates for BSR (Hoebel,1969). The selectivity of the estrogen treatment is difficult to assess, however, since the period of estrus in Hoebel's (1969) study was associated with a decrease in food intake. Since food deprivation is also reported to cause response increments for BSR, it may be that the response enhancing properties of estrogen on BSR may be more directly related to the resulting hypophagia rather to a sex hormone interaction with the central reward circuitry. Furthermore the results of Hoebel's (1969) study are at odds with an earlier report showing no effect of estrogen on BSR (Hodos & Valenstein, 1960). The discrepancy between Hoebel's (1969) and Hodos and Valenstein's (1960) results may be due to the fact that the effects of estrogen were assessed on responsiveness to stimulation of different reward sites (septum vs MFB). The anatomical specificity of drive manipulations on BSR is an issue that will be dealt with later.

iv) Conclusions. The effects of food-related post-ingestional factors on response rates for BSR appear to parallel their effects on feeding behavior. Food deprivation and

insulin treatment cause response increments for BSR, whereas stomach loading and glucagon and glucose treatment have the opposite effect. The selectivity of post-ingestional factors on BSR is consistent with the idea that electrical stimulation produces its rewarding effect by activating the substrate for food reward. Although they have not been as thoroughly analyzed, the effects of water-related post-ingestional factors on BSR also appear to be consonant with the idea that the stimulation activates a water reward system. The effects of sex-related manipulations on BSR, at least in the female rat, are not clear and at times seriously confounded; therefore no firm conclusions can be made as to whether BSR activates a sex reward substrate. The same can be said of sex-related manipulations on BSR in the male rat. Of all the drive manipulation studies reviewed, those pertaining to the effects of castration and testosterone administration on BSR have yielded the least robust effects and have not been subjected to any significant amount of independent analysis. It therefore remains to be seen if sex related factors have any significant bearing on the responsiveness to BSR.

Finally, several points must be raised concerning the selectivity of drive manipulations on BSR. The first point has to do with the dependent measure used to assess the effects of motivational factors on what is presumed to be the rewarding impact of the electrical stimulation. Care has been taken throughout this part of the review to avoid making any implicit assumptions about the meaning of a response rate increment or

decrement following a particular drive manipulation. This is made necessary by the fact that response rate measures are relatively poor indices of the rewarding impact of electrical stimulation (Valenstein, 1964). Caution must therefore be used in interpreting the effects of motivational variables on BSR— in particular those that cause response decrements. A decrease in responsiveness to BSR can be due to a variety of factors which are unrelated to a decrease in the rewarding impact of the stimulation (i.e. motor incapacitation). All of the studies reviewed have used changes in rate (lever pressing rate, running speed etc.) as a measure of the effects of motivational variables on BSR. Therefore, although the studies reviewed above do suggest that responsiveness to BSR is affected by changes in a drive state, they do not unequivocally prove that drive manipulations selectively affect the rewarding effect of the stimulation.

Neither can it be said that drive manipulations selectively affect the first stage system for BSR. If one assumes for a moment that drive manipulations do alter the rewarding impact of MFB electrical stimulation, it does not necessarily follow that they do so at the first stage system of the BSR circuitry. Motivational variables may exert their influence on the rewarding effect of electrical stimulation at several synapses away from the first stage system. Hence, inasmuch as some drive manipulations appear to selectively affect an animal's responsiveness to rewarding MFB electrical stimulation, there is

very little substantial evidence in the literature reviewed to indicate that they do so by altering the reward-relevant signal propagated by the first stage system or, for that matter, by any other component of the BSR circuitry.

b) Anatomical specificity of drive manipulations on BSR.

In the previous section the effects of different motivational variables on BSR were described with special reference to their physiological selectivity. In the present section the anatomical specificity of drive manipulations on BSR will be discussed. The issue of anatomical specificity of drive manipulations on BSR is important to the present investigation because it probably constitutes the most important line of evidence suggesting the idea that MFB electrical stimulation produces its rewarding effect by activating anatomically distinct MFB fiber systems which, under normal circumstances, carry the neurophysiological signal generated by independent conventional rewards. If anatomically distinct MFB motivational systems are assumed, then it follows that electrical activation of a particular motivational system (e.g. food reward system) should be specifically affected by the appropriate motivational variables (food deprivation, supersatiation, etc.), and that activation of another system (e.g. sex reward system) should be affected by another set of variables (hormones).

The lateral hypothalamic MFB had been viewed in the past as



the center responsible for food reward. As we have seen previously, this view stemmed in large part from the widely held contention that feeding is elicited exclusively from the lateral hypothalamic MFB. This view has also been reinforced by studies suggesting that food-related motivational variables specifically affect lateral hypothalamic self-stimulation. Hoebel (1968) reported that stomach loading with food produced response decrements for lateral hypothalamic self-stimulation but had no effect on septal self-stimulation rates. Response increments for BSR produced by food deprivation have also been reported to be site dependent. Goodall and Carey (1975) reported that lateral hypothalamic rates of self-stimulation increased during food deprivation whereas medial prefrontal cortex and substantia nigra self-stimulation remained comparatively unaffected by this treatment. Taken together the data from the above two studies appear to suggest that rewarding lateral hypothalamic MFB stimulation may be specifically activating the substrate for food reward.

Anatomical specificity of drive manipulations on BSR is also suggested by the differential effects of castration and testosterone treatment on lateral and posterior hypothalamic BSR. Caggiula (1970) reported in two rats, both with lateral and posterior hypothalamic electrodes, that castration and subsequent testosterone replacement respectively decreased and reinstated pre-castration response rates for posterior hypothalamic stimulation, but left lateral hypothalamic self-stimulation

relatively unaffected. Although they were statistically significant, Caggiula's (1970) castration and testosterone effects on posterior hypothalamic MFB self-stimulation were by no means robust, and, since lateral hypothalamic MFB self-stimulation appears at times to be affected by sex-related drive manipulations, the apparent anatomical specificity of castration and testosterone effects on posterior hypothalamic BSR has to be interpreted with caution. Nonetheless, Olds (1958) reported that there was an inverse relation between food deprivation effects and castration and testosterone effects on BSR, in that self-stimulation sites that were sensitive to food deprivation effects seemed insensitive to the effects of castration and subsequent testosterone replacement and vice versa. However, contrary to the later claim of Caggiula (1970), the effectiveness of sex and food related drive manipulations in Olds' experiment (1958) did not appear to depend on the anterior-posterior location of the stimulating electrodes within the MFB, but rather appeared to be determined by the medial-lateral location of the electrodes; self-stimulation of the medial aspect of the MFB seemed to be selectively affected by food deprivation, whereas self-stimulation of the lateral aspect appeared to be selectively affected by castration and testosterone treatment.

There are, however, other studies indicating that the effects of food- and sex-related motivational variables on BSR may not be as tied to particular electrode placements as was

suggested by Olds and Caggiula. Consider the case of food related drive manipulations on BSR. Gallistel and Beagley (1971) reported that rats, implanted with two MFB stimulating electrodes, had different electrode preferences depending on whether they were food or water deprived. In one arm of a T-maze, lever pressing triggered rewarding stimulation to one MFB electrode while lever pressing in the other arm triggered rewarding stimulation to a more caudally situated MFB electrode. When thirsty, some rats preferred to run to one arm of the T-maze and self-stimulate one part of their MFB, whereas during a hunger state they preferred the other arm to self-stimulate another part of their MFB. Taken at face value, Gallistel and Beagley's (1971) data suggest that rewarding electrical stimulation of the MFB is subserved by at least two anatomically distinct motivational MFB fiber systems; one subserving food reward and another subserving water reward. However, with self-stimulation sites extending along the full anterior-posterior axis of the MFB, Gallistel and Beagley's (1971) study did not uncover any clear anatomical delineation between those MFB self-stimulation sites that were sensitive to water deprivation and those that were sensitive to food deprivation. Both hunger-induced and thirst-induced response increments were observed for ventral tegmental area, posterior and lateral hypothalamic MFB and for septal self-stimulation. Hence, although Gallistel and Beagley's (1971) study does provide evidence that different MFB self-stimulation sites can be specifically affected by different drive manipulations, it does not support the idea that

self-stimulation sites that are sensitive to the effects of food- or water-related motivational variables are restricted to any particular anterior-posterior level of the MFB.

In fact, in the majority of studies reviewed, a lack of anterior-posterior specificity of food-related drive manipulations on BSR appears to be the rule rather than the exception. In addition to influencing responding for rewarding lateral hypothalamic MFB stimulation, food-related drive manipulations will selectively affect responding for caudate (Brady et al., 1957), septal (Brady et al., 1957; Gallistel & Beagley, 1971; Hodos & Valenstein, 1960; Olds, 1958) posterior hypothalamic MFB (Becker-Rose et al., 1972; Gallistel & Beagley, 1971) and ventral tegmental area (Gallistel & Beagley, 1971) rewarding stimulation. In this regard it is somewhat interesting to note that the apparent lack of anatomical specificity of food related drive manipulations on BSR is paralleled by the lack of anatomical specificity for stimulation induced feeding as discussed by Cox and Valenstein (1969).

#### c) Summary and conclusions

The studies reviewed in the previous two sections have examined the effects of drive manipulations on BSR as a means of determining whether the MFB contains anatomically distinct motivational fiber systems. The question that these studies set out to answer was whether BSR is subserved by a single or several

anatomically distinct MFB first stage systems. The basic assumption underlying this type of study is that MFB electrical stimulation produces its rewarding effect by activating the fibers that are normally activated by conventional rewards.

The reported differential effects of various drive manipulations on BSR has been used in the past to argue for distinct motivational systems which are confined to different anterior-posterior levels of the MFB. From these studies emerged the contention that the reward experience produced by the stimulation depended on the placement of the electrode in the MFB. Hence self-stimulation of the lateral hypothalamus was thought to be related to the food reward experience whereas posterior hypothalamic self-stimulation was thought to be related to the sex reward experience. The intersection of the reward substrate with food- and sex-related motivation systems as depicted in Figure 1a, illustrates the type of anatomical arrangement that would underlie the specialization of motivational function of different anterior-posterior levels of the MFB. The purpose of the present review was to critically examine the empirical support for such an anatomical arrangement.

The issues of physiological selectivity and anatomical specificity were identified as being important for the interpretation of drive manipulations on BSR. The effects of motivational variables on BSR as reported in the literature appear to be selective, in that their effects on the rate of responding

for rewarding electrical stimulation parallel their effects on rate of responding for conventional rewards. However the selectivity of drive manipulations on the rewarding impact of electrical stimulation has not been unequivocally demonstrated. There is even less reason to believe that the studies reviewed have demonstrated that drive manipulations selectively alter the rewarding impact of MFB electrical stimulation at the first stage system of the reward circuitry.

Drive manipulations of BSR do not appear to show any clear anatomical specificity. Although some self-stimulation sites appear to be specifically sensitive to the effects of water deprivation, while others appear to show specific sensitivity to the effects of food deprivation there does not appear to be any consistent evidence that the effects of drive manipulations in general are specific to self-stimulation of any particular region of the MFB.

If one assumes for a moment that drive manipulations do alter the effectiveness of rewarding stimulation by selectively changing the excitability of the first stage system of the food reward circuitry, then certain conclusions can be drawn concerning the anatomy of the MFB first stage system for SIF. The fact that food related drive manipulations appear to alter self-stimulation at sites which extend along the full anterior-posterior axis of the MFB, is not consistent with the contention held by many in the past that distinct motivational

systems are restricted to different anterior-posterior levels of the MFB. Such an anatomical arrangement, which is depicted in Figure 1a, cannot account for the lack of anatomical specificity of food related drive manipulations on BSR found by most of the studies reviewed above.

If the effects of various motivational variables on MFB self-stimulation are truly specific and reflect the presence of distinct motivational systems, then the data from the studies that have been reviewed point to anatomically distinct MFB first stage systems which course through the longitudinal axis of the MFB. Such an arrangement is consistent with the anatomical models depicted in Figures 1b. According to model 1b, rewarding electrical stimulation of MFB would specifically activate anatomically distinct first and second stage systems. This model could explain Olds' (1958) data where the effectiveness of food and sex related motivational variables was correlated with the medial to lateral position of self-stimulation sites. In this case the food reward first stage system would course through the MFB in a position medial to the sex first stage system. This hypothesis would, of course, need empirical support based on stronger evidence than that which has been presented in the studies examined in the present review.

- 5) Post-stimulation excitability characteristics of the first stage system for stimulation induced feeding.

Very little reliable information has been obtained about the electrophysiological characteristics of the MFB first stage system for stimulation induced feeding (SIF). However, as we have seen earlier, a great deal of information has been obtained concerning the electrophysiological and anatomical characteristics of the MFB first stage system for BSR. In an attempt to determine if SIF and BSR are subserved by a common or separate MFB first stage systems, it would be logical to first compare the electrophysiological characteristics of the fibers underlying the two behaviors.

The post-stimulation excitability cycles of the first stage fibers for MFB self-stimulation have been estimated to range from 0.4 to 2.0 msec (Bielajew et al., 1981; Rompre and Miliaressis, 1980; Yeomans, 1975). These refractory period estimates have been obtained behaviorally using a trade-off procedure. The applications of trade-off procedures to the elucidation of the quantitative properties of the substrate for MFB self-stimulation have already been discussed. However, as a brief reminder, a trade-off procedure involves determining the value of one parameter that is necessary to offset the effects produced by a change of a second parameter. Hence, experiments using a trade-off procedure are designed to determine the various combinations of two sets of parameters that will produce a constant level of behavior. To estimate the refractory periods of reward-relevant fibers, the frequency of pairs of pulses necessary to maintain a predetermined rate of lever pressing is



compared to the frequency of single pulses required to maintain the same rate of lever pressing across a range of intra-pulse intervals. Hence, the relative effectiveness of the T-pulse in eliciting a second action potential in reward-relevant fibers is derived from the decreased pulse pair frequency requirements relative to the single pulse frequency requirements. The values of relative T-pulse effectiveness at different C-T intervals therefore reflect the weighed distribution of MFB reward-relevant fibers which are no longer refractory to the T-pulse.

Trade-off procedures can also be used to behaviorally estimate the post-stimulation excitability cycles of the first stage system of SIF. If SIF were to result from the activation of a population of MFB fibers which had a different distribution of refractory periods than that which was seen for BSR, then it could be concluded that the first stage system of SIF comprised, in whole or in part, fibers which were not common to the first stage system of BSR. If, however, the distribution of refractory periods for both SIF and BSR are similar then two interpretations would remain possible. On the one hand, SIF and BSR might be subserved by a common population of first stage MFB fibers. On the other hand, SIF and BSR might be subserved by distinct populations of first stage fibers which happen to have very similar refractory period distributions.

Several attempts have been made to compare estimates of refractory periods of MFB feeding-relevant fibers to those of

reward-relevant fibers. Early studies, which did not use trade-off procedures, concluded that the substrates underlying these two behaviors had different refractory period characteristics. It appears now that the observed differences can be ascribed to faulty methodology. The results of these early refractory period estimates and a critical analysis of the methods used to obtain these results follows.

a) Early estimates of refractory periods of feeding-relevant fibers

Rolls (1973) and Halboth and Coons (1973) estimated the refractory periods for the SIF substrate to be in the 0.6 to 1.6 msec range. Hawkins and Chang (1974) later compared estimates of the refractory periods of the MFB substrate for SIF and BSR. The distribution of refractory periods for SIF, estimated by Hawkins and Chang (1974), differed markedly from the distribution of refractory periods for BSR. The estimates of refractory periods of fibers underlying BSR ranged from 0.9 to 2.0 msec, whereas the estimates of refractory periods of fibers underlying SIF ranged from 0.6 to 1.2 msec. Hawkins and Chang (1974) interpreted their results as suggesting that different populations of fibers composed the first stage systems for BSR and SIF. Despite the consistency of their refractory period estimates for SIF, all three of these studies contain serious methodological flaws which undermine the reliability of their results. Although Halboth and Coons' (1973) study also contains refractory period data for SIF

using more appropriate methodology it is still subject to criticisms which will be explained later.

Hawkins and Chang (1974), Halboth and Coons (1973) and Rolls (1973) used changes in the rate (latency) of feeding as an index of the effectiveness of the T-pulse in eliciting a second action potential in the feeding-relevant fibers. Estimates of refractoriness based directly on an animal's latency to feed are subjected to the effects of variables which are unrelated to the post-stimulation excitability cycles of the feeding-relevant fibers. Yecmans (1975) has convincingly demonstrated, in the case of BSR, that refractory period estimates which are based on changes of lever pressing rate produced by varying the C-T interval are dependant on the base frequency used to obtain these estimates. Yecmans' (1975) criticisms also apply to Hawkins and Chang's (1974), Rolls' (1973) and Halboth and Coons' (1973) refractory period data for SIF.

To understand how behavioral output measures can distort refractory period estimates for SIF, it is necessary to understand the relationship between the rate of elicited feeding and the frequency of stimulation. The latency to initiate feeding within a 20 sec train of pulses of constant intensity is dependent on the pulse frequency; the function relating pulse frequency to latency to feed resembles an inverted ogive (Figure 3). The linear portion of the latency-frequency curve is bound at sub-threshold pulse frequencies by feeding latencies that exceed the 20 sec

## FIGURE 3

The latency versus frequency curve.

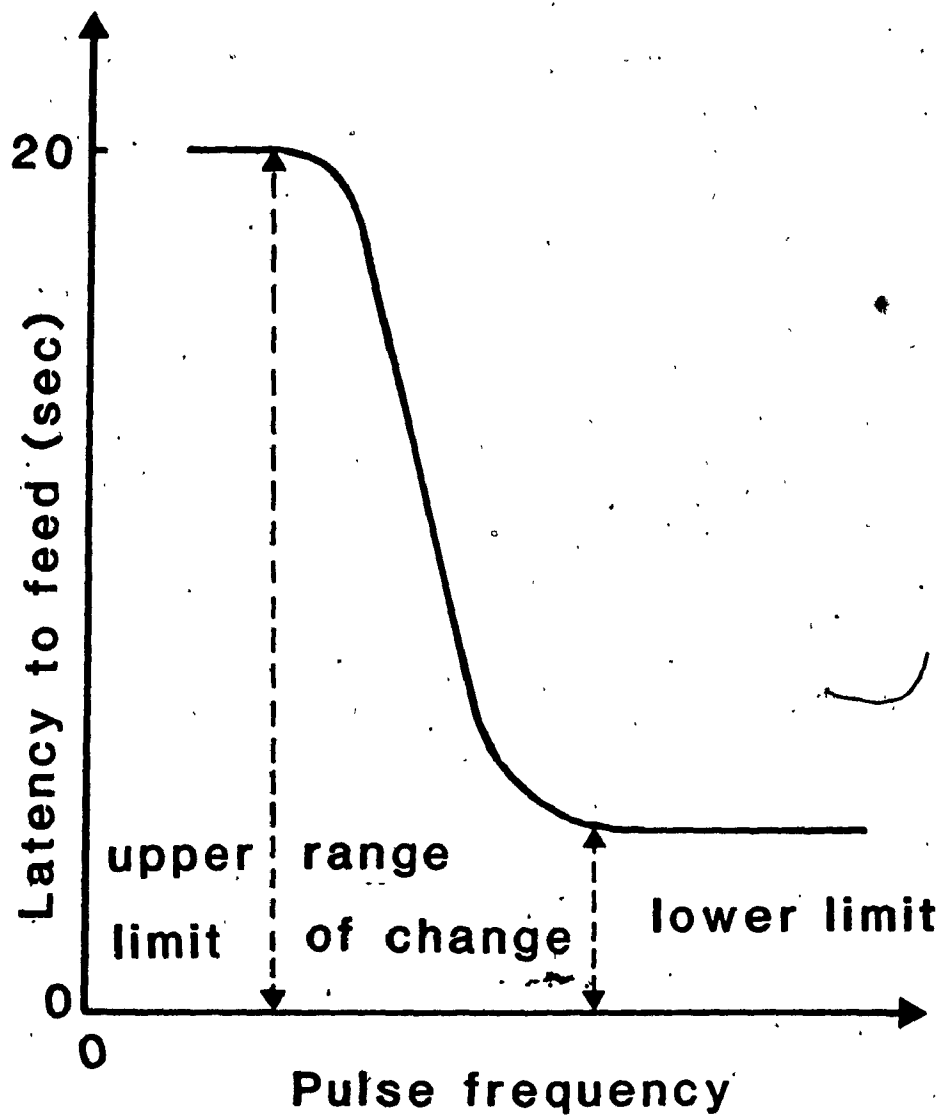


Figure 3

cut-off point (upper limit) and by asymptotic feeding latencies at maximal pulse frequencies (lower limit). In essence the upper and lower limits of the frequency-latency curve define the limits of observable changes of an animal's latency to feed in response to variations in stimulation frequency. The lower limit defines the portion of the curve where any further increase in pulse frequency affords no further significant decrease in feeding latency, whereas the upper limit defines that portion of the curve where the frequency of stimulation is insufficient to elicit a feeding response within an arbitrarily chosen time period (20 sec). Hence, in order to observe the effects of a particular manipulation on feeding latency, a pulse frequency should be chosen that will (a) elicit a feeding response and (b) elicit feeding at a latency which will allow the effects of the manipulation to be observed. The choice of an appropriate pulse frequency, therefore, will depend on the expected effect of the manipulation on the feeding latency. If, for example, a particular manipulation is expected to lengthen feeding latencies, then a base pulse frequency that elicits short latency feeding should be used.

In the case of a refractory period experiment, the addition of T-pulses to a train of C-pulses will, if the C-T interval is long enough, elicit shorter latency feeding than that which is seen when only C-pulses are applied. The choice of an appropriate base frequency is therefore critical. If a frequency of single pulses is chosen which elicits asymptotic feeding

latencies, then the addition of a T-pulse will not produce any decrease in feeding latency regardless of the C-T interval used and therefore no estimate of the feeding-relevant refractory periods can be obtained. If a frequency of single pulses is used which is below the threshold for feeding, then the best refractory period estimate of the feeding-relevant fibers will be that C-T interval which is sufficiently long to produce double firings in enough feeding-relevant fibers to exceed the feeding substrate's activation threshold and generate an observable feeding response.

The manner in which refractory period estimates vary according to the frequency of single pulses used is illustrated in Figure 4. The four hypothetical curves in Figure 4 express the relationship between latency to feed and eight different C-T intervals using four different single pulse frequency conditions (10, 15, 20 and 30 Hz). At single pulse frequencies of either 10, 15 or 20 Hz no feeding response is elicited within the 20 sec. period, whereas 30 Hz elicits feeding at close to asymptotic latencies. It is obvious that estimates of the shortest refractory periods (indicated by arrows) of the feeding-relevant fibers vary according to the base frequency that is used and that they do so in a predictable fashion. The three upper curves using base frequencies of 20, 15 and 10 Hz result in different refractory period estimates of 0.6, 0.8 and 1.0 msec respectively. However, when a base frequency which produces close to asymptotic feeding latencies is used (30 Hz), the

## FIGURE 4

Theoretical curves illustrating the errors of estimation of refractory periods that may result when behavioral output (latency to feed) is used to assess the effectiveness of the T-pulse. See text for further explanations.



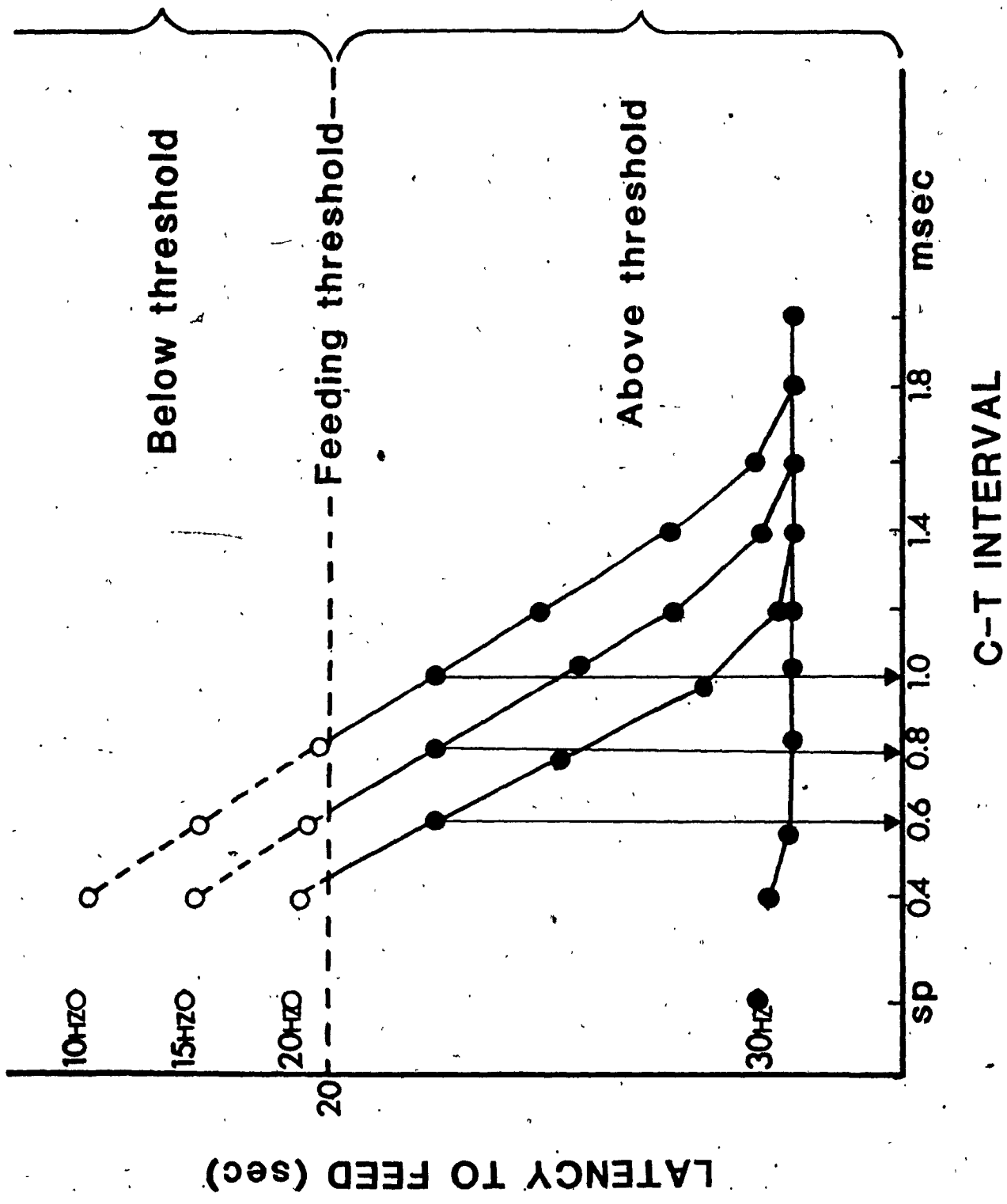


Figure 4

addition of a T-pulse produces at best a questionable decrease in feeding latencies and a dubious estimate of refractoriness. Hence, when subthreshold single pulse frequencies are used, an underestimation of the shortest refractory periods ensues. The severity of this underestimation will depend on how much the base frequency deviates below the feeding threshold.

Herein lies the problem with Hawkins and Chang's (1974), Rolls' (1973) and Halboth and Coons' (1973) estimates of the refractory periods of feeding-relevant fibers. These investigators used the restricted range of behavioral changes produced by varying the pulse frequency to measure the effectiveness of the T-pulse. Hence, it becomes difficult to assess whether their refractory period estimates are reliable or if they are subjected to the frequency induced biases described above. Furthermore, this problem is exacerbated when refractory period data for SIF is compared to the refractory period data for BSR which was obtained using the same faulty methodology. Given these methodological considerations, one can ask if the refractory period estimates for SIF provided in the above three studies are reliable and if the differences in the refractory period distributions for SIF and BSR observed by Hawkins and Chang (1974) are real or artefactual.

As mentioned earlier Halboth and Coons (1973) also obtained refractory period estimates for MFB feeding-relevant fibers using more appropriate methodology. Their refractory

period estimates for SIF, which also ranged from 0.5 to 1.2 msec, were based on changes in the stimulation intensity necessary to elicit feeding at various C-T intervals. Halboth and Coons (1973) showed that as the C-T interval is lengthened, the current intensity required to elicit a feeding response at a constant latency decreases. This is equivalent to saying that as the number of feeding-relevant fibers which fire to both the C- and T-pulses increases, the total number of feeding-relevant fibers that must be fired to maintain a feeding response at a constant latency decreases. This method of measuring the effectiveness of the T-pulse involves a trade-off procedure whereby the latency to feed is kept constant and therefore is not subject to the criticisms levelled at studies which use changes in latency to feed as a measure of T-pulse effectiveness.

However, Halboth and Coons' (1973) estimates of refractory periods for SIF may still be distorted because their methodology involves varying the current intensity of the stimulation. Hence, Halboth and Coons (1973) chose current intensity as the parameter they varied to offset the increased excitation produced by applying pairs of pulses. We have already seen that varying the current intensity changes the size of the field of effective stimulation and, therefore, the number of fibers that are fired by the stimulation. It therefore follows that measurements of T-pulse effectiveness based on changes in current intensity thresholds result in refractory period estimates of different populations of feeding-relevant fibers. The reliability of

refractory period estimates obtained in this manner is based on the assumption that the distribution of refractory periods of feeding-relevant fibers excited by the stimulation does not vary systematically as the number of feeding-relevant fibers recruited by the stimulation is changed. In other words, Halboth and Coons (1973) assumed that the distribution of refractory periods of feeding-relevant fibers deviated around a common mean with equal variance regardless of the size of the population of feeding-relevant fibers being excited by the stimulation. This may or may not be the case.

Recovery from refractoriness following the application of a C-pulse is a temporal event in that it allows the neuron to fire to the subsequent application of a T-pulse. It follows then that the parameter that should be varied to offset a doubling of the firing frequency produced by an effective T-pulse should be the frequency of paired pulses. Yeomans (1975) and Yeomans and Davis (1975) have shown that trade-off experiments using pulse frequency as the offsetting variable provide the most reliable estimates of refractory periods of MFB reward-relevant fibers. Up to now, only one study has provided refractory period estimates for SIF using pulse frequency as the offsetting variable.

b) Recent refractory period estimates of feeding-relevant fibers.

Hawkins et al. (1983) showed that the distribution of refractory periods of MFB feeding-relevant fibers ranged from 0.6 to 2.0 msec. As suggested by Yeomans (1975) and Yeomans and Davis (1975), Hawkins et al. (1983) used pulse and paired pulse frequency as the offsetting variable to determine the relative effectiveness of the T-pulse and provided empirical evidence that refractory period estimates for SIF using a frequency measure are not subjected to any systematic scaling distortions. The refractory period estimates for SIF obtained by Hawkins et al. (1983) correspond closely to their own refractory period estimates for BSR and to those obtained by others using current methodology (Bielajew et al., 1981; Rompre and Miliaressis, 1980; Yeomans, 1975; Yeomans, 1979).

The fact the refractory periods for SIF and BSR have basically the same distribution suggests one of two possibilities. On the one hand it may be that BSR and SIF are subserved by a common MFB first stage system. Such an arrangement would correspond to either of the anatomical models depicted in Figures 2a and 2b. On the other hand the data of Hawkins et al. (1983) may mean only that SIF and BSR are subserved by fibers which have very similar refractory period distributions. Such an interpretation would be consistent with the anatomical model shown in Figure 1b.

c) Summary and conclusions.

Very little is known of the electrophysiological properties of the first stage system for SIF. Although four studies have obtained estimates of the refractory periods of feeding-relevant fibers, only one (Hawkins et al., 1983) has provided, what appear to be reliable estimates. The refractory period estimates presented in the three other studies are based on faulty methodology and therefore cannot be assumed to be valid. It is however interesting to note that, despite their methodological flaws, these three studies have found refractory period distributions for SIF which are surprisingly similar to those obtained by Hawkins et al. (1983).

The main conclusion that arises from the literature reviewed in this section is that the differences in refractory period distributions between SIF and BSR originally reported by Hawkins and Chang (1974) are not observed when the appropriate methodology is used. Although the data of Hawkins et al. (1983) are suggestive of a common first stage system for BSR and SIF, the fact remains that similar refractory period distributions for SIF and BSR do not constitute undisputable proof that the same population of MFB fibers underlie the motivating as well as the rewarding effects of electrical stimulation. The present research is aimed at shedding additional light on this question.

#### 6) General summary and conclusions

The first part of the general introduction to this thesis

provided a review of our current knowledge concerning the electrophysiological, anatomical and pharmacological characteristics of the MFB substrate for BSR. The second part was a review of the literature pertaining to the MFB substrate of SIF and its relationship, if any, to the MFB substrate of BSR. The question of interest throughout this part of the introduction has been whether or not SIF and BSR are subserved by a common population of MFB fibers. Based on the existing model of the MFB substrate for BSR several assumptions and models concerning the anatomy of the MFB substrate for SIF were made. It was assumed that electrical stimulation which elicits a feeding response activates a first stage fiber system which inputs into a second stage system or integrator. It was further assumed that the functions of the first and second stage systems for SIF were the same as those of the first and second stage systems for BSR. Four anatomical models of the MFB substrate for SIF were then presented. Two models depicted the first stage system for SIF as being anatomically separate from the first stage system for BSR, whereas two other models depicted a common first stage system for both SIF and BSR.

Using these anatomical models as conceptual guidelines, the literature relevant to the anatomical specificity of SIF and BSR at the level of the first stage system was reviewed. Considering the fact that the notion of anatomically distinct SIF and BSR substrates appeared to predominate the literature, there is seemingly little compelling evidence to support such a

contention. It is true that such a conclusion rests comfortably, and somewhat complacently on the benefits of hindsight. Nonetheless the strongest evidence reviewed tends to suggest that SIF and BSR may be subserved by a common population of MFB fibers. Contrary to the widely held view, SIF does not appear to be restricted to any particular region of the MFB. As is the case with BSR, stimulation sites from which a feeding response can be elicited are distributed all along the longitudinal axis of the MFB. An animal that feeds in response to MFB stimulation will also find the stimulation rewarding and, if it is given the opportunity, will also exhibit other consummatory behaviors in response to stimulation of the same site. Finally, when the appropriate methodology is used, estimates of refractory periods for BSR and SIF are similar.

The studies that suggest anatomically distinct first stage systems for BSR and SIF are considerably weaker, mostly because they are based on faulty assumptions or they used unsound methodology. The effects of various drive manipulations on BSR do not exhibit the degree of anatomical specificity that would warrant the conclusion that the MFB comprises anatomically distinct first stage reward systems. While the effects of drive manipulations on BSR parallel their effects on responding maintained by conventional rewards, there is no compelling evidence to suggest that changes in responsiveness to BSR produced by various drive states operate on the reward-relevant signal propagated by the first stage system for BSR.



Up to now, very few studies have attempted to analyse the anatomical specificity of SIF and BSR by comparing the neurophysiological properties of the directly activated substrates underlying these two behaviors. The use of psychophysical techniques has yielded a considerable amount of data on the neurophysiological properties of the MFB substrate for BSR. It is fair to expect a similarly fruitful outcome when these techniques are used to elucidate the neurophysiological properties of the MFB substrate for SIF.

#### 7) Purpose of the present investigation

In general, the present investigation addresses the long standing question of whether or not SIF and BSR are subserved by a common neuronal substrate. Although the question appears to be simple, the task of determining whether the feeding-relevant circuitry is common to the reward-relevant circuitry is too ambitious for any single investigator to embark upon. The specific goals of this investigation are therefore considerably more modest. The experiments described in this thesis have documented some of the electrophysiological and anatomical characteristics of the directly activated MFB fibers underlying SIF and compared them to those which underlie BSR.

The first experiment is basically a replication of the study of Hawkins et al. (1983) with the added feature that

refractory period estimates for SIF and BSR were obtained from the same stimulation site using a fine grain analysis. The second experiment is an attempt to elucidate the pharmacological properties of the first stage system for BSR. The third experiment deals with the anatomy of the first stage system for SIF. This experiment was designed to test the hypothesis that the first stage system for SIF comprises fibers which course along the longitudinal axis of the MFB, between the lateral hypothalamus and the ventral tegmental area.

## EXPERIMENT 1

## Introduction

Hawkins et al. (1983) have argued that the MFB fibers subserving SIF have post-stimulation excitability cycles that are similar to those of neurons activated by rewarding stimulation, suggesting that the two behaviors are subserved either by the same population of fibers or by two distinct populations of fibers with very similar refractory period characteristics. Their work, however, provided a between-animal comparison of excitability cycles of the substrate for these two behaviors. Because refractory period estimates may show slight variations from one site to another, it is important to provide a within-animal comparison of refractory period estimates of the SIF and BSR substrates. The present experiment was designed to provide refractory period estimates for neurons subserving SIF and BSR at the same site of stimulation. If the same population of fibers or a common subset of fibers were directly activated by both rewarding stimulation and by stimulation that induces feeding, then the distribution of refractory periods or a component of the distribution should be similar for both behaviors.

The present investigation also provides refractory period estimates for SIF and BSR using a fine grain analysis of T-pulse effectiveness. Unlike previous studies, which did not test very many C-T intervals, the present investigation provides more

accurate estimates of the distribution of refractory periods for SIF and BSR by varying C-T intervals in small increments. It was reasoned that a fine grain analysis of the changes in T-pulse effectiveness values would increase the chances of uncovering any differences between the distribution of refractory periods for SIF and that of BSR.

Finally, SIF and BSR are subserved by a common set of fibers, feeding should be obtained all along the MFB and not only in the lateral hypothalamic MFB. Data of Cox and Valenstein (1969) and of Waldbillig (1975) make it clear that sites supporting SIF are not as localized as was once thought. The present study also provides estimates of refractory periods for SIF and BSR at different anterior-posterior levels of the MFB.

#### METHOD

##### Animals and surgery

The subjects were male, Long-Evans rats weighing, on average, 500 grams at the time of surgery. The animals were individually housed, maintained on a 12 hour day/ 12 hour night cycle and had ad libitum access to water and food (Purina rat chow). Under sodium pentobarbital (Somnotol; 60mg/kg), anesthesia, two rats were implanted with unipolar fixed electrodes aimed at the lateral hypothalamic MFB (DeGroot plane: A.P.=0.8mm behind bregma, Lat.=1.6mm from midline, D.V.=8.9mm

below dura), while the remaining rats were implanted with unipolar moveable electrodes aimed at various antero-posterior levels of the MFB. The electrode was 254 micrometer stainless steel wire insulated with Formvar and exposed only at its square cross-section. Fixed electrodes were soldered to Amphenol<sup>®</sup> male mini-pins. Moveable electrodes were concentrically soldered to male Amphenol pins which had been threaded with a 2-56 die. The threaded Amphenol pins were each screwed into a cylindrical piece of nylon which had been threaded with a 2-56 tap. A complete rotation of the Amphenol pin resulted in a 0.454 mm vertical movement of the electrode. Current return was through a stainless steel wire soldered to an amphenol pin and wrapped around two stainless steel screws imbedded in the cranium. The entire assembly was held together and anchored to the skull screws with dental acrylic cement.

#### Apparatus

The temporal parameters of the stimulation were controlled by a digital pulse generator, whereas the pulse amplitude was controlled by constant current generator (Mundl, 1980). An oscilloscope (Gould OS300) was used to monitor the current intensity by reading the voltage drop across a 1 kohm resistor in series with the rat's electrode. The build-up of electrical charge at the electrode-tissue interface was shunted to the current return at the offset of each pulse through a 1 kohm resistor. The impedance at the electrode-tissue interface was

monitored by applying to the rat's depth electrode a continuous train of 0.1 msec pulses at 20 Hz and 200 microamps and measuring the voltage drop across the stimulating electrode and current return. Rats with an electrode-tissue impedance higher than 16 kohms were not tested. To allow unimpeded movements of the rats, the stimulation was delivered through a flexible lead which was connected to a mercury commutator.

#### Procedure

##### a) Screening and preliminary testing

All rats were first tested for stimulation induced feeding during daily two hour sessions. Immediately before the testing session, the rats were placed for 30 minutes in a bucket containing fresh rat chow to assure recent satiation. They were then placed in a 25x35 cm wooden box with a plexiglass facade, the floor of which was covered with 45 mg Noyes pellets. Once they had habituated to the surroundings, the rats received, every 40 seconds, a 20 second train of 0.1 msec cathodal pulses at a frequency of 40 Hz. The current intensity was increased in 10 microamp increments until the rats demonstrated moderate levels of forward approach behavior. The stimulation strength was then held constant at this level for the remainder of the screening session. The rats were tested during daily sessions for a maximum of 10 days or until feeding behavior emerged. A rat was considered to be a feeder when it consistently ate at least 4

pellets during the stimulation period and when feeding terminated immediately upon offset of the stimulation. The frequency threshold for feeding was then determined for each rat and was defined as the pulse frequency at which the rats would eat 4 pellets in 20 seconds. Because the latency to meet criterion decreases as a function of increasing the pulse frequency, frequency threshold determinations simply involved decreasing in 5% increments the pulse frequency from high values that produced short latency feeding to low values which elicited feeding at latencies greater than 20 sec. The frequency threshold for feeding was then derived from the resulting latency-frequency function by graphical interpolation. Figure 5a illustrates the manner in which frequency thresholds are derived from the latency-frequency function.

Once frequency thresholds for feeding had stabilized (usually after 5-10 days of testing), the rats were placed in standard operant cages and screened for brain stimulation reward (BSR). Depression of the lever initiated a 500 msec train of 0.1 msec cathodal pulses at the same intensity used for stimulation-induced feeding. The frequency threshold for BSR was determined in much the same manner as for feeding. Pulse frequency was decreased in 5% steps from initial values which sustained maximal lever pressing rates to a pulse frequency for which the rats would not respond. The pulse frequency necessary to maintain 10% of the maximal lever pressing rate was derived from the rate-frequency function by graphical interpolation. The

manner in which frequency thresholds are derived from the rate-frequency curve is illustrated in Figure 5b. The frequency threshold for BSR was generally higher than the frequency threshold for SIF. However current intensity was always adjusted so as to obtain frequency thresholds for both SIF and BSR that were in the 20-60 Hz range.

#### b) Refractory period tests

Refractory period tests were conducted for SIF first; however the same testing protocol was used for both BSR and SIF. A session always began by determining the rats' frequency threshold under a single pulse (SP) condition. Threshold determinations were then conducted under 15-18 different paired pulse conditions, where the delay between the two, equal amplitude, constituent pulses (C and T pulses) of each pulse pair was varied. The same C-T intervals were tested for both behaviors. A testing session usually consisted of 5-6 blocks of 3 paired pulse and 6-7 single pulse threshold determinations. Within each block one short, one medium and one long C-T interval were tested and a single pulse threshold determination preceded and followed each block of paired pulse conditions tested. The testing order of each block was randomized across days. The averaged frequency thresholds for the two single pulse conditions was used to estimate the relative effectiveness of the T-pulse under the intervening paired pulse conditions. Estimates of T-pulse effectiveness for each C-T interval tested were obtained

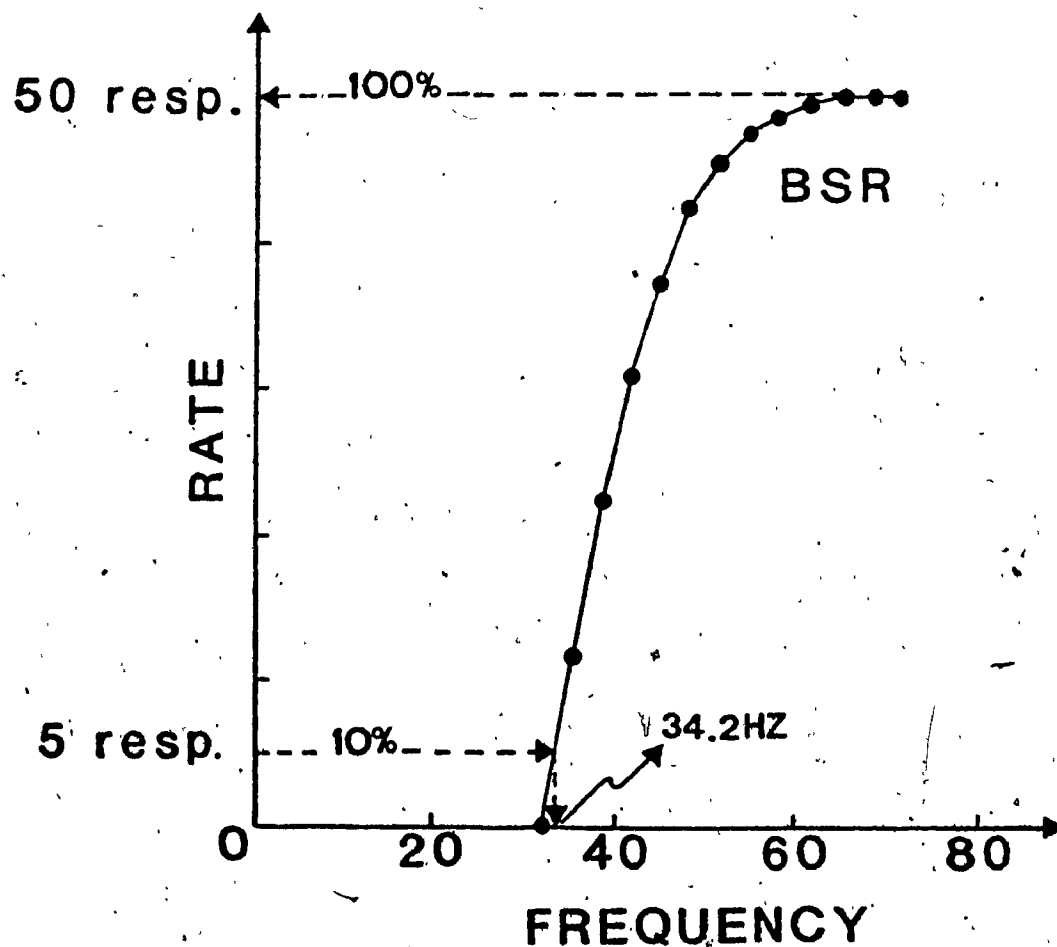
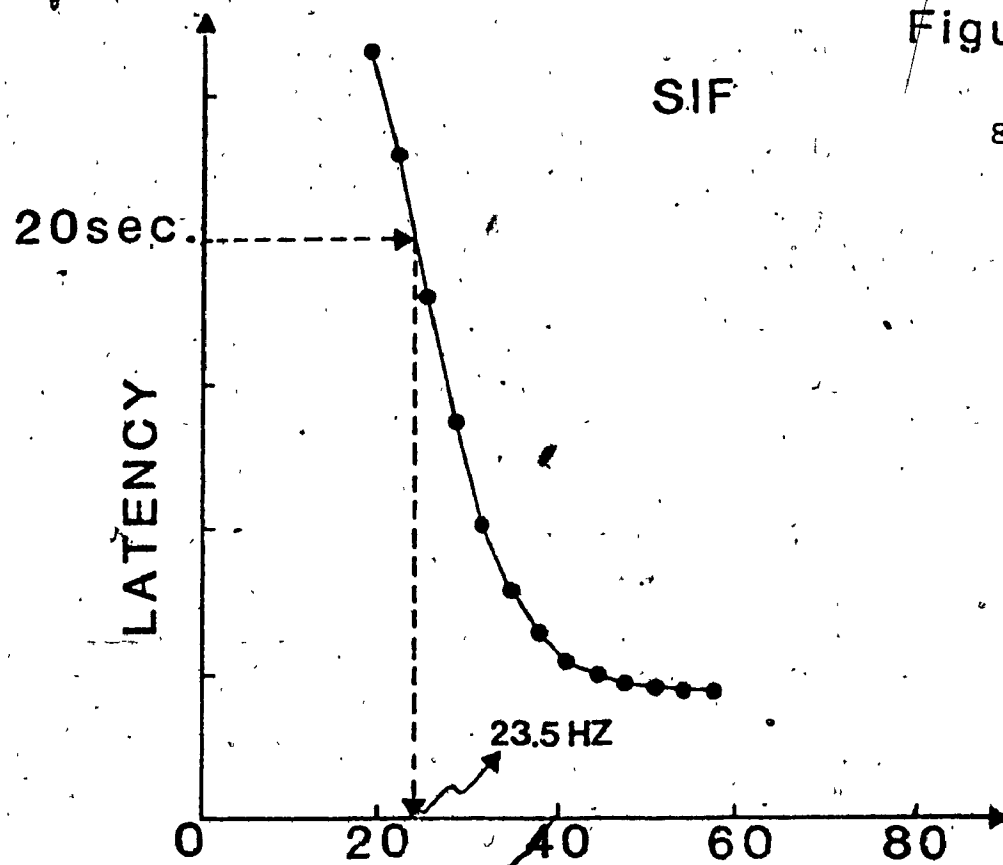


**FIGURE 5**

Determination of frequency thresholds for (a) SIF and (b) BSR  
by graphical interpolation. See text for further explanations.

Figure 5

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by using Yeomans' (1975) equation:

$$\text{TPE} = \frac{\text{RHz sp}}{\text{RHz c-t}} - 1$$

where TPE = T-pulse effectiveness.

RHz sp = the required frequency of single pulses.

RHz c-t = the required frequency of paired pulses at a given C-T interval.

A minimum of four replications were performed at the following C-T intervals:

0.2, 0.4, 0.44, 0.48, 0.5, 0.52, 0.56, 0.6, 0.7, 0.8, 1.0, 1.2, 1.6, 2.0, 2.5, 3.0, 3.5, 4.0 msec.

### c) Mapping and histology

After the refractory period experiments had been completed those rats with moveable electrodes were tested for SIF at more ventral sites. The electrodes were lowered 110 micrometers and frequency threshold determinations for the new site were performed at a fixed intensity of 200 microamps. Frequency threshold determinations were also obtained for BSR in two rats. The electrodes in these two rats were lowered in 230 micrometer increments. The electrodes were lowered only when frequency thresholds deviated by no more than 10% on three consecutive days.

The maximum frequencies tested never exceeded 250 Hz. When the maximum vertical travel of the electrode (approx. 2.5 mm) had been reached or when a change in electrode position resulted in a loss of feeding or self-stimulation, the rats were anesthetized with chloral hydrate and transcardially perfused with 0.9% saline followed by a 10% formalin solution. The brains were then sliced in 40 micrometer sections and stained with formol-thionin for purpose of electrode localization. The position of the electrodes were compared and transposed to diagrams of coronal slices taken from Pellegrino, Pellegrino and Cushman's (1979) stereotaxic atlas. Five rats (AG2, AG4, AG6, AG7 and AG918) from the present experiment were used in a second experiment. However, following the completion of Experiment 2, the mapping study and subsequent histological analysis were performed according to the procedure just described.

#### d) Statistical analysis of refractory period curves

A statistical treatment of the data was chosen which would help determine whether or not the rates of recovery from refractoriness of the feeding-relevant fibers are the same as that of the reward-relevant fibers. To do so, a comparison of the slope of the regression lines for the SIF and BSR refractory period curves was performed for each rat used in the experiment. Before the regression analysis could be performed the data were subjected to preliminary treatments designed to (a) define the rising portion of the refractory period curves and (b) eliminate

any extraneous variables which would have a disproportionate effect on the slope of the rising portion of the refractory period curve. A Student's t-test for correlated samples was first used to determine the range of C-T intervals which produced significant increases in T-pulse effectiveness (TPE). The C-T interval which produced the lowest average TPE value was compared to the TPE values at successively shorter C-T intervals until a C-T interval that did not produce a significant increase ( $\alpha=0.05$ , one-tail t-test) in TPE was found. The same procedure was employed to determine the C-T interval which produced no further significant increase in TPE (asymptote) when compared to the C-T interval at which the highest average TPE value occurred. Data points at C-T intervals longer and shorter than these critical C-T intervals were excluded from the analysis.

The averaged TPE values for the C-T intervals which contributed significantly to the rising portion of the refractory period curve were then rescaled so that each curve extended over the entire range of TPE values from 0 to 1.0. In order to do so Bielajew's et al. (1981) transformation procedure was used according to the following equation:

$$\text{TPE transformed} = \frac{\text{TPE c-t} - \text{TPE min}}{\text{TPE max} - \text{TPE min}}$$

where TPE transformed = the T-pulse effectiveness rescaled

to a range of TPE values from 0 to 1.0.

TPE  $c-t$  = the untransformed TPE value at a given C-T interval

TPE  $_{min}$  = the lowest untransformed TPE value obtained

TPE  $_{max}$  = the highest untransformed TPE value obtained

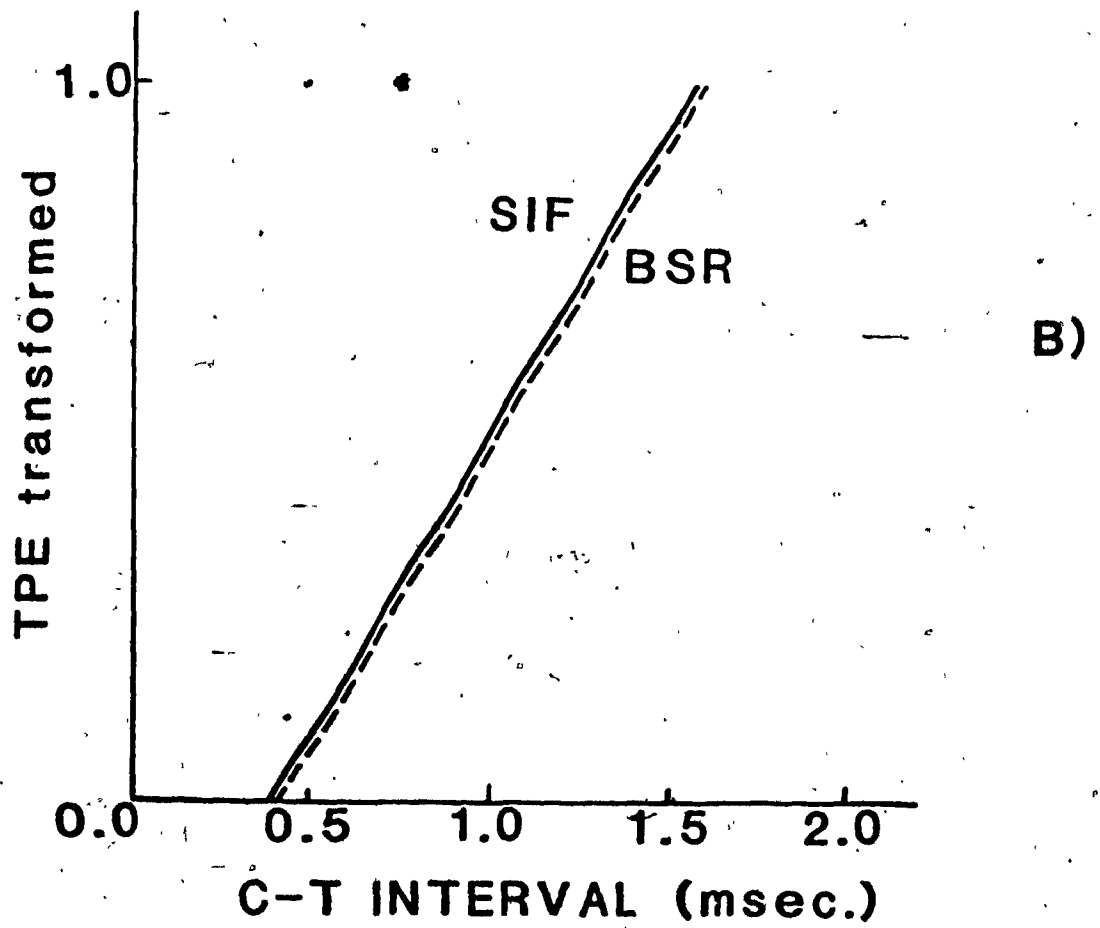
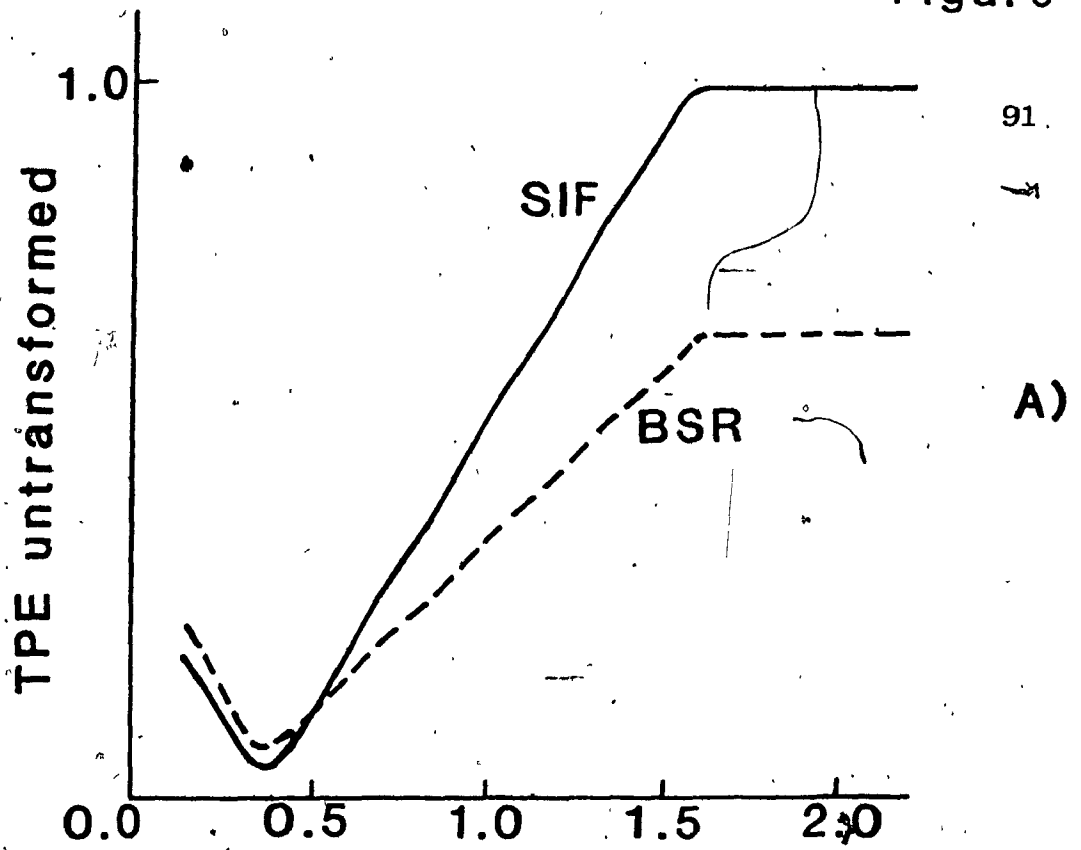
Bielajew et al. (1981) have argued that rescaling the data in this manner offers a more precise comparison of two refractory period curves by eliminating factors that may affect the slope of the refractory period function but which have little bearing on the actual rate of recovery from refractoriness of the underlying substrate. Theoretically refractory period curves should level off at TPE values of 1.0; in practice however they do not always do so. If SIF and BSR refractory period curves approach asymptote at the same C-T interval but at different TPE values, one would be left with the erroneous impression that the fibers subserving SIF and BSR have different rates of recovery from refractoriness. This situation is illustrated in Figure 6a. However by allowing the rising portion of both refractory period curves to extend from 0 to 1.0 one can see that both curves show proportionately similar rates of recovery from refractoriness (Figure 6b).

The slope of the regression lines fitted to the transformed refractory period data for SIF and BSR were compared by using a  $t$ -test statistic for parallelism (Kleinbaum and Kupper, 1978).

**FIGURE 6**

(a) Hypothetical example of an apparent difference in the slopes of two refractory period functions that reach asymptote at different TPE values and (b) the expected refractory period functions after the TPE values have been transformed using the scaling procedure of Bielajew et al. (1981). See text for further explanation.

Figure 6





The level of significance was set at 0.05. Therefore a non-significant t-test indicates that the rate of recovery from refractoriness of feeding-relevant fibers does not differ from the rate of recovery from refractoriness of reward-relevant fibers.

## RESULTS

### a) Histology and mapping of SIF sites

Data were collected from a total of 9 rats. Refractory period estimates for both SIF and BSR were obtained from 8 rats implanted with stimulating electrodes at different anterior-posterior levels of the MFB. An additional rat provided mapping data for SIF; however no refractory period data were collected. The histological reconstructions of the electrode placements in the 9 animals used are shown in Figures 7a to 7h. Two rats (Figure 7a) were implanted with fixed electrodes in the perifornical (AG4) and the midlateral (AG2) regions of the lateral hypothalamic area. The electrode tips in rats AG2 and AG4 were both located 0.8 mm behind bregma.

The 7 other rats (AG6, AG7, AG18, AG19, AG30, AG917, AG918) were implanted with moveable electrodes. Hence the graph on the right side of each brain slice plots the frequency threshold determinations for each successive penetrations of the electrode tip. The most anterior electrode placement (AG6) was

0.4 mm anterior to bregma at the level of the anterior hypothalamic nucleus (Figure 7c). Sites positive for SIF extended over 1.75 mm in the dorsal-ventral axis; from the ventral boundary of the reticular thalamic nucleus to the ventral-lateral tip of the fornix. Frequency threshold determinations for SIF were obtained at each site at an intensity of 200 microamps and, and in the case of AG6 only, at intensities of 150 and 400 microamps as well. At 200 microamps, the lowest frequency thresholds were found at the more dorsal SIF sites, while frequency thresholds progressively increased as the electrode was lowered to more ventral sites. Stimulation of the most ventral site tested appeared to be aversive as evidenced by shuffling of the food pellets. Increasing the current intensity to 400 microamps resulted, as expected, in lower frequency thresholds for SIF at each site tested whereas higher frequency thresholds were obtained when the current intensity was decreased to 150 microamps. Increasing the current intensity used for SIF increased the number of more ventral sites which supported SIF; 6 sites supported SIF at 150 microamps, 10 at 200 microamps and 13 at 400 microamps.

The most posterior electrode placement (AG 918, Figure 7b) was 3.0 mm behind bregma on the midline of the ventral tegmental area. Only the most ventral of the 3 sites tested supported SIF. The electrode placement in AG30 was only slightly more rostral to that of AG918 (2.6 mm behind bregma) but approximately 1.2 mm off the midline. The electrode tip in AG30 (Figure 7h) traversed the

VTA; positive sites for SIF extended over 0.875 mm (7 sites).

Only the most ventral site in AG30 failed to support SIF whereas the most dorsal site supported SIF at the lowest pulse frequency.

Electrical stimulation in both AG918 and AG30 elicited a feeding response which was accompanied by forward locomotion. These rats would usually shovel the food into their mouth as they walked around the perimeter of the cage. This is marked contrast with feeding elicited from more rostral MFB electrode placements. In this case the stimulation usually causes the rat to stop locomoting and initiate feeding.

Two other moveable electrode placements (AG7, AG917) lay in the posterior regions of the MFB, approximately midway between the lateral hypothalamic area and the VTA. The electrode in AG7 (Figure 7f) was 1.8 mm behind bregma: where the mammillothalamic tract emerges from the mamillary nuclei. Four sites, extending from the ventral boundary of the zona incerta to the medial tip of the cerebral peduncle supported SIF. Stimulation at the most ventral of the 13 sites tested in AG7 elicited an erratic feeding response which was contaminated by forced head movements; no stable frequency threshold determination could be obtained from this site. The electrode placement in AG917 (Figure 7g) was slightly more caudal and medial to the electrode placement in AG7. Sites positive for SIF in AG917 extended over 1.125 mm: from the medial tip of the zona incerta to the medial tip of the cerebral peduncle. The lowest frequency threshold was obtained at the most dorsal site; successive electrode penetrations

resulted in progressively higher frequency thresholds for SIF. Here again, stimulation at the most ventral site produced an erratic feeding response which was accompanied by forced movements.

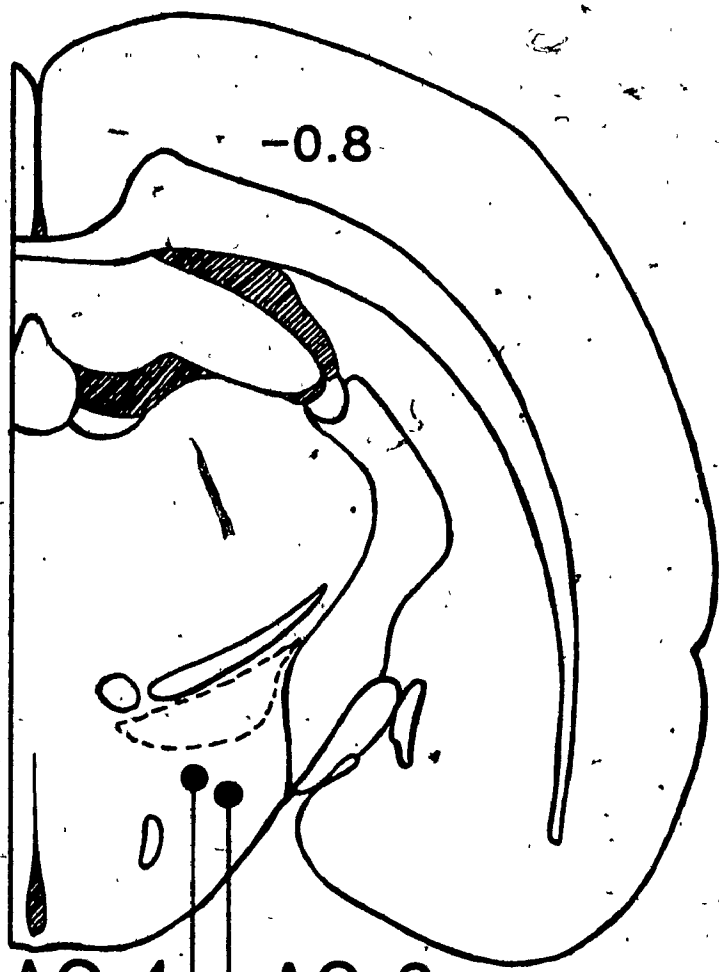
Rats AG18 and AG19 had moveable electrode placements in the perifornical region of the lateral hypothalamic area (Figures 7d & 7e). In both of these rats frequency threshold determinations were performed for BSR and SIF at the same intensity (200 microamps). However, in the case of these two rats, the size of the electrode movement increments was doubled to 227 micrometers.

In both cases, all the sites which supported SIF also supported BSR, whereas the most ventral site which was negative for SIF was also negative for BSR. Although the frequency thresholds for BSR were higher than that which was observed for SIF, the changes in frequency threshold for SIF caused by successive electrode penetrations were matched by proportionately similar changes in frequency thresholds for BSR. This is equivalent to saying that the ratio of pulse frequency requirements for SIF to the pulse frequency requirements for BSR is constant across the stimulation sites tested. This relationship is depicted in the insets of Figures 7d and 7e.

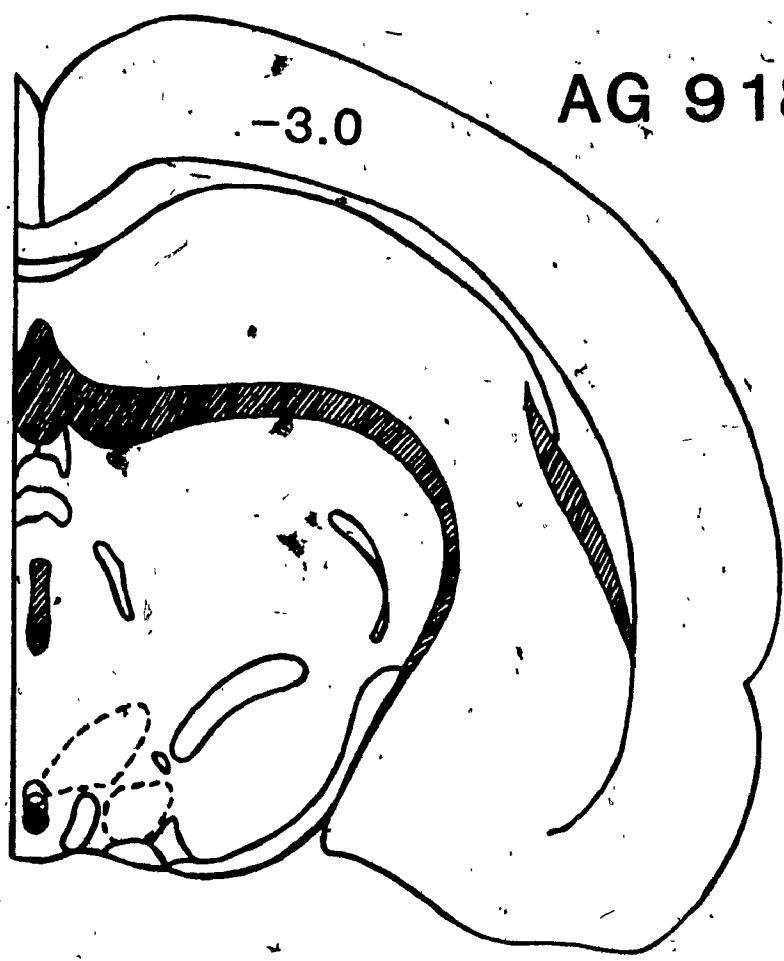
In summary, the sites positive for SIF were not restricted to the lateral hypothalamic MFB. The most rostral sites from which feeding was elicited were from the anterior portion of the lateral hypothalamic area (AG6), whereas the most caudal feeding site was in the midline of the ventral tegmental area (AB918).

## FIGURE 7

Histological localisation of electrode tracks of animals used in Experiment 1. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino and Cushman (1979). The number on each slice is the electrode distance from bregma. Negative numbers are posterior to bregma and positive numbers are anterior to bregma. Figure 7a shows positions of fixed electrodes of rats AG2 and AG4. Figure 7b shows the three VTA sites that were tested in AG918; only the most ventral site (filled circle) supported SIF. Figures 7c to 7h show the mapping data collected in the 6 other animals. Sites positive for SIF are represented by filled circles and sites negative for SIF are represented by circles. The graphs on the right plot the frequency thresholds for SIF as a function of electrode sites. See text for further explanations.



AG 4 | AG 2



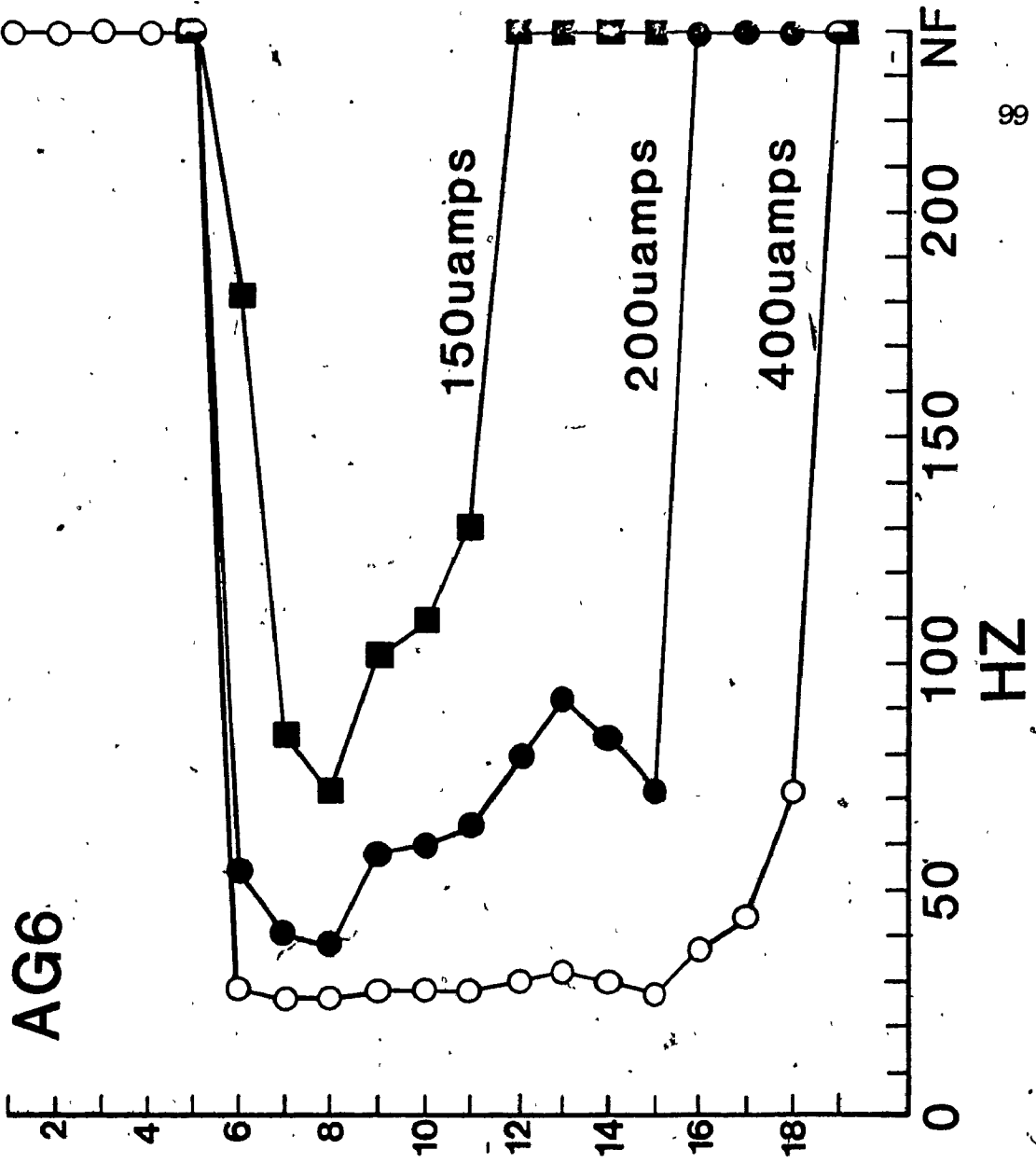
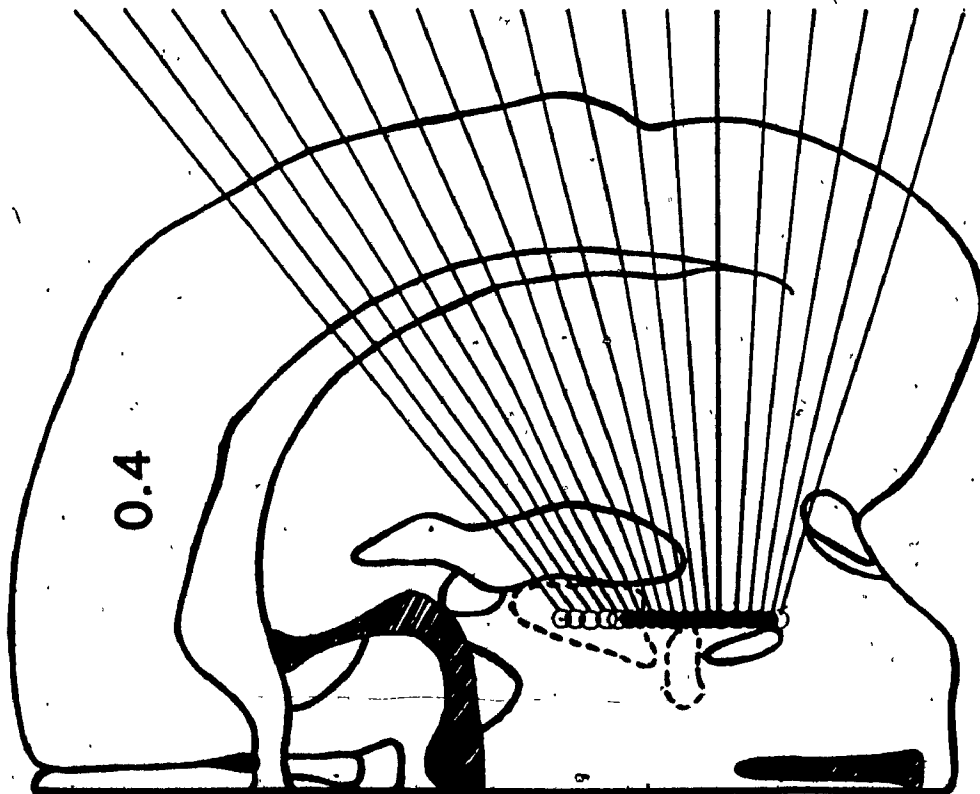
AG 918



FIGURE 7c

Histology and mapping data for rat AG6.



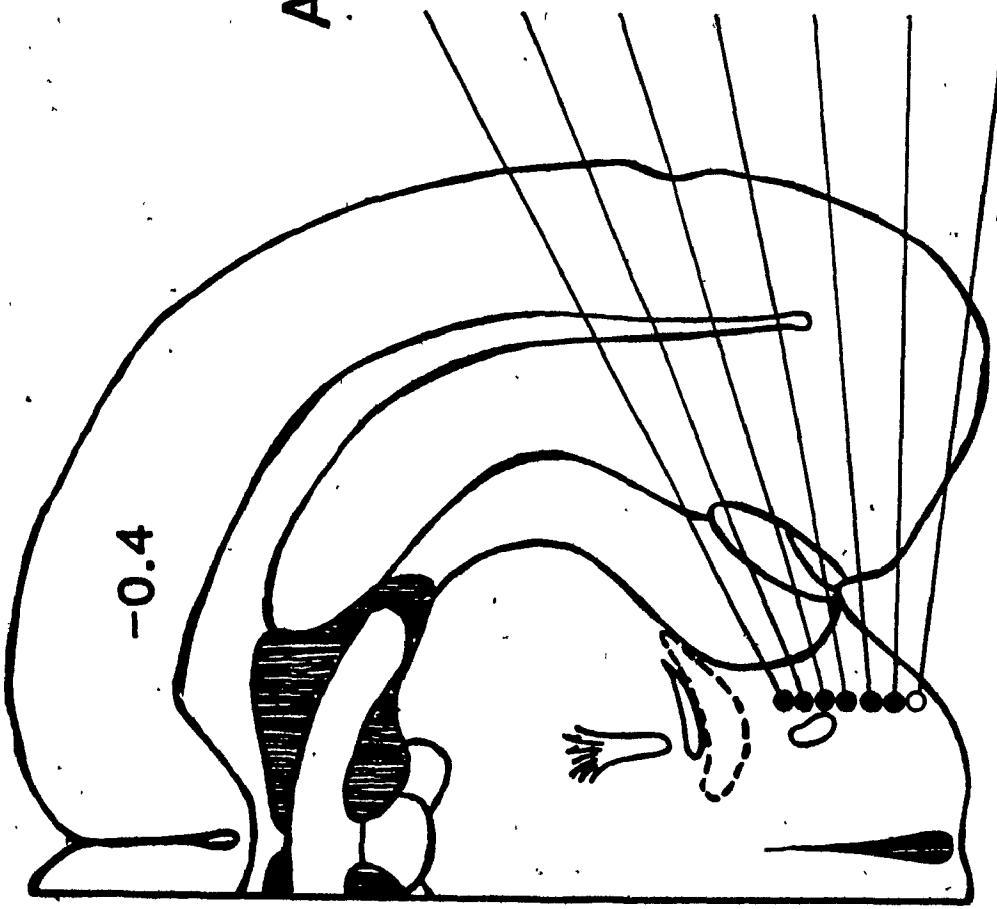




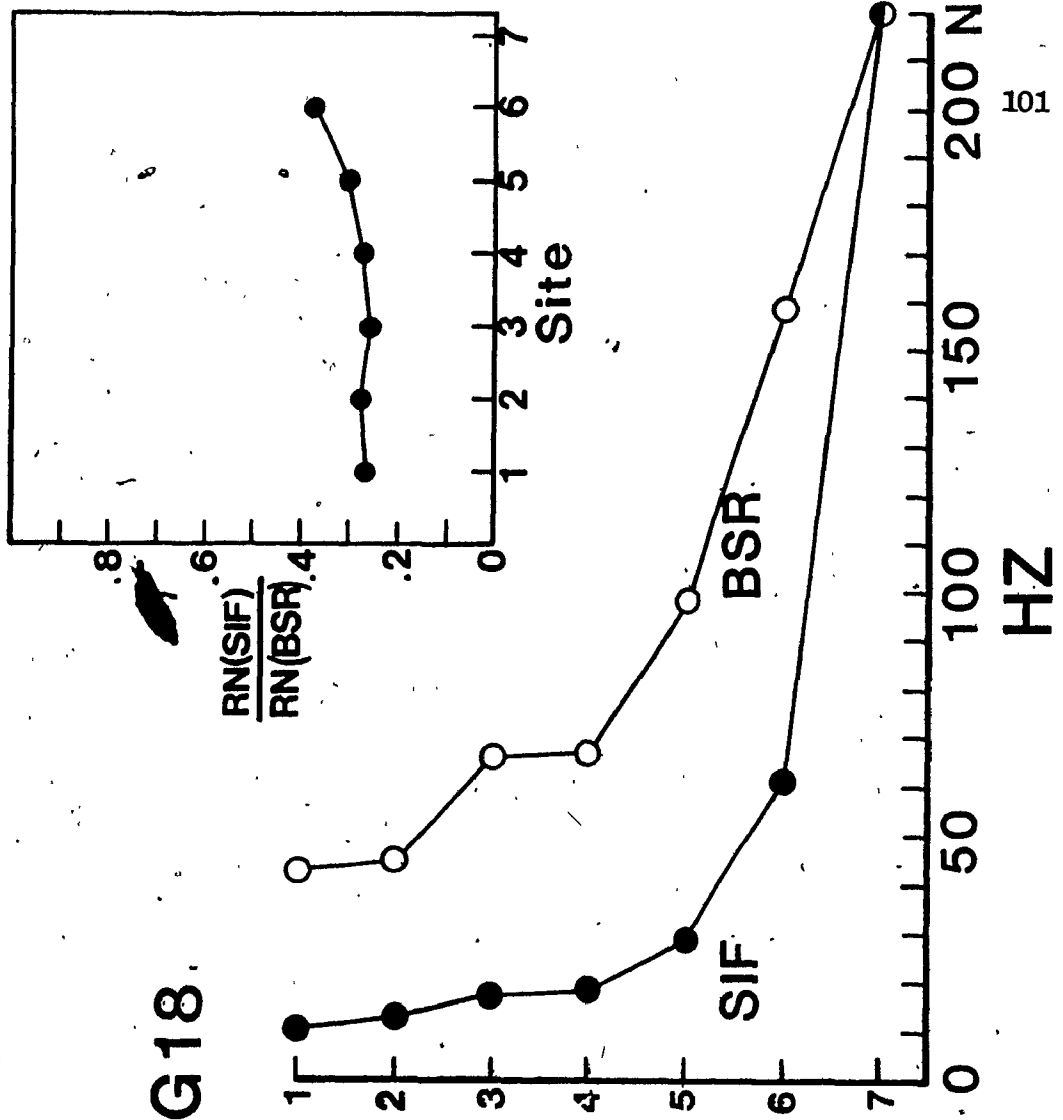
## FIGURE 7d

Histology and mapping data for rat AG18.

Inset plots the ratio of frequency threshold for SIF to that of BSR as a function of electrode position. See test for further explanations.

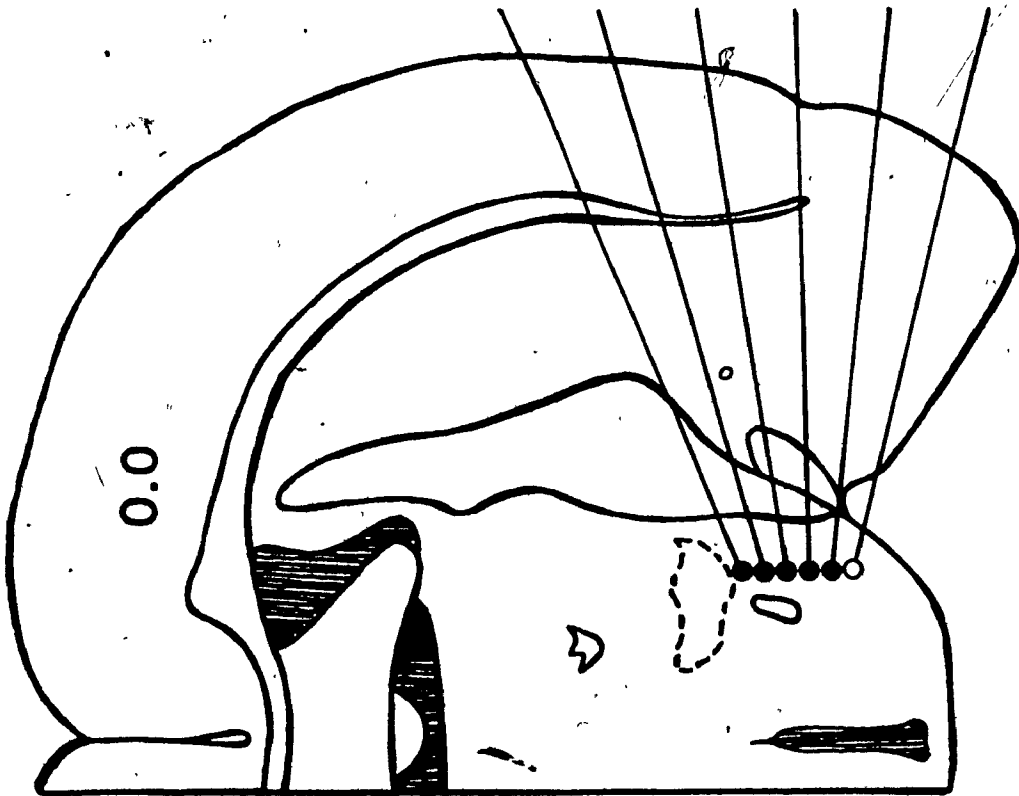


AG18.



**FIGURE 7e**

**Histology and mapping data for rat AG19.**



AG19

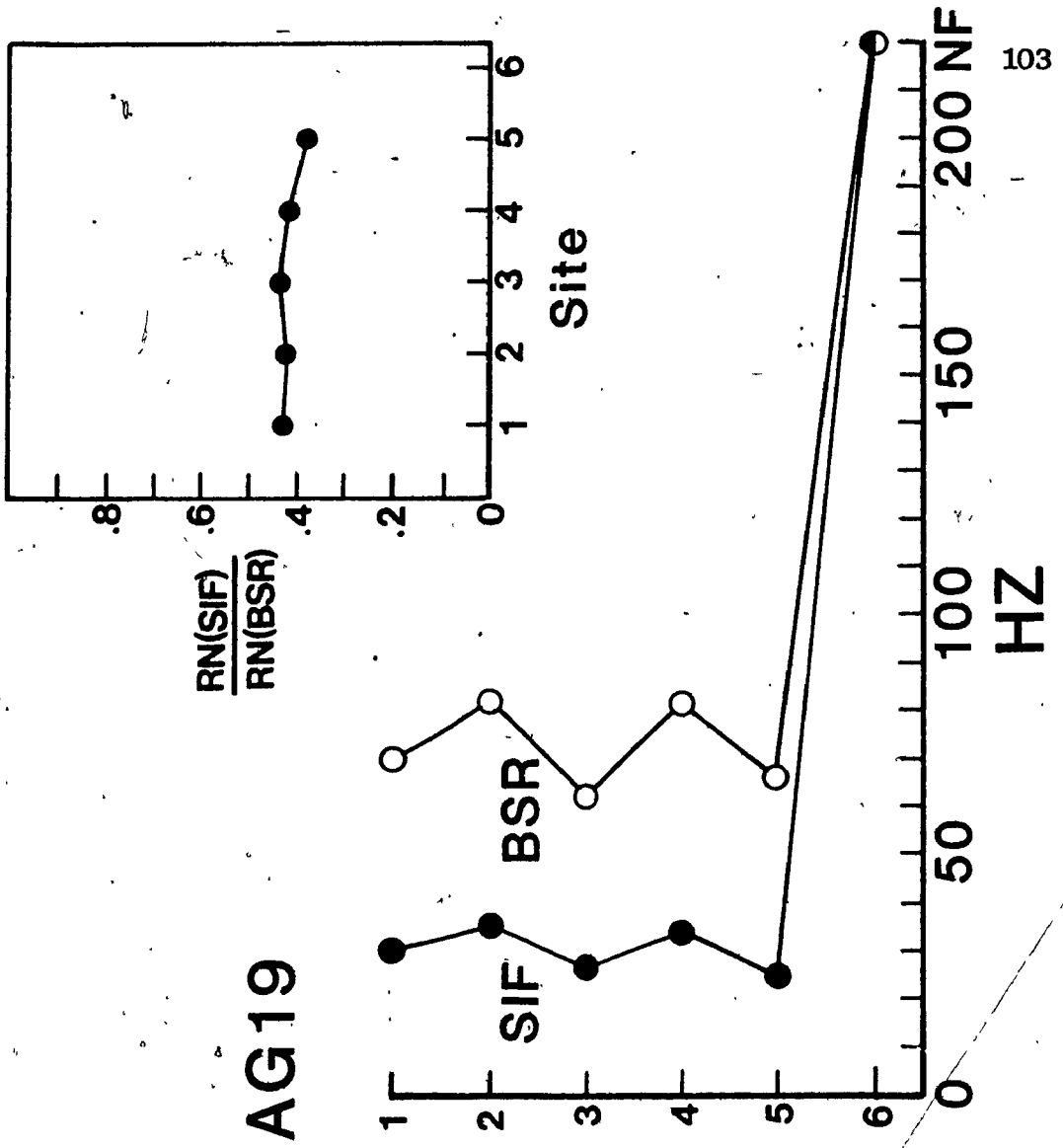
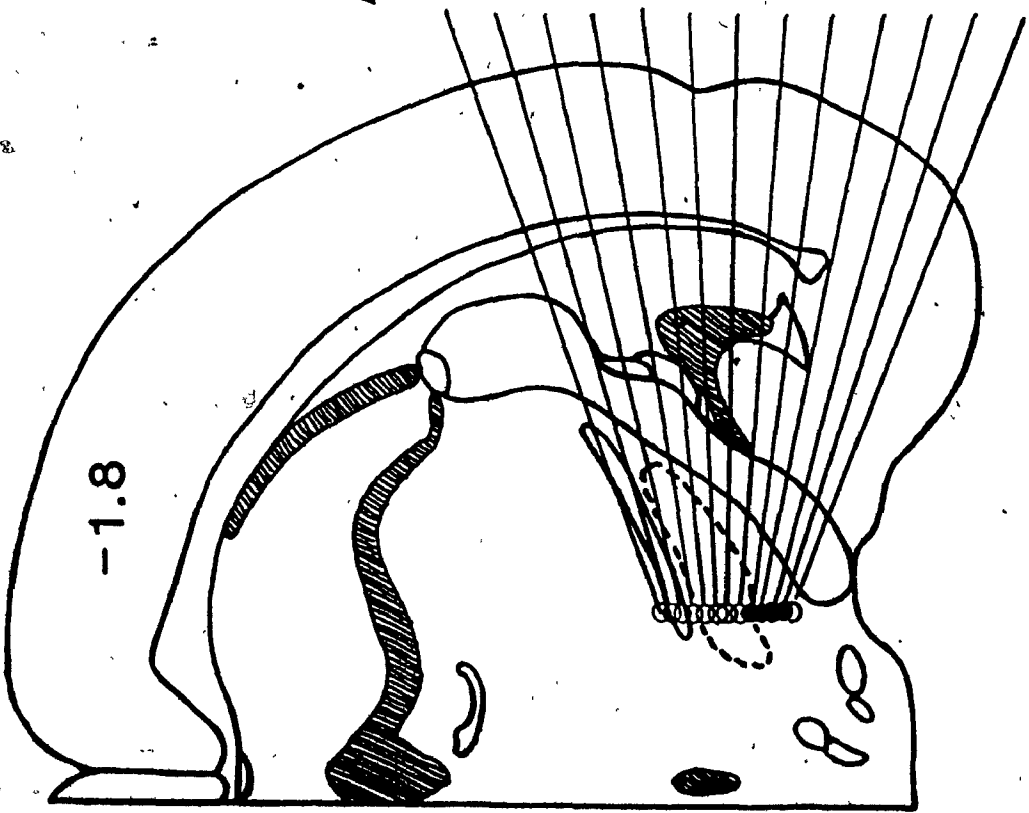


FIGURE 7f

Histology and mapping data for rat AG7.



AG 7

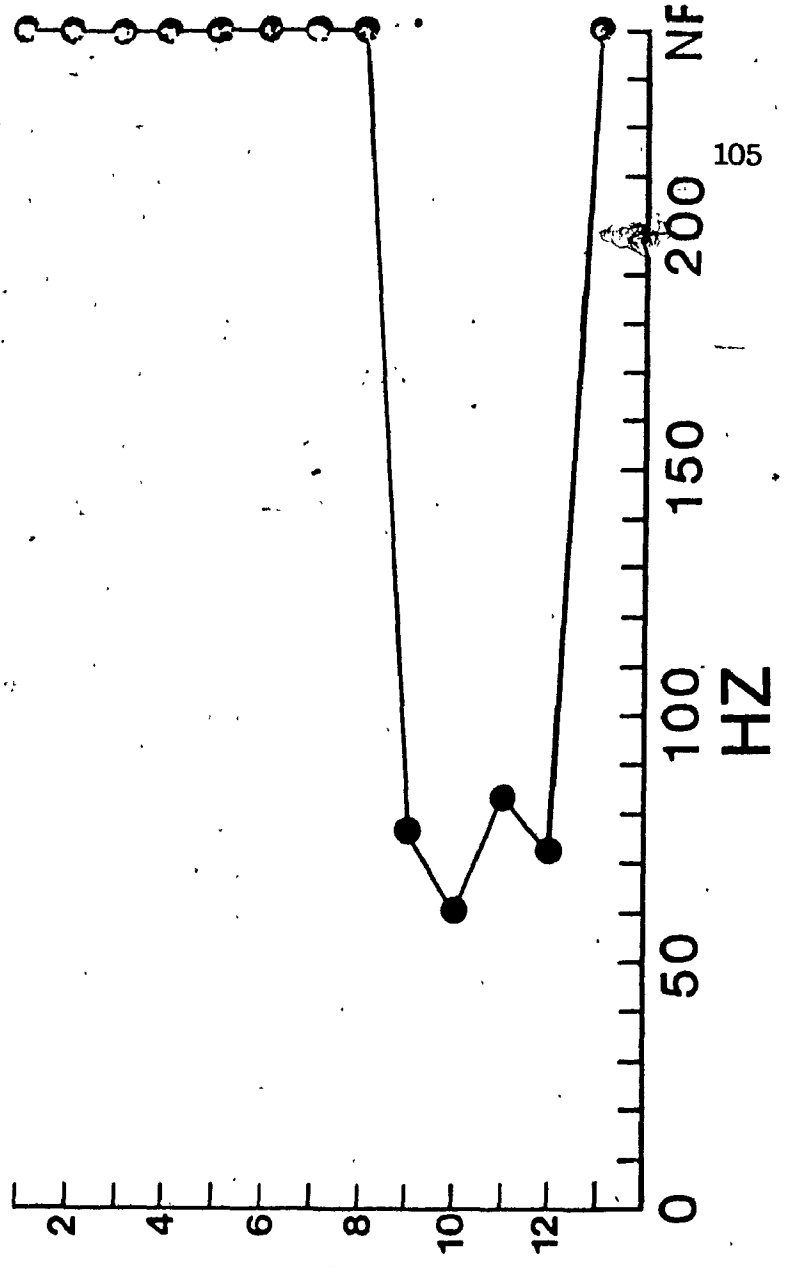
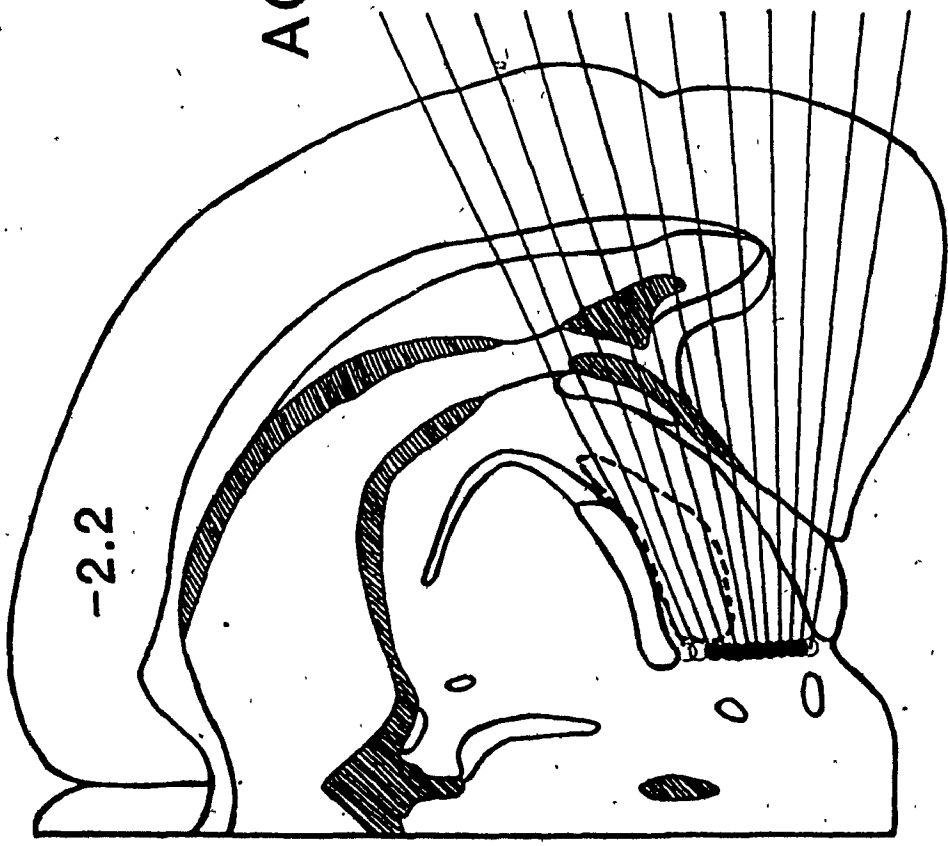
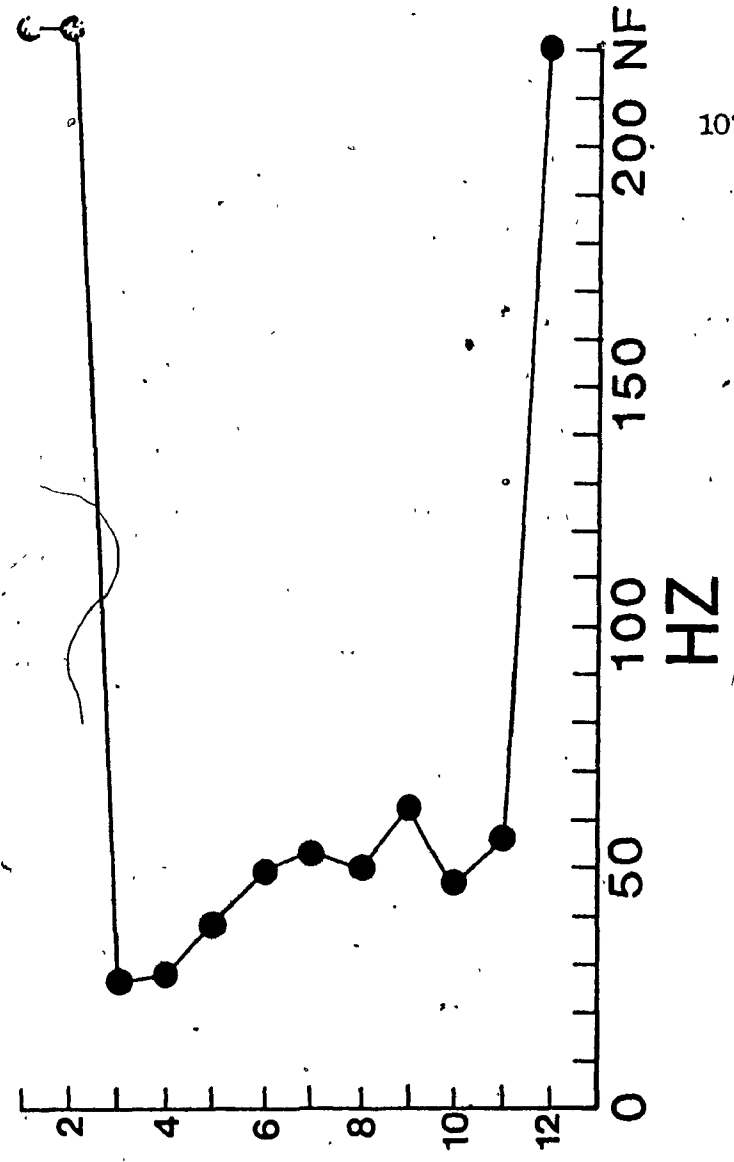


FIGURE 7g

Histology and mapping data for rat AG917.



AG 917





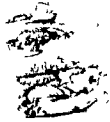
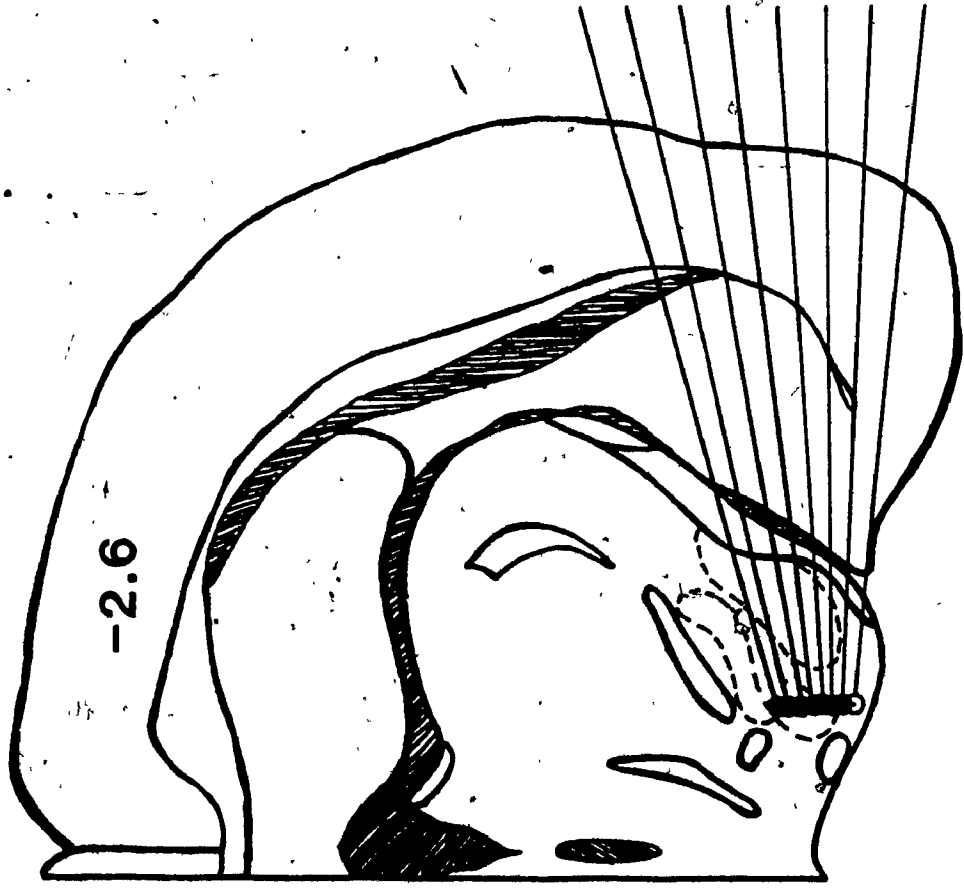
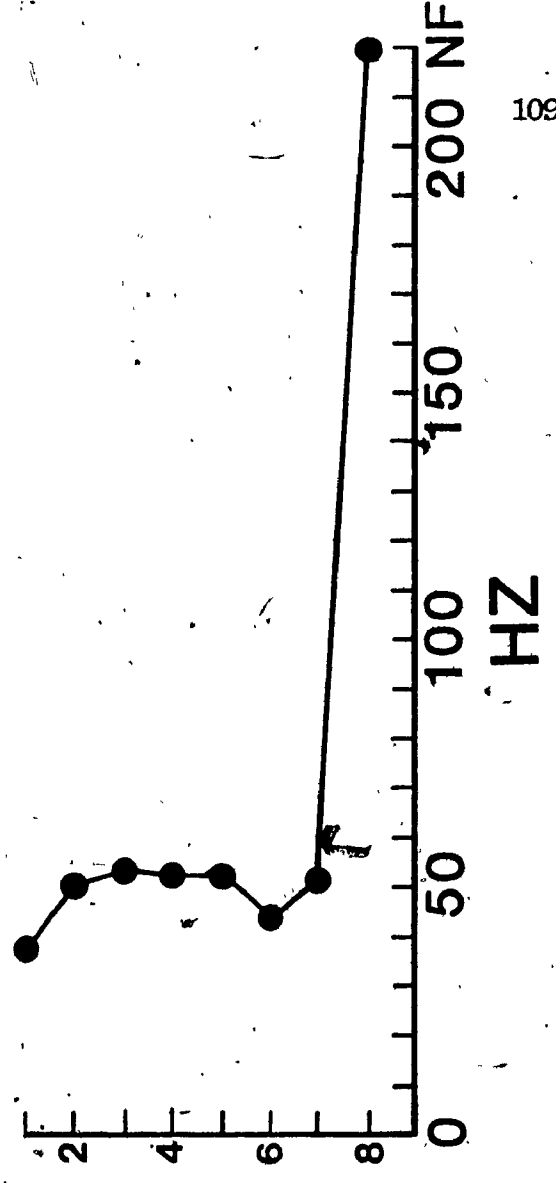


FIGURE 7h

Histology and mapping data for rat AG30.



AG 30



Approximately 3.4 mm separate the most rostral feeding sites from the most caudal feeding sites .

b) Refractory period estimates for SIF and BSR

Refractory period estimates for SIF and BSR were obtained from rats AG2, AG4, AG6, AG7, AG18, AG19, AG917, AG918. It should be noted that rats AG18 and AG19 were not tested at all the C-T intervals tested in the 6 other rats. Table 1 lists for each rat the intensity of the stimulation, the average single pulse frequency for SIF and BSR, the ratio of SIF to BSR single pulse frequency requirements and, in the case of rats implanted with moveable electrodes, the site at which the refractory period estimates were obtained. Figures 8a to 8h present the refractory period curves for BSR and SIF of individual rats. In the left panel of each figure, the untransformed TPE values are shown as a function of C-T intervals. The regression lines fitted to the transformed TPE values for BSR and SIF are shown in the right panel of each figure.

In all rats, TPE values for BSR and SIF increased over approximately the same range of C-T intervals; the lowest TPE values occurred at C-T intervals of 0.4-0.5 msec while the highest values were found at C-T intervals of 1.6-2.5 msec. Table 2 lists for each rat the range of C-T intervals which produced significant increases in TPE for BSR and SIF. For the most part, increases in TPE occurred at shorter C-T intervals for

SIF than for BSR. This was the case in those rats (AG2, AG4, AG6, AG7, AG917, AG918) in which TPE values were obtained at several C-T intervals in the 0.4 to 0.6 msec range. In these animals significant increases of TPE values for SIF occurred at C-T intervals which were on average 0.061 msec ( $\pm 0.0061$  msec) shorter than that which was observed for BSR. In the two other animals (AG18 & AG 19) the C-T intervals in the 0.4-0.6 msec range were only varied in 0.1 msec increments. In both these animals, the TPE values for BSR and SIF increased significantly at the same C-T interval (0.5 msec).

The slopes of the regression lines fitted to the transformed BSR and SIF refractory period data did not differ significantly in 6 of the 8 animals tested. In two animals (AG6 & AG918), the slope of the SIF regression line was significantly steeper than the slope of the BSR regression line. Table 3 presents the correlation coefficients and regression equations for each refractory curve and the results of the t-test comparisons of parallelism between the regression lines fitted to individual BSR and SIF refractory period curves.

Finally, an interesting feature of the refractory period curves for both SIF and BSR should be pointed out. The refractory period curves for SIF and BSR of all eight animals showed a plateau between C-T intervals of 0.6 and 0.7 msec. In other words, the slope of the refractory period curves between 0.6 and 0.7 msec was, for both BSR and SIF, closer to zero than the slope

Stimulation intensity (I); single pulse (SP) frequency requirements for SIF and BSR (+/- s.e.m.), SP(sif) to SP(bsr) ratio and site of stimulation of 8 rats used in refractory period experiment.

RAT	I(microamps)	SP(sif)	SP(bsr)	ratio	site
AG2	500	30.88 Hz +/- 0.52	52.05 Hz +/- 0.74	0.593	fixed
AG4	200	19.70 0.59	52.31 0.98	0.376	fixed
AG6	250	28.54 1.58	67.01 1.33	0.425	site 8
AG7	400	29.63 0.66	55.82 0.72	0.531	site 12
AG18	200	16.86 0.70	48.47 1.14	0.348	site 1
AG19	400	20.54 0.48	45.75 1.09	0.448	site 1
AG917	175	36.13 0.59	76.25 1.57	0.473	site 4
AG918	350	32.27 0.62	66.40 1.00	0.484	site 3
Mean	309.3	26.8	57.9	0.432	
s.e.m.	42.24	2.43	3.77	0.031	

Shortest and longest C-T intervals producing significant increases in T-pulse effectiveness for SIF and BSR.

Rat:	SIF		BSR	
	shortest	longest	shortest	longest
AG2:	0.48 msec	2.5 msec	0.52 msec	2.5 msec
AG4	0.48	1.6	0.56	1.6
AG6	0.48	1.6	0.52	2.0
AG7	0.44	2.0	0.5	2.0
AG18	0.5	2.0	0.5	2.0
AG19	0.5	2.0	0.5	2.0
AG917	0.44	1.6	0.48	1.6
AG918	0.48	1.6	0.52	2.0
Mean.	0.475	1.863	0.513	1.963
s.e.m.	0.008	0.115	0.008	0.100

Correlation coefficients, regression equations and t-test for parallelism values for SIF and BSR transformed refractory period data.

## AG 2

corr. SIF:0.9395/ corr. BSR:0.9424  
 reg. equation SIF:  $TPE=48.4571 \times C-T + (-5.0766)$   
 reg. equation BSR:  $TPE=51.6331 \times C-T + (-11.724)$   
 t-test:  $t=0.3945$ ,  $df=20$ ,  $t\text{-crit} (\alpha=0.05)=2.086$

## AG 4

corr. SIF:0.9921/ corr. BSR:0.9820  
 reg. equation SIF:  $TPE=85.8492 \times C-T + (-32.3576)$   
 reg. equation BSR:  $TPE=85.9530 \times C-T + (-32.5064)$   
 t-test:  $t=0.0154$ ,  $df=15$ ,  $t\text{-crit} (\alpha=0.05)=2.131$

## AG 6

corr. SIF:0.9571/ corr. BSR:0.9910  
 reg. equation SIF:  $TPE=85.4251 \times C-T + (-22.1428)$   
 reg. equation BSR:  $TPE=62.2088 \times C-T + (-26.0940)$   
 t-test:  $t=2.7105$ ,  $df=17$ ,  $t\text{-crit} (\alpha=0.05)=2.110$  (sig.)

## AG 7

corr. SIF:0.9629/ corr. BSR:0.9612  
 reg. equation SIF:  $TPE=59.7085 \times C-T + (-10.6424)$   
 reg. equation BSR:  $TPE=63.7509 \times C-T + (-15.7054)$   
 t-test:  $t=0.5170$ ,  $df=20$ ,  $t\text{-crit} (\alpha=0.05)=2.086$

## AG 18

corr. SIF:0.9563/ corr. BSR:0.9896  
 reg. equation SIF:  $TPE=61.8074 \times C-T + (-12.5228)$   
 reg. equation BSR:  $TPE=60.0990 \times C-T + (-16.5612)$   
 t-test:  $t=0.2207$ ,  $df=14$ ,  $t\text{-crit} (\alpha=0.05)=2.145$

## AG 19

corr. SIF:0.9807/ corr. BSR:0.9750  
 reg. equation SIF:  $TPE=63.2096 \times C-T + (-17.9494)$   
 reg. equation BSR:  $TPE=62.7483 \times C-T + (-14.2428)$   
 t-test:  $t=0.0640$ ,  $df=14$ ,  $t\text{-crit} (\alpha=0.05)=2.145$

## AG 917

corr. SIF:0.9444/ corr. BSR:0.9666  
 reg. equation SIF:  $TPE=84.4662 \times C-T + (-16.4918)$   
 reg. equation BSR:  $TPE=87.8721 \times C-T + (-26.7387)$   
 t-test:  $t=0.2785$ ,  $df=19$ ,  $t\text{-crit} (\alpha=0.05)=2.093$

## AG 918

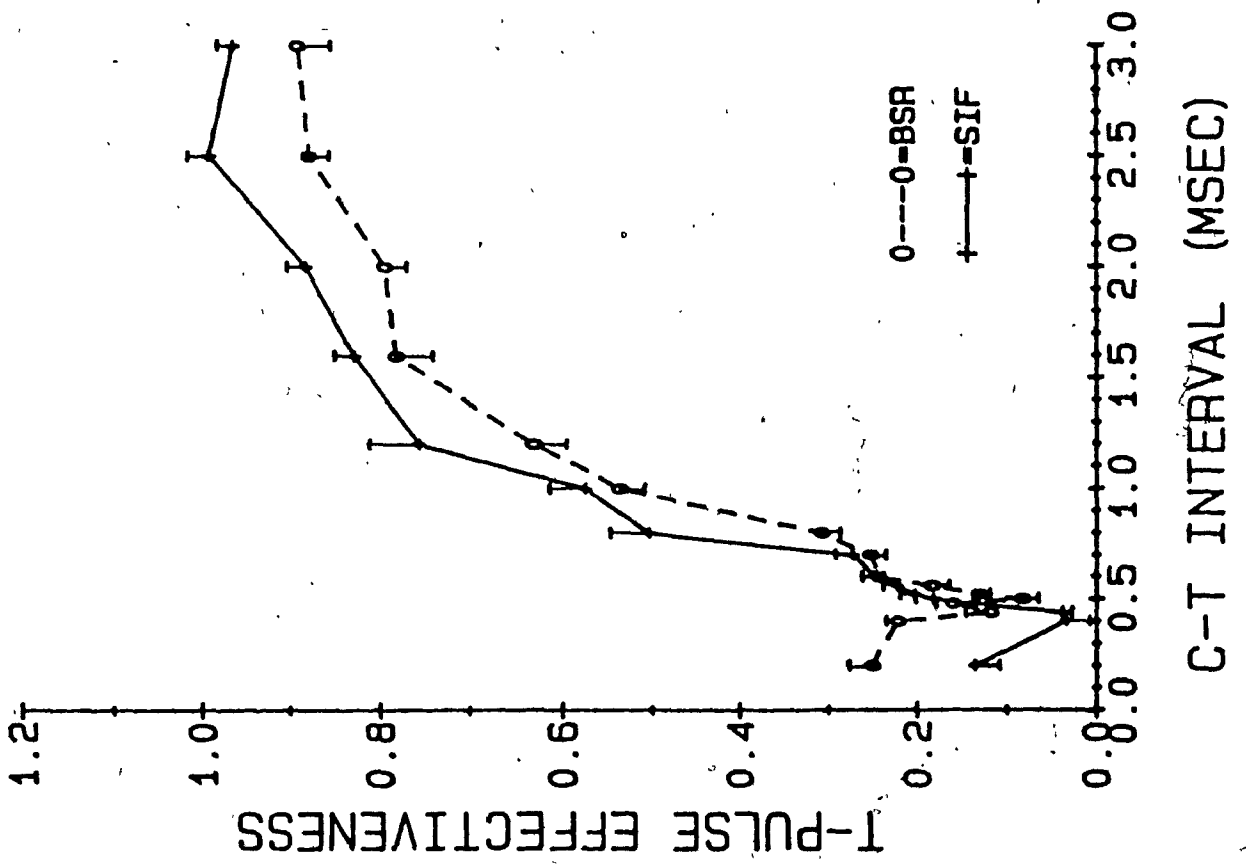
corr. SIF:0.9612/ corr. BSR:0.9969  
 reg. equation SIF:  $TPE=79.5962 \times C-T + (-20.1280)$   
 reg. equation BSR:  $TPE=64.0884 \times C-T + (-31.3558)$   
 t-test:  $t=2.1393$ ,  $df=17$ ,  $t\text{-crit} (\alpha=0.05)=2.110$  (sig.)

## FIGURE 8a

Refractory period data for AG2. The left panel shows the untransformed data and the right panel shows the transformed data. Refractory period data for SIF are represented by solid lines and plus signs while the data for BSR are represented by dashed lines and circles.



Untransformed data AG2



Transformed data AG2

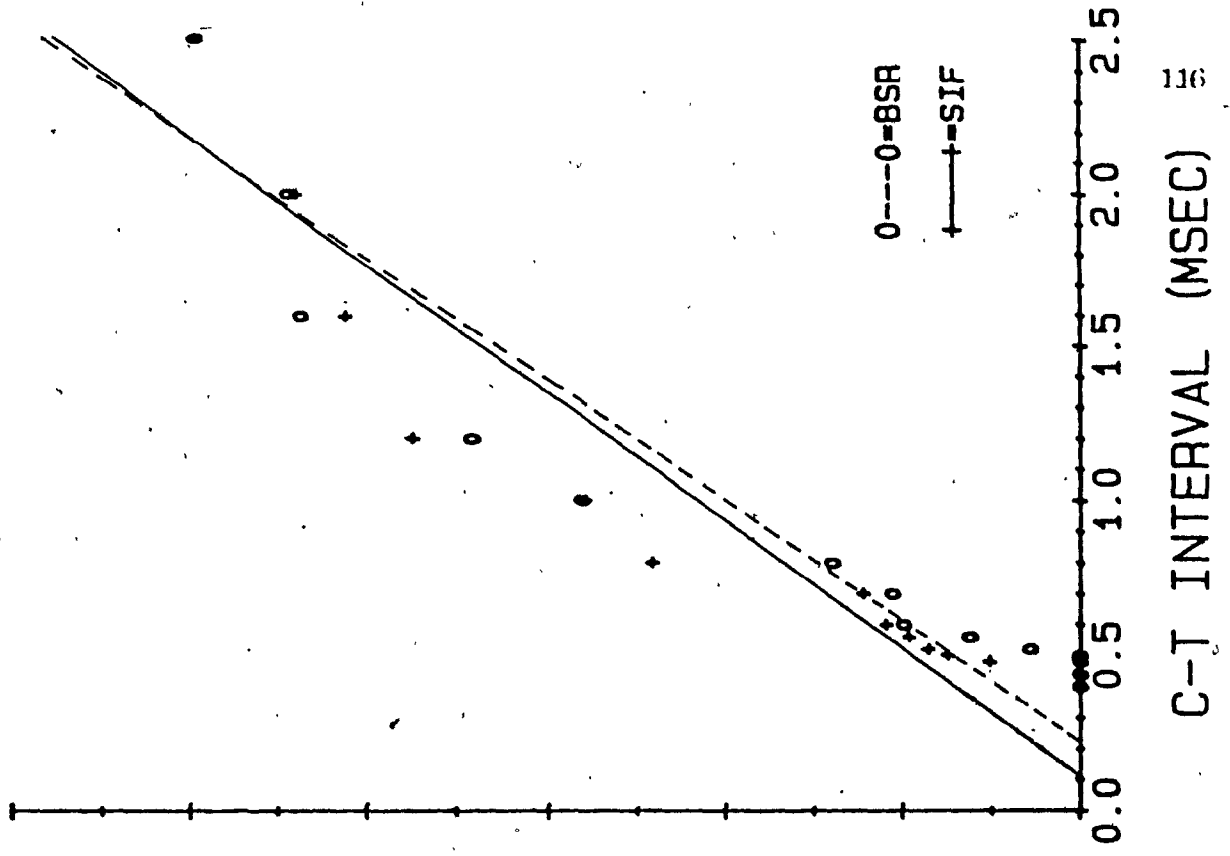
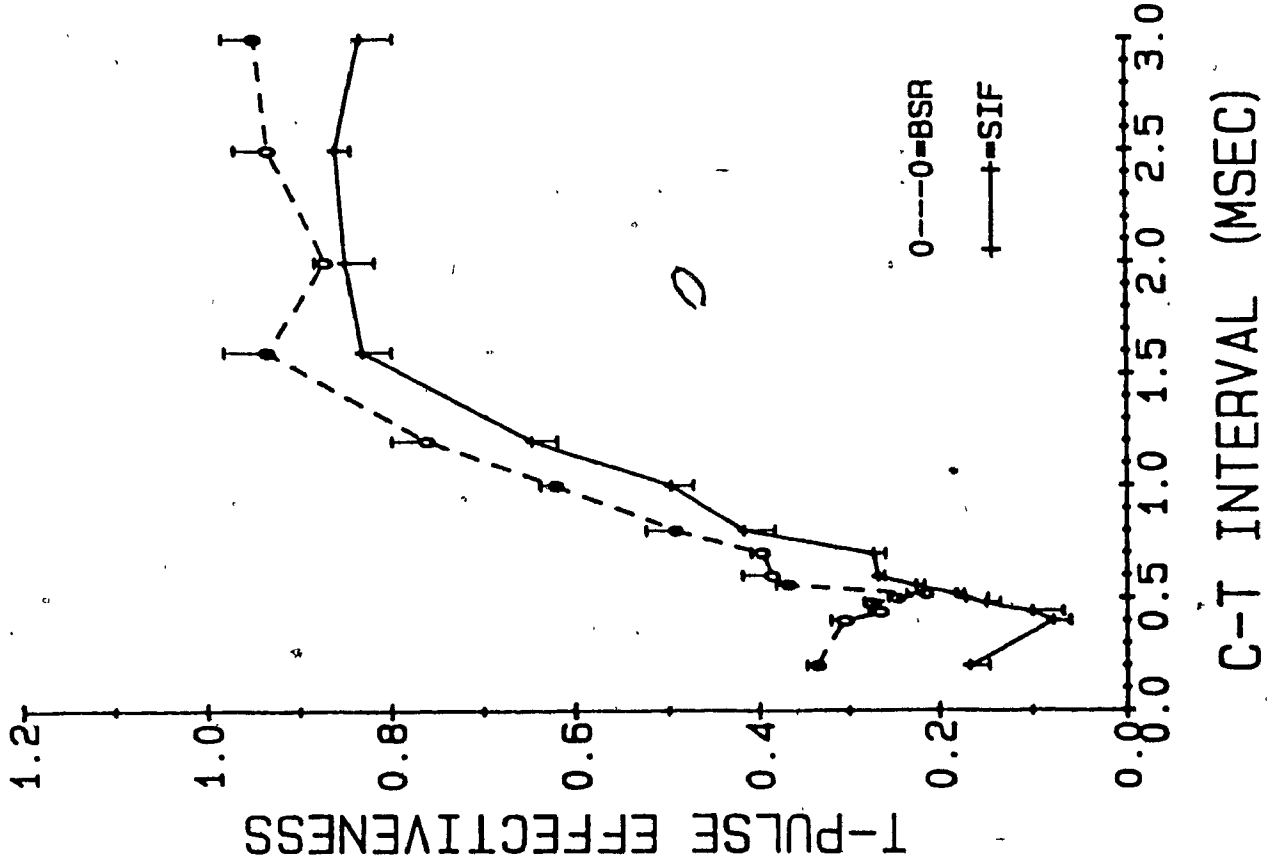


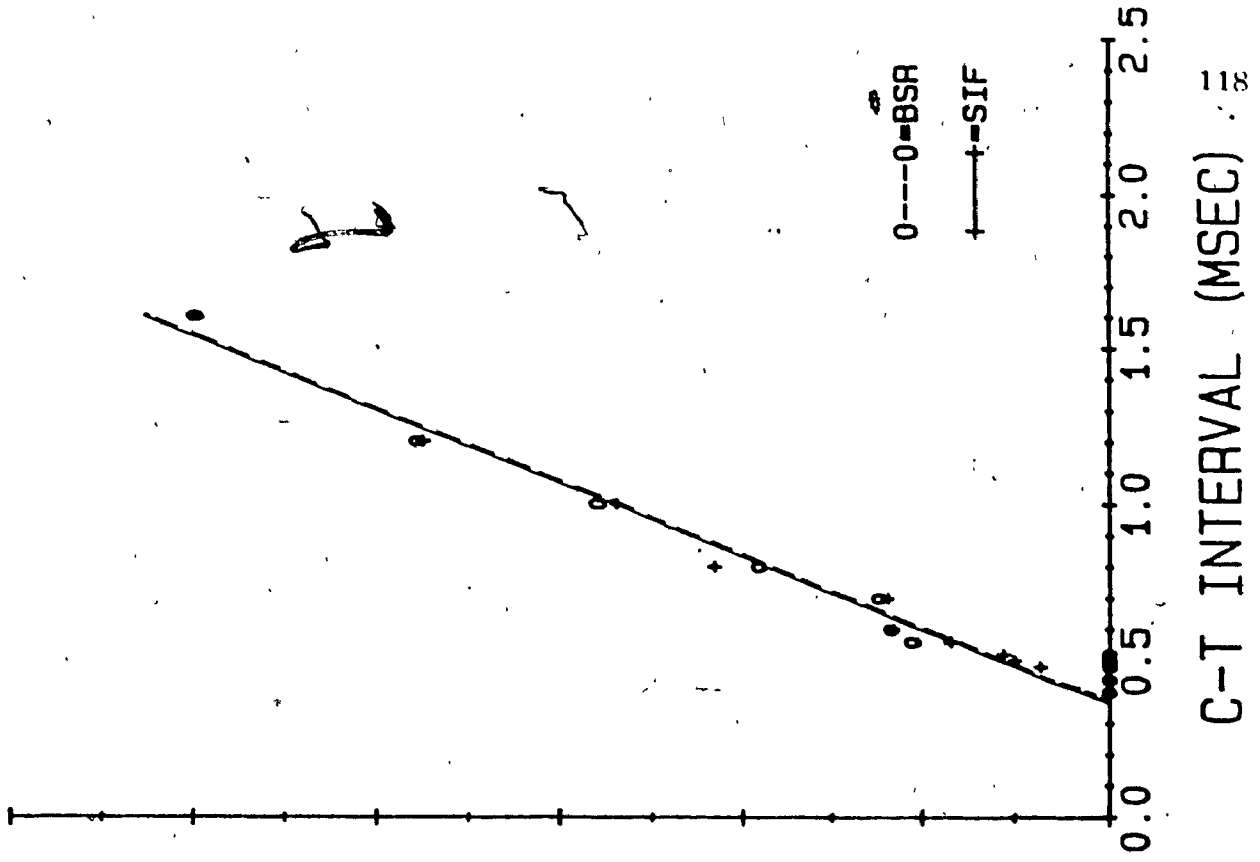
FIGURE 8b

Refractory period data for AG4.

Untransformed data AG4



Transformed data AG4



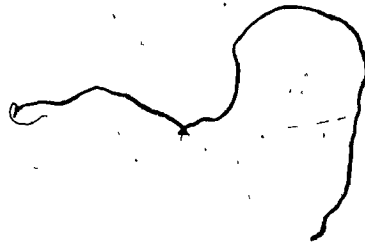
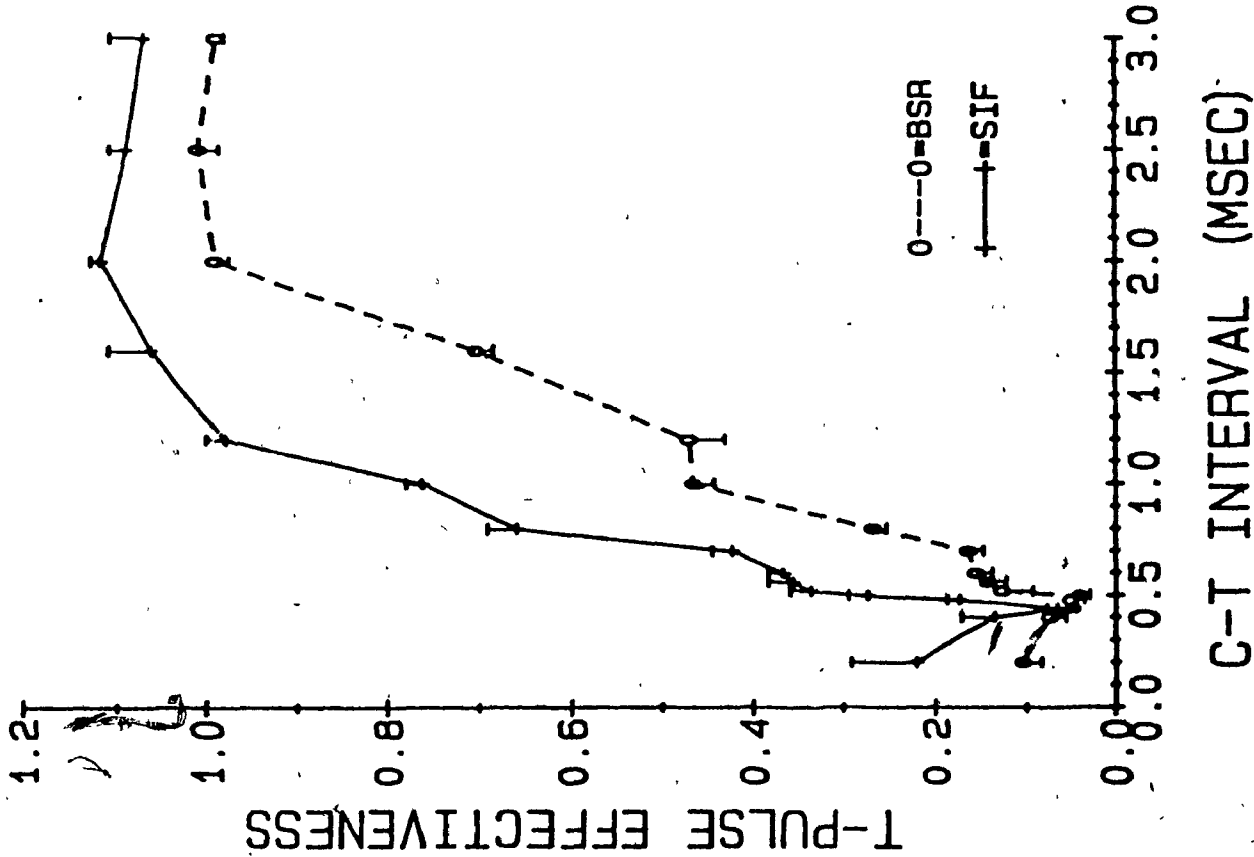


FIGURE 8c

Refractory period data for AG6.

Untransformed data AG6



Transformed data AG6

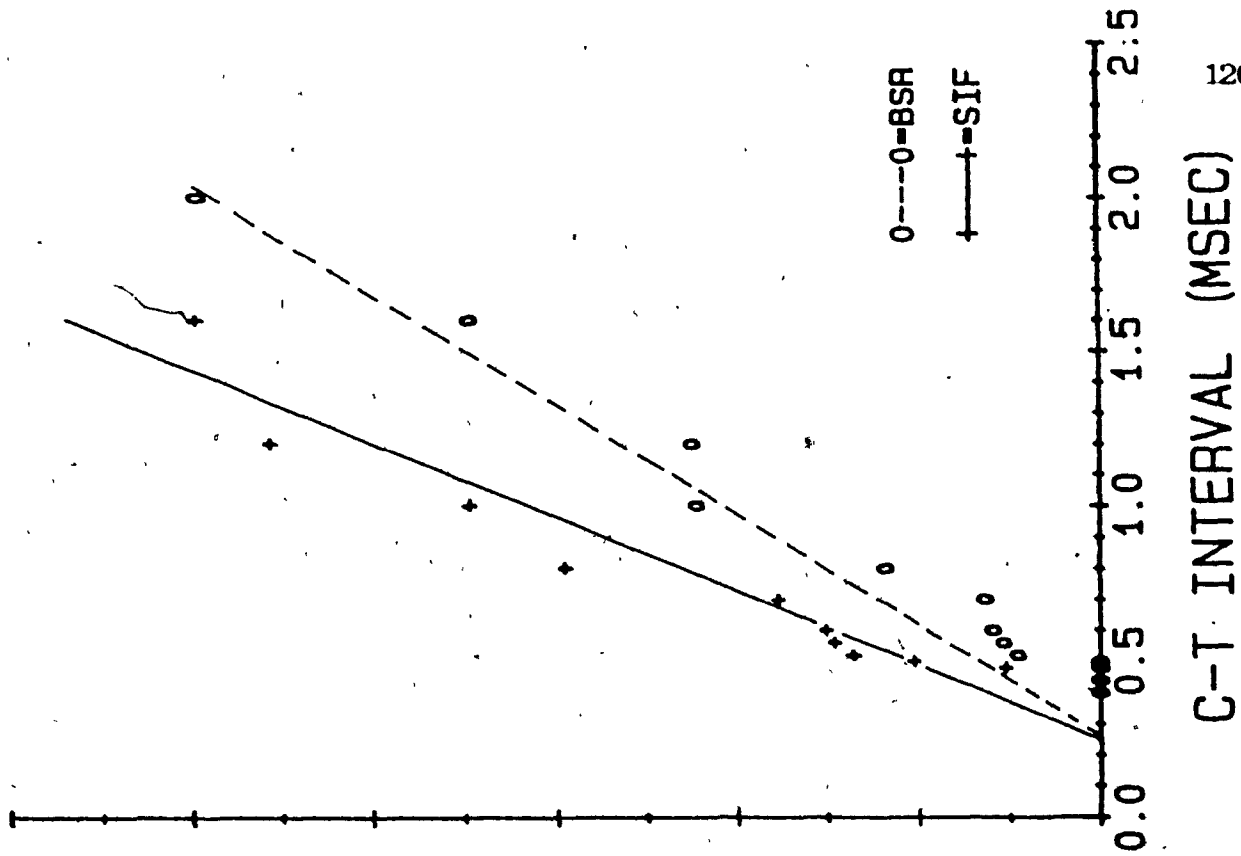
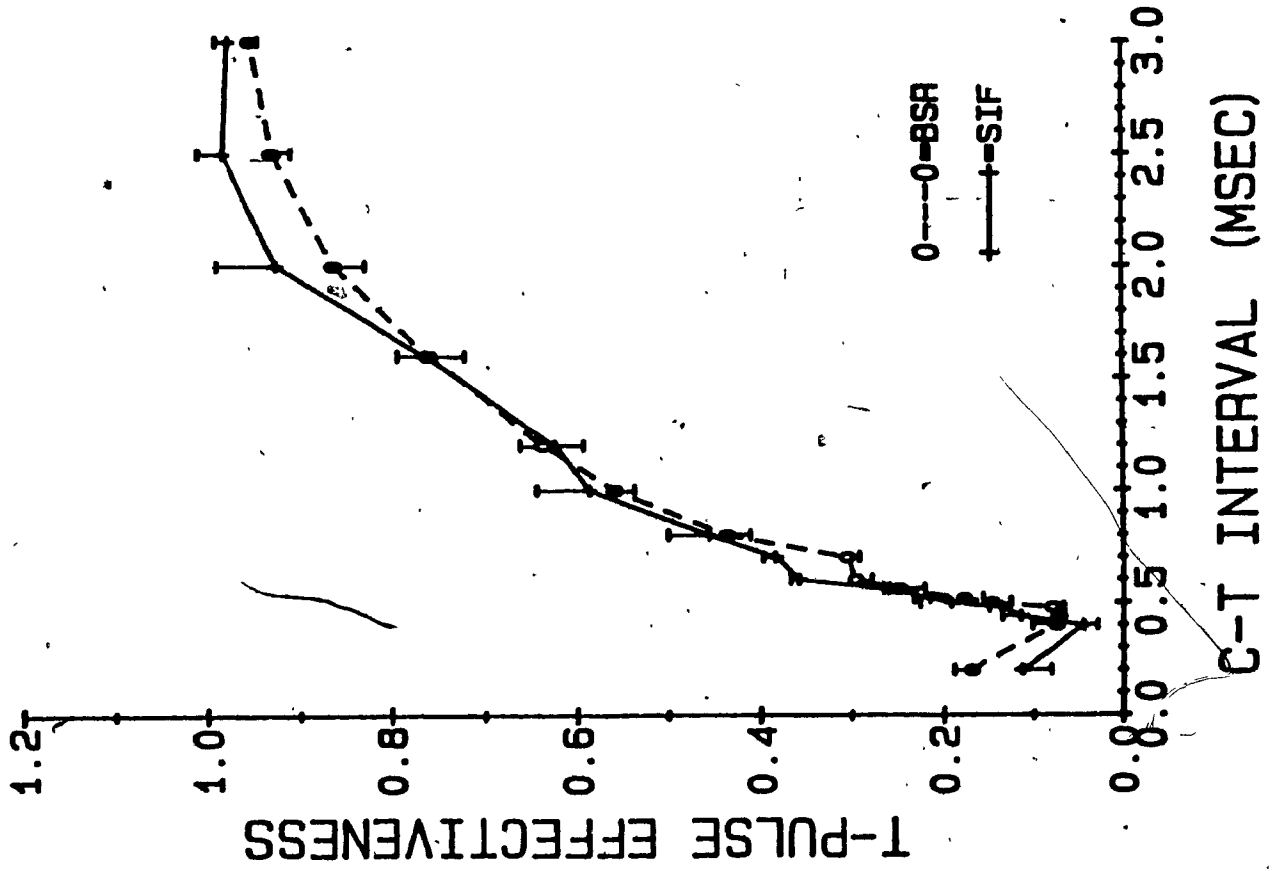


FIGURE 8d

Refractory period data for AG7.

Untransformed data AG7



Transformed data AG7

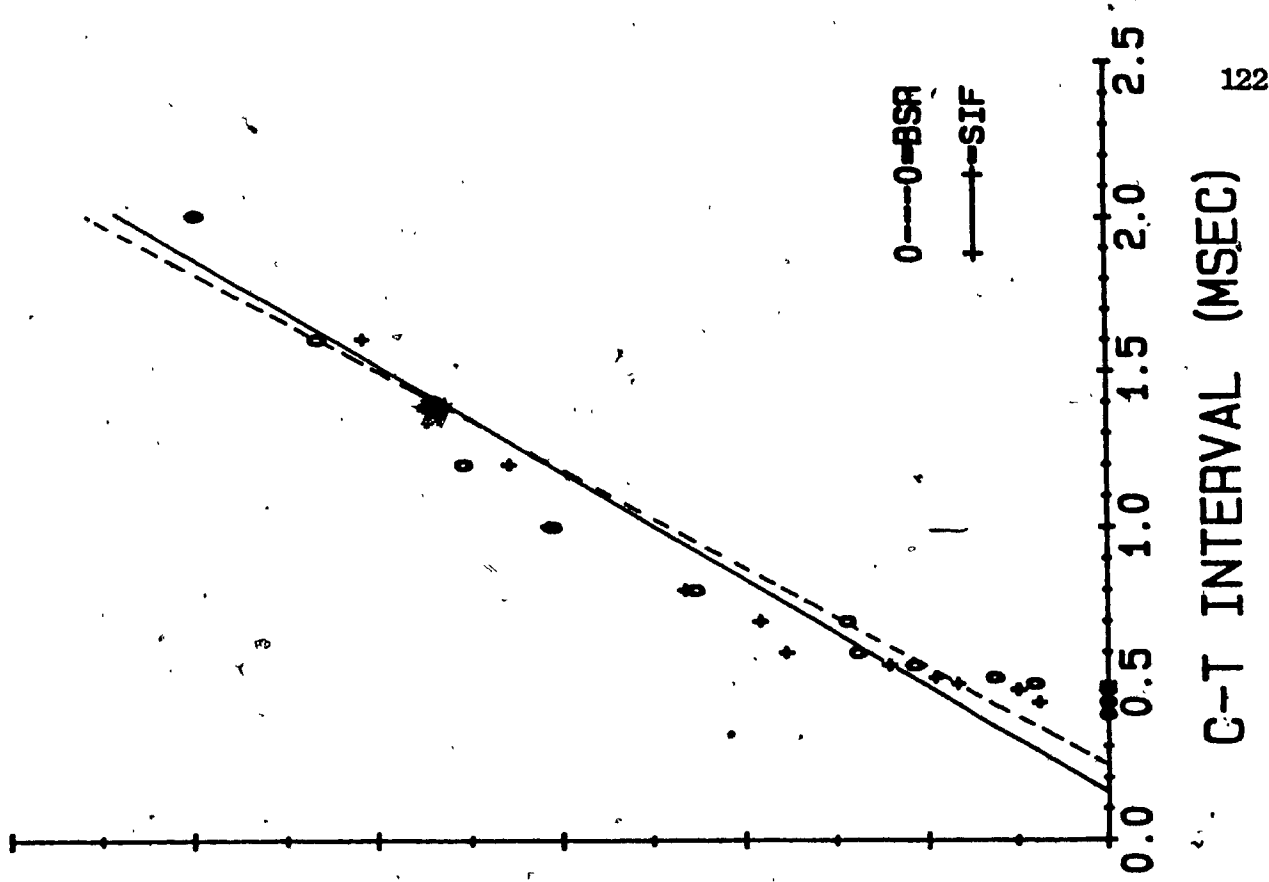
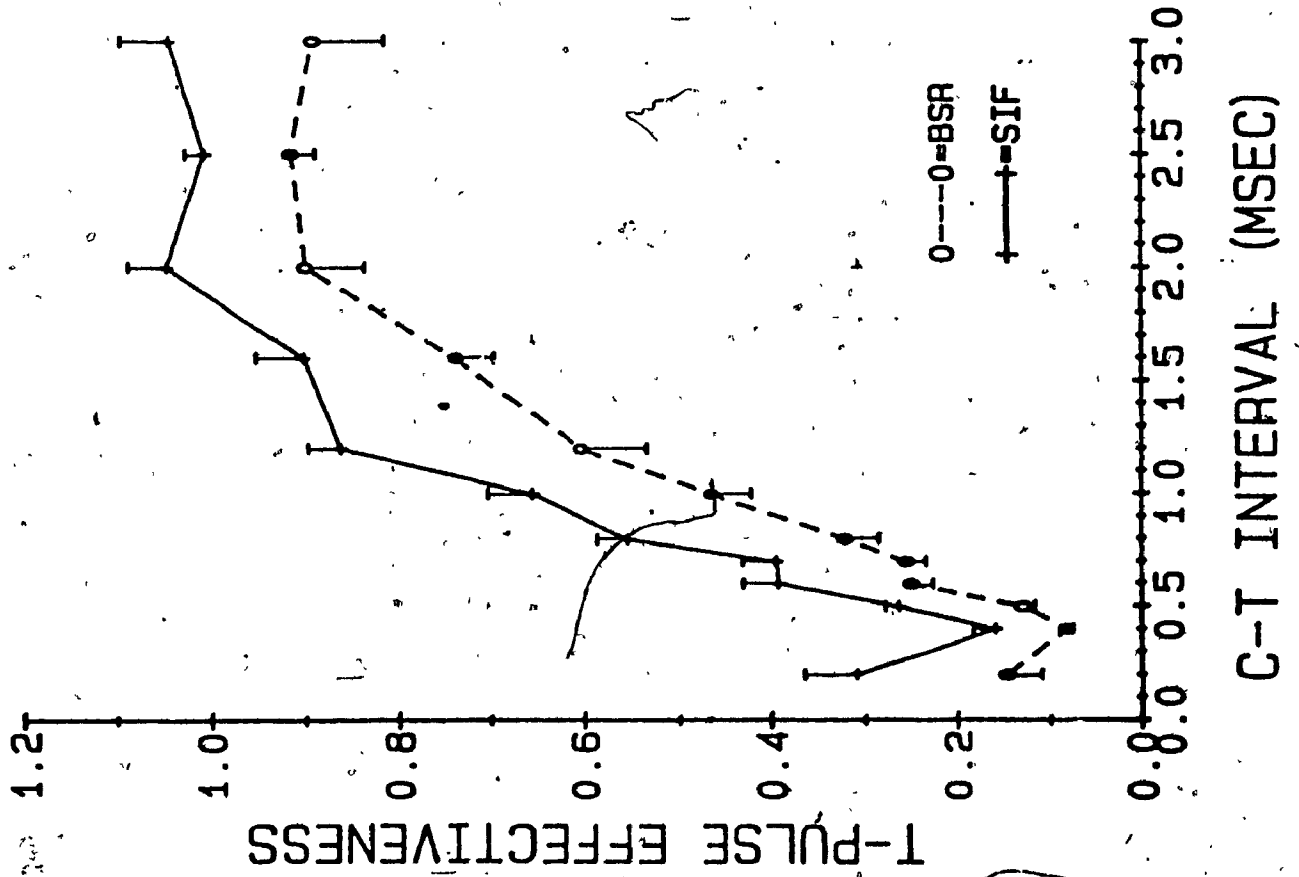


FIGURE 8e

Refractory period data for AG18.



Untransformed data AG18



Transformed data AG18

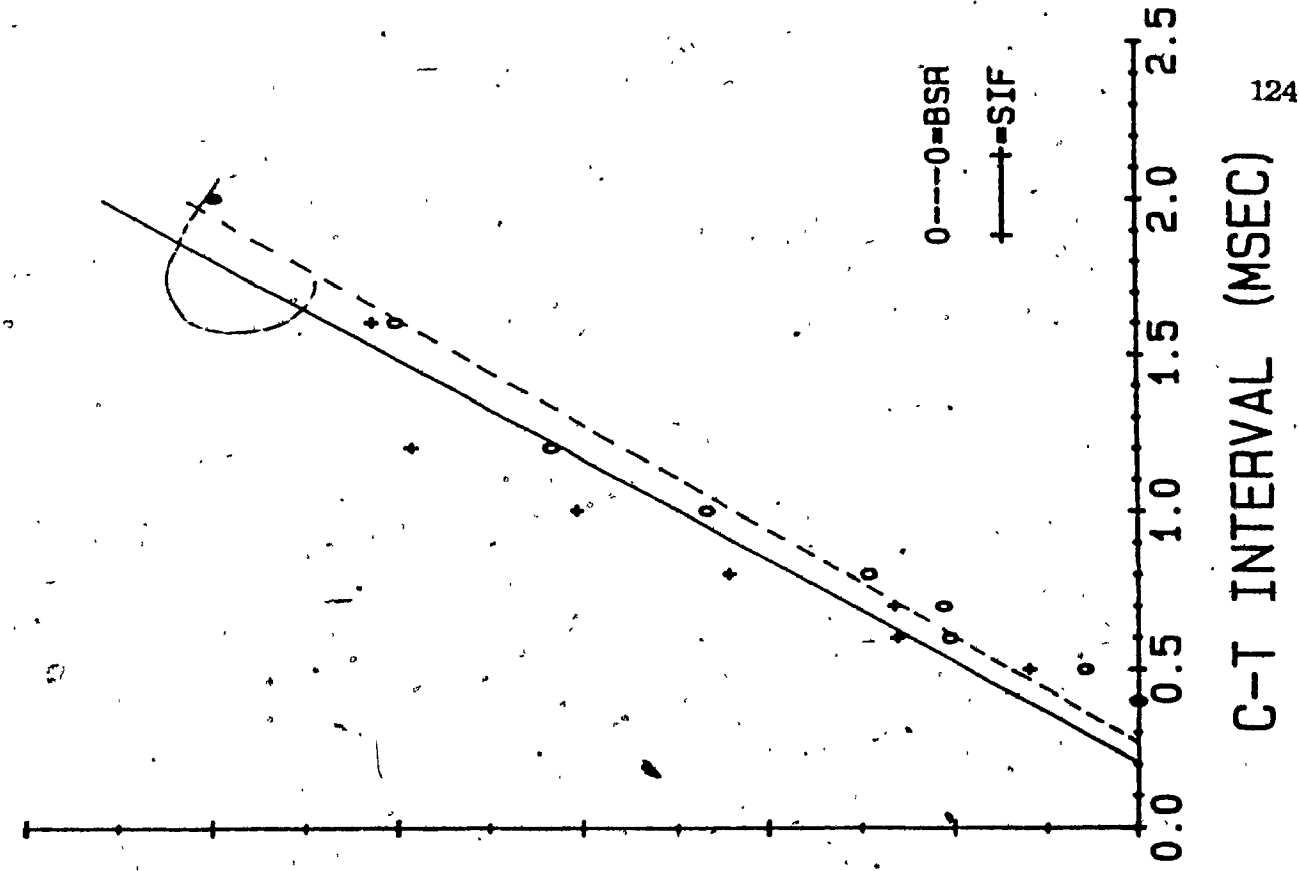
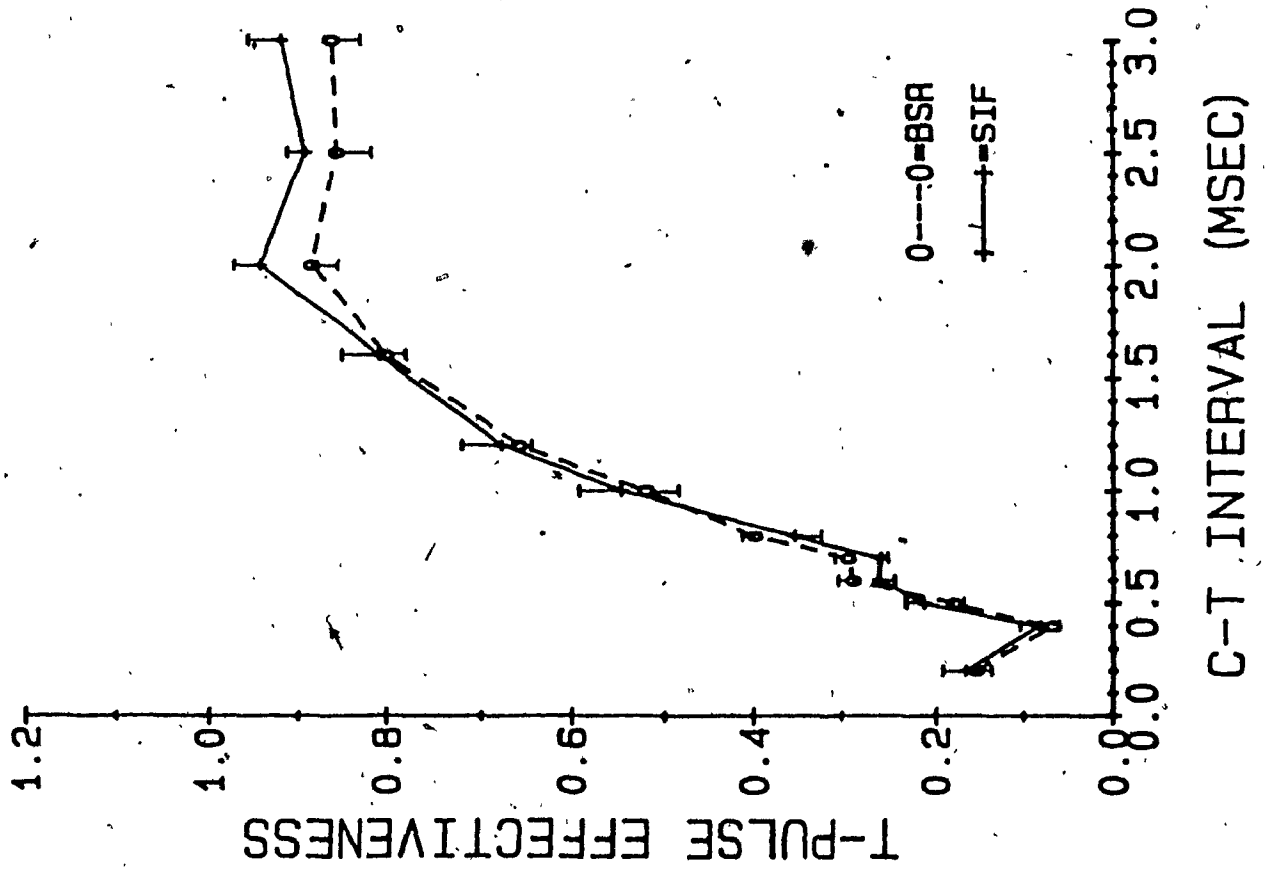


FIGURE 8f

Refractory period data for AG19.

Untransformed data AG19



Transformed data AG19

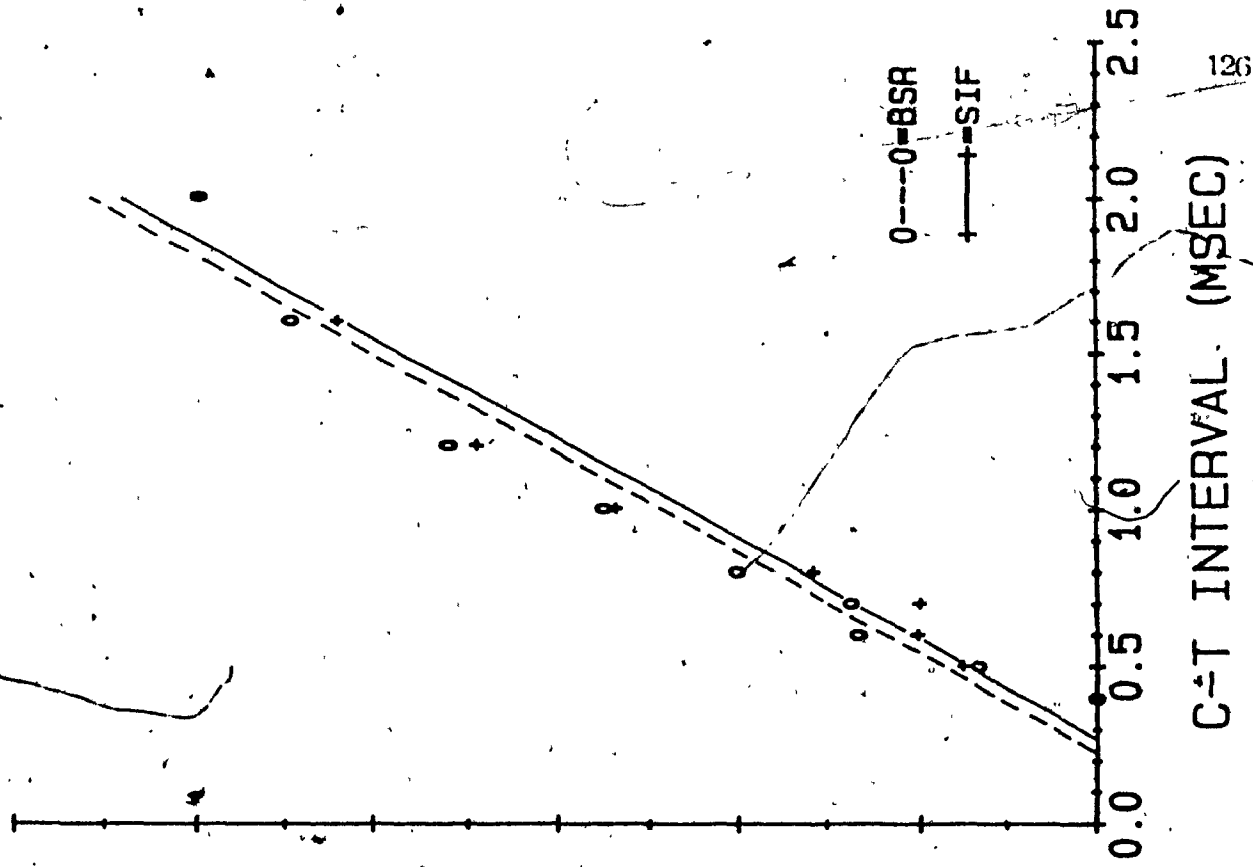
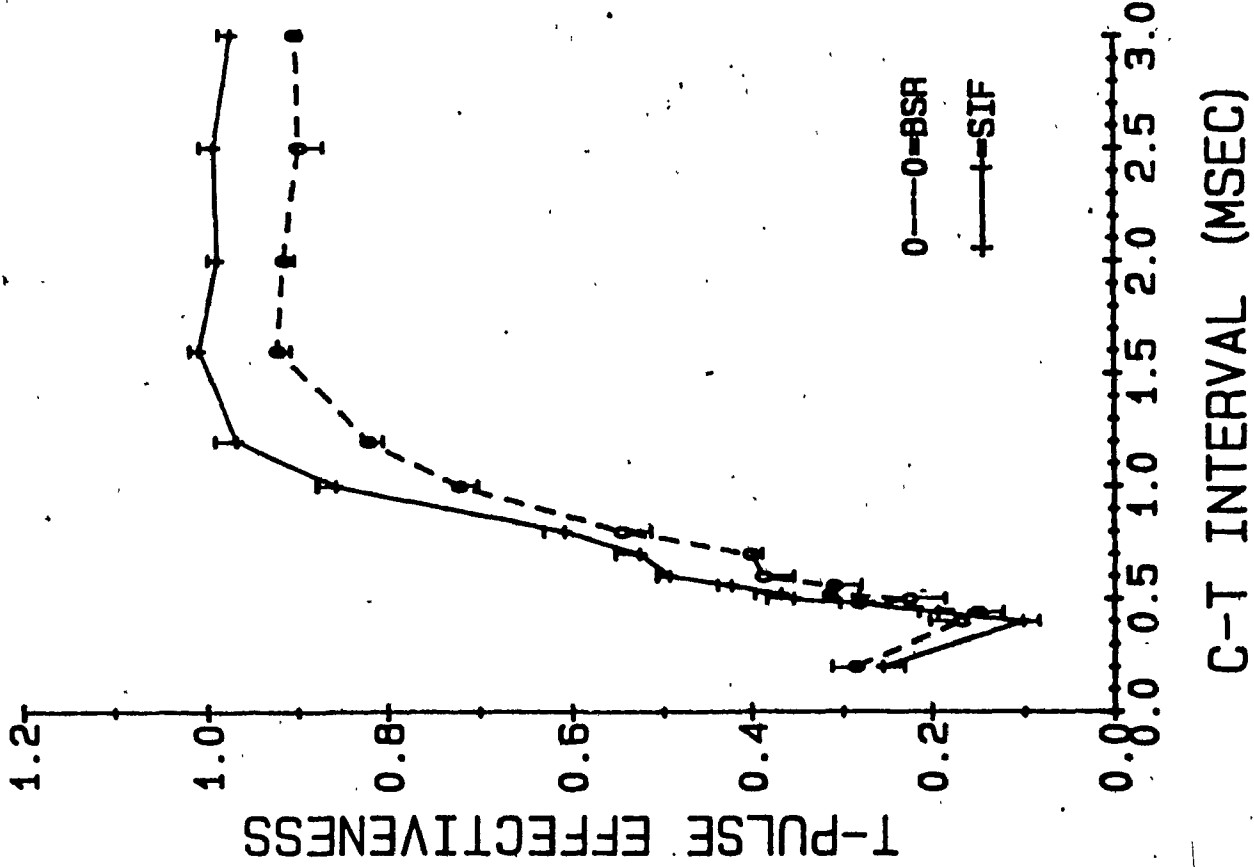


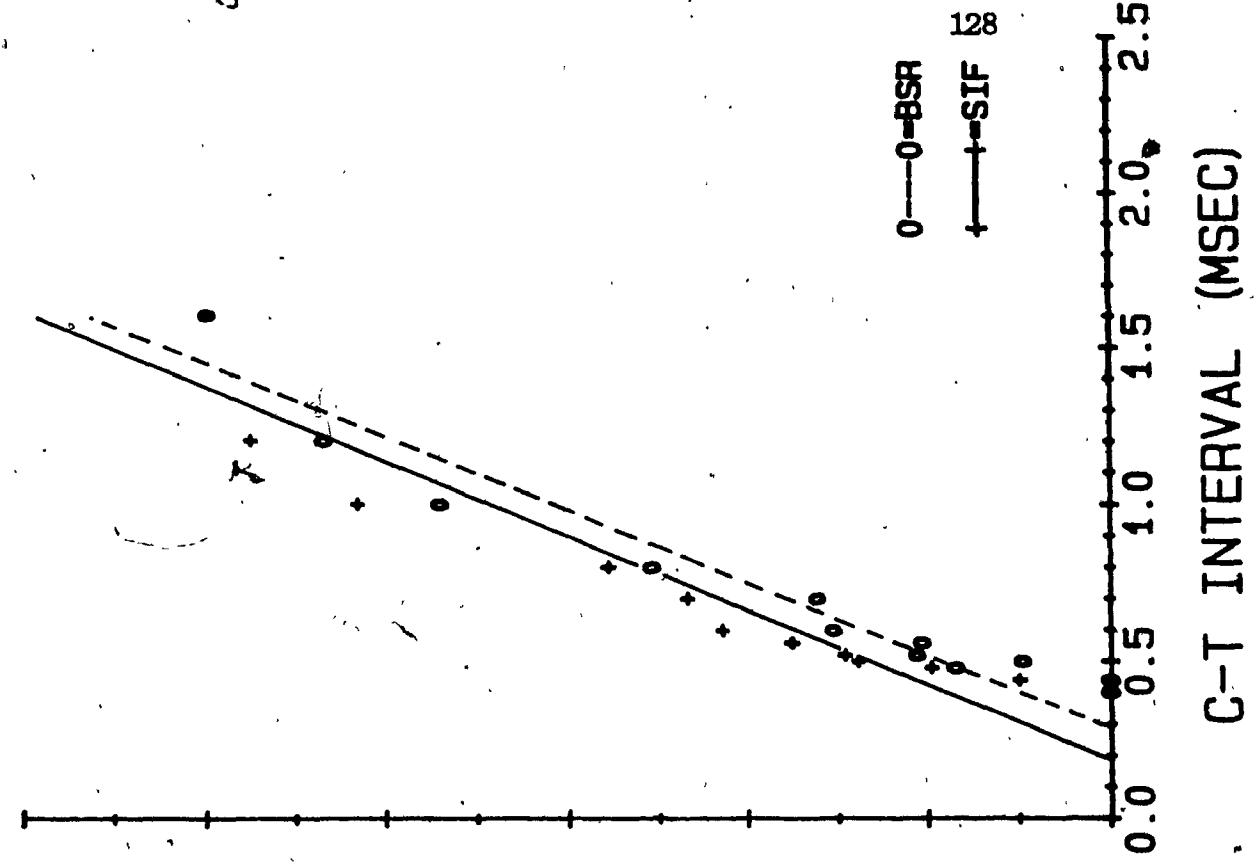
FIGURE 8g

Refractory period data for AG917.

Untransformed data AG917



Transformed data AG917



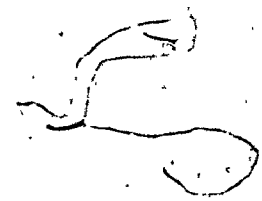
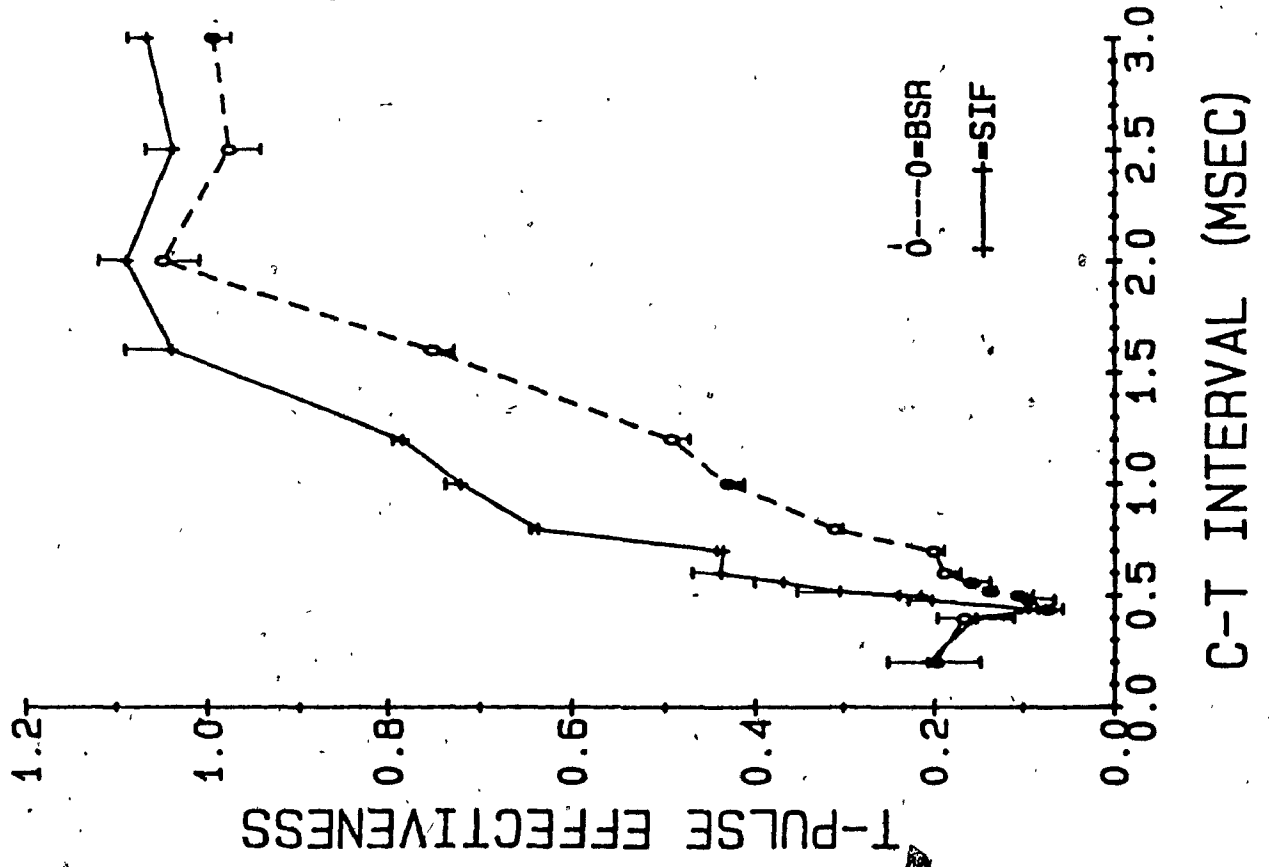


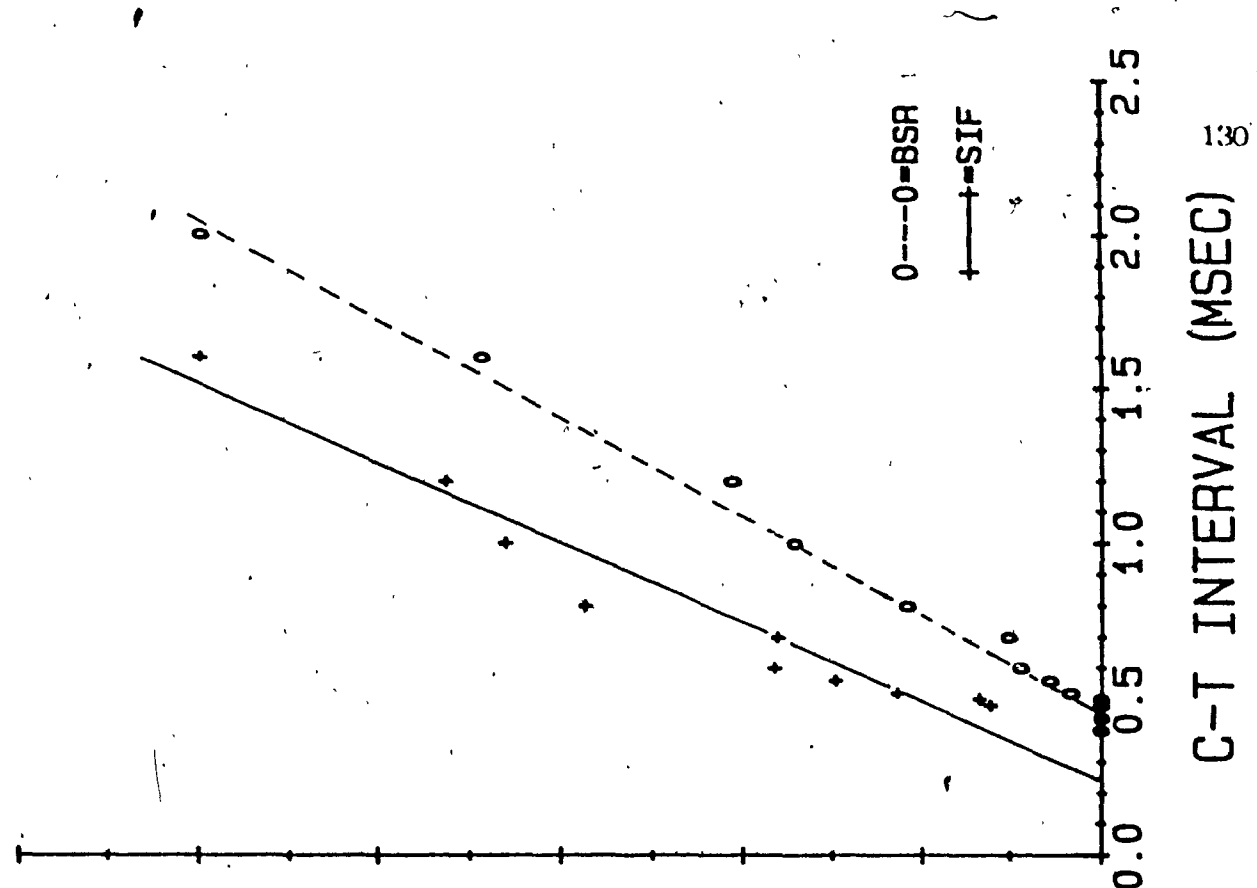
FIGURE 8h

Refractory period data for AG918.

Untransformed data AG918



Transformed data AG918



of adjoining segments of the curves.

## DISCUSSION

### a) Mapping of SIF sites

In the present investigation feeding was elicited by electrical stimulation at several different anterior-posterior levels of the MFB: from the anterior portion of the lateral hypothalamic area to the ventral tegmental area. This finding confirms Cox and Valenstein's (1969) study which showed a similar anterior-posterior spread of MFB electrode placements that supported SIF. The present mapping of MFB SIF sites is not consistent with the widely held view that SIF is restricted to electrode placements in the lateral hypothalamic MFB (e.g. Hoebel, 1969) and suggests that feeding-relevant fibers course along the longitudinal axis of the MFB.

The present data also suggest that the dorsal and ventral boundaries of the feeding-relevant substrate are well defined. With stimulation intensities of 200 microamps, which has been estimated to excite tissue to within a 0.2 to 0.3 mm radius (Fouriezos and Wise, 1985), a movement of 0.110 mm carried the electrode tip from a site which was negative for SIF to a site which supported SIF at relatively low pulse frequencies. The same can be said of the ventral boundary of the feeding-relevant substrate; small electrode movements lowered the electrode tip



from a low frequency threshold SIF site to a site that was negative for SIF. However, the clear localization of the feeding-substrate's dorsal and ventral boundaries may depend on other factors. In at least three cases (AG6, AG7 & AG917) stimulation at the most ventral of the penetration elicited either forced movements or aversive effects which may have impeded the normal elaboration of a feeding response. Hence it may be that the recruitment of motor or aversion fibers artificially sharpened the ventral boundary of the feeding-relevant substrate. Defining the dorsal boundary of the feeding-relevant substrate is also complicated by the fact that SIF usually emerges only after the animal has had extensive stimulation experience. Thus one is never really sure whether a site which proves to be negative for SIF after 7 daily sessions of repeated stimulation might not have supported SIF had the animal been subjected to more extensive stimulation experience. Taken together the above considerations imply that the dorsal-ventral distribution of SIF sites mapped in the present study may in fact represent a conservative estimate of the size of the MFB feeding-relevant substrate.

The present mapping study also provides additional information on the distribution of SIF sites in relation to sites that support BSR. The frequency thresholds for both SIF and BSR were assessed at several sites using a moveable electrode. The data from two rats show that sites supporting BSR are co-extensive with sites that support SIF. The present data also

confirm what has been routinely observed in previous studies; the pulse frequency requirements for BSR are higher than the pulse frequency requirements for SIF. This observation has long been interpreted as an indication that different populations of MFB fibers might subserve BSR and SIF (Huston, 1971). Assuming a scalar relationship between the pulse frequency and the number of times reward- and feeding-relevant fibers fire, and given the fact that pulse frequency requirements for both behaviors were assessed at the same intensity, then the differential pulse frequency requirements for BSR and SIF could suggest that two populations of MFB fibers with different densities underlie these two behaviors. However the present data show that the magnitude of frequency threshold changes resulting from successive electrode penetration is proportionately the same for both BSR and SIF. Hence, although BSR has a higher frequency threshold than SIF the ratio of pulse frequency requirements for SIF to that for BSR is constant across the sites tested within an electrode penetration. The between site constancy of the SIF to BSR pulse frequency requirement ratio suggests that the same population of MFB first stage fibers underlies both behaviors. Although it is possible, it is unlikely that the density of a distinct population of reward-relevant fibers varies in exactly the same manner as the density of feeding-relevant fibers.

If BSR and SIF do share a common MFB first stage system, then why do BSR and SIF have different pulse frequency requirements? The present data do not answer this question but

at least one study has suggested that no differential stimulation requirements between SIF and BSR exist when the train duration is equated (Ball, 1968). It may be, however, that the differential pulse frequency requirements between BSR and SIF depend more directly on the differences in the behavioral criteria used to assess the animal's sensitivity to the rewarding and motivating effects of the stimulation. The present investigation determined the pulse frequency required to eat 4 pellets in 20 seconds. Had the behavioral criterion been 8 pellets in 10 seconds, the required pulse frequencies would undoubtedly have been higher and closer to the pulse frequency requirements for BSR. Conversely, had the rewarding effect of the stimulation been assessed by using an easier operant task, such as nose poking (Ettenberg et al., 1981), the pulse frequency requirements for BSR could be expected to be closer to the frequency requirements for SIF.

b) Refractory period estimates for SIF and BSR

The progressive increase of T-pulse effectiveness as a function of increasing the C-T interval observed for BSR and SIF in the present investigation suggests that a population of reward- and feeding-relevant fibers with a heterogeneous distribution of refractory periods recovered from the loss of excitability that followed the application of the C-pulse. The refractory period curves obtained for SIF and BSR can be taken to reflect the proportional contribution of first stage feeding- and reward-relevant fibers with different refractory period

characteristics. According to the present data, the MFB first stage system for SIF contains fibers which have refractory periods ranging from 0.4 to 2.0 msec. These estimates are similar to those obtained by Hawkins et al. (1983). The refractory period estimates for BSR obtained in the present study are consistent with those of previous reports (Bielajew et al., 1982; Rompre and Miliaressis, 1980; Yeomans, 1975; Yeomans, 1979).

The present study compared the refractory period distributions of first stage fibers for SIF and BSR at the same site of stimulation and at the same current intensity. It was assumed that any differences in the distribution of refractory periods of feeding- and reward-relevant fibers could not be ascribed to regional variations of refractory period distributions which may occur in a between-animal or between-site comparison. Therefore, any systematic differences between refractory period curves for BSR and SIF using the present design would be a strong indication that SIF and BSR are subserved by different populations of fibers.

The time course of recovery from refractoriness of feeding- and reward-relevant fibers appear to be quite similar in at least 6 of the 8 animals tested. In three rats (AG2, AG7 & AG19) the untransformed curves are almost superimposed, and in all but two cases (AG6 & AG918), the BSR and SIF refractory period curves reach asymptotic recovery at the same C-T intervals. However, with the exception of rats AG18 and AG19 which were not

tested at a wide variety of C-T intervals, SIF curves in the other 6 rats consistently recovered from refractoriness at shorter C-T intervals than BSR curves. This finding suggests that the feeding-relevant substrate contains fibers with short refractory periods which are not activated by rewarding stimulation. However, in the case of AG2, AG4 and possibly AG7 the retarded recovery of the BSR curve could be due to a local potential summation effect which could mask the recovery from refractoriness of reward-relevant fibers with the shortest refractory periods. This argument could explain the differences in the initial point of recovery from refractoriness between BSR and SIF in AG2 and AG4 where local potential summation effects for BSR are considerably larger than for SIF. Although local potential summation effects dissipate rapidly at C-T intervals in the 0.4 to 0.6 msec range (Yeomans, 1979), there may be enough residual summation to distort the initial point of recovery of the BSR curve. The question remains, however, as to why in most animals local potential summation effects were larger for BSR than for SIF. No simple answer to this question has yet been proposed.

The statistical analysis of the slope of the regression lines fitted to the transformed refractory period curves for BSR and SIF confirms what is seen by a simple visual inspection of the untransformed data. In 6 of the animals the two regression lines either overlapped perfectly (AG4), were reasonably parallel (AG19 AG917) or showed slow divergence (AG18) or convergence (AG2 &

AG7). The slopes of the two regression lines in AG6 and AG918, diverged significantly from one another; the rate of recovery from refractoriness of SIF fibers appears to be faster than that of BSR fibers. The obvious explanation for the different rates of recovery between the two curves in AG6 and AG918 is that SIF and BSR are subserved by different populations of fibers. If this were the case, then the apparent lack of differences between SIF and BSR in the other 6 animals would suggest that, at some points in the MFB, the SIF and BSR substrates overlap extensively and that both substrates comprise fibers with remarkably similar excitability characteristics.

There is, however, an additional feature of the refractory period curves obtained in the present study suggesting that BSR and SIF may indeed share a common substrate. In all the animals tested, both the SIF and BSR curves exhibit a plateau between C-T intervals of 0.6 and 0.7 msec; negligible increases in T-pulse effectiveness occur when the C-T interval is increased from 0.6 to 0.7 msec. The fact that refractory period curves for SIF and BSR both exhibit near-zero slopes between 0.6 and 0.7 msec may not be coincidental and may be interpreted as additional evidence that SIF and BSR are subserved by the same population of MFB first stage fibers.

Step-like BSR refractory period curves have already been reported (Gratton and Wise, 1985; Rompre, 1984). The reason step-like BSR refractory period curves have not been observed more

often is due to the fact that most previous studies have not varied the C-T intervals in the small increments that were used in the present study. There are several ways of interpreting the meaning of a plateau in a refractory period curve. It may be that at C-T intervals in the 0.6 to 0.7 msec range, neurons that have inhibitory inputs to BSR and SIF neurons recover from refractoriness and cause a portion of the feeding-and reward-relevant fibers to become less excitable. A more straightforward explanation is that both in the case of SIF and BSR, the stimulation activates two sub-populations of fibers with non-overlapping distributions of refractory periods: one population with refractory periods ranging from 0.4 to 0.6 msec and a second population of fibers with refractory periods ranging from 0.7 to 2.0 msec.

The contribution to BSR of two populations of MFB fibers with different refractory period distributions was first suggested by Deutsch (1964) and later supported by Gallistel et al. (1969). Based on behaviorally inferred refractory period data, both these authors suggested that the rewarding effect of MFB stimulation was subserved by fibers with refractory periods in the 0.5 to 0.7 msec range whereas a different population of MFB fibers with refractory periods in the 0.9 to 1.2 msec range were responsible for the motivating (priming) effect of the stimulation. Although the studies of Deutsch (1964) and Gallistel et al. (1969) studies have since been criticized on procedural grounds (Yeomans, 1975), the distribution of

refractory periods they reported for the rewarding and motivating effects of MFB stimulation correspond surprisingly well with the ranges of refractory periods for the first and second components of the BSR and SIF refractory period curves of the present investigation. Hence it may be that the two components of the refractory period curves reported in the present investigation, reflect the contribution of functionally distinct fibers systems to the elaboration of SIF and BSR.

Despite the above considerations, the "two sub-population" explanation of the plateau observed in the present refractory period curves is not easily predicted by our current understanding of cellular neurophysiology. The "two sub-population" hypothesis implies that the first, short refractory period, component of the function reflects exclusively a contribution of the absolute refractory period of BSR and SIF relevant fibers. In other words, the increase in TPE values from 0.4 to 0.6 msec reflects the contribution of fibers for which the C-T interval exceeds their absolute refractory periods and for which the T-pulse current intensity is great enough to eliminate the contribution of the relative refractory period. It is true that the contribution of relative refractory periods of reward-relevant fibers does not appear to be great (Bielajew et al., 1982; Yeomans, 1979). Nonetheless, it remains that for such a situation to occur, most of the feeding- and reward-relevant fibers must lie very close to the electrode tip, where the current density is high enough to overcome the decreased membrane



excitability associated with the relative refractory period.

Although unlikely, it may be that there is something special about the anatomy of the MFB fibers at sites which support both SIF and BSR. Nevertheless, if the substrate for SIF and BSR truly comprises two sub-populations of fibers with distinct refractory period distributions, then it may be possible to dissociate them pharmacologically. This the purpose of the second experiment.

## EXPERIMENT 2

## Introduction

The purpose of the present experiment was to examine the hypothesis that the step-like refractory period curves observed for BSR and SIF in the previous experiment reflect the contribution of two sub-populations of reward- and feeding-relevant fibers. It was reasoned that if this were the case and that if each sub-population had a different neurochemical identity, then pharmacological manipulations could selectively affect the relative contribution of one or the other of the sub-populations.

The neurochemistry of the MFB first stage system for BSR is unknown. It was once thought that catecholaminergic systems, which course through the MFB, were the fibers directly activated by rewarding stimulation. Psychophysical studies make it clear now that the electrophysiological and anatomical characteristics of the major portion of the first stage system are incompatible with those of dopaminergic or noradrenergic neurons (Gallistel et al., 1981). While pharmacological studies (Wise, 1978) do implicate dopaminergic systems in the reward circuitry it is apparent now that dopamine's involvement in BSR must occur at least one synapse away from the fibers that are directly activated by the rewarding stimulation.

Recently, however, Yeomans et al. (1985) reported that infusions of the muscarinic antagonist, atropine sulfate, into the ventral tegmental area attenuated lateral hypothalamic self-stimulation. Yeomans et al. (1985) interpreted their results as suggesting that cholinergic muscarinic receptors in the VTA play a role in the MFB reward circuitry. The data of Yeomans et al. (1985) also suggest that the first stage system for BSR may comprise cholinergic fibers which synapse onto cell bodies of the VTA. Alternatively, it may be that the output of first stage reward fibers is in some way modulated by a cholinergic system.

If a portion of the first stage system for BSR comprises cholinergic fibers which have a distinct distribution of refractory periods, then the administration of an anticholinergic drug should affect, in some way or another, the refractory period curve. In order to evaluate this possibility the effects of systemic atropine sulfate on the BSR refractory period curves were assessed. The effects of the quaternary ammonium derivative of atropine —atropine methyl nitrate— which does not readily cross the blood-brain barrier, were also assessed to control for the effects of parasympathetic cholinergic blockade.

The effects of the dopamine receptor blocker, pimozide, on the refractory period curves for BSR were also examined. In order to ascertain that any effect of atropine sulfate on the refractory period curves for BSR truly reflects a pharmacological action on the first stage system for BSR, it is necessary to show

that a drug, that affects a component of the reward circuitry other than the first stage system, does not affect the refractory period curves for BSR. As mentioned earlier, dopamine containing fibers are thought to be involved in BSR, but not at the level of the first stage system. If this is the case, then pimozide should have no effect on the refractory period curves for BSR.

#### METHOD

##### Subjects

Rats AG2, AG4, AG6, AG7, AG918 were used in the present experiment. Refractory period estimates were collected from the same stimulation sites and at the same current intensities that were used in Experiment 1. Hence the refractory period estimates for BSR obtained from these five rats in Experiment 1 served as baseline data for the present investigation.

##### Apparatus

The same apparatus as in Experiment 1 was used.

##### Procedure

Except for the following changes, the procedure used to obtain refractory period estimates for BSR in Experiment 1 was used in the present experiment. A session started by testing a

few representative C-T intervals (usually 0.44, 0.6, 1.0 and 2.0 msec) as a pre-drug control of T-pulse effectiveness. One of three drugs was then administered. Equimolar doses of 0.6 mg/kg of atropine sulfate or atropine methyl nitrate (Sigma) were injected intraperitoneally (i.p.) 15 to 20 minutes before testing, whereas pimozide (Janssen) was injected 4 hours before testing at a dose of 0.25 mg/kg i.p. For each rat, refractory period estimates for BSR were obtained under atropine sulfate treatment on a least three daily sessions, whereas the effects of atropine methyl nitrate and pimozide were tested only once. At least 48 hours separated each drug test. Except for C-T intervals 0.2, 0.4, 0.48, 0.52 msec., which were not tested, TPE values were obtained at the same C-T intervals that were tested in Experiment 1 according to the same testing protocol.

## RESULTS

Figure 9 shows the refractory period curves for BSR under the non-drug condition of the five rats used in the present experiment. These data have already been presented in Experiment 1, except that the data are now presented so as to highlight the changes in the slope of the curves as a function of varying the C-T interval. In order to do so the first derivative (slope) of each line segment of the refractory period curve, starting at the lowest average TPE value, was obtained using the following equation:

$$\text{Slope} = \frac{\Delta \text{TPE}\%}{\Delta \text{C-T}}$$

where  $\Delta \text{TPE}\%$  = the difference in T-pulse effectiveness values, expressed as a percentage, between two consecutive C-T intervals

$\Delta \text{C-T}$  = the difference between two consecutive C-T intervals.

The dashed lines in panels C to F of Figure 9 depict the first derivative of the overlying refractory period functions. The values plotted by the dashed lines do not correspond to those on the ordinate. As can be seen the slope of the refractory period curves invariably fall to zero or near-zero values between C-T intervals of 0.6 and 0.7 msec. In all rats tested, the slope analysis of the refractory period curves results in a bimodal distribution of slope values, while in two rats AG6 & AG918 the distribution appears to be trimodal. The consistency of the plateau between 0.6 and 0.7 msec can also be appreciated by examining panel A of Figure 9, where each replication of the refractory period curves for all 5 rats is depicted.

The effects of atropine sulfate on the refractory period curves for BSR are depicted in the left panels of Figures 10a to 10e, whereas the effects of atropine methyl nitrate and pimozone are shown in the right panels of the corresponding figures. It

should be noted that AG918 was not tested under pimozide treatment; this animal's electrode became dislodged. Under atropine sulfate treatment, significant (one-tail t-test,  $\alpha=0.05$ ) increases in TPE values occurred at a longer C-T interval than under either the non-drug condition or under atropine methyl nitrate and pimozide treatment: although there was some suggestion of recovery in one rat (AG7). Atropine sulfate consistently delayed the initial rise in TPE values from an average C-T interval of 0.524 msec ( $\pm 0.009$  msec.) to 0.8 msec.

In contrast, asymptotic increases in TPE values occurred at the same C-T as under the baseline condition in all but one animal (AG7). The shortest and longest C-T intervals to produce significant increases in TPE values are listed in Table 4. Since pimozide and atropine methyl nitrate were tested only once, the shortest and longest C-T intervals that resulted in significant increases in TPE values could not be determined statistically. However, a simple visual inspection of the pimozide and atropine methyl nitrate curves gives no reason to suspect that these drugs produced any changes in the time course of recovery from refractoriness for BSR in the any of the rats tested. Figure 10f depicts the averaged changes in the slope of the refractory period curves as a function of C-T interval under baseline condition (solid line) and atropine sulfate (dashed line) treatment.

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Hence, Figure 10f merely serves to highlight the relative lack of change in the slope of the refractory period curve between C-T intervals of 0.4 and 0.7 msec under atropine sulfate treatment.

The averaged single pulse frequency thresholds under baseline and drug conditions are listed for each rat in Table 5. Atropine sulfate did not appear to produce any dramatic change in single pulse frequency thresholds, although in 4 of the 5 rats tested the frequency thresholds under atropine sulfate tended to be lower than under the no-drug condition. Atropine methyl nitrate also produced slight decreases in single pulse frequency requirements in 3 rats, while in the two other rats the pulse frequency requirements increased. The single pulse frequency threshold in AG918 under the atropine methyl nitrate condition was unusually high. Since this animal's electrode assembly became dislodged shortly after the atropine methyl nitrate test, it may be that the increase in pulse frequency requirements was due to a change in the electrode's position. Hence the refractory period data obtained from AG918 under atropine methyl nitrate treatment may not be reliable.

As expected, pimozide treatment resulted in substantial increases in single, as well as paired pulse frequency requirements. Whereas pimozide produced moderate increases in pulse frequency requirements at the beginning of the testing session, the pulse frequency threshold steadily increased during the course of the session until the end of the session where some rats had to be primed with non-contingent stimulation to lure them to the lever.

#### DISCUSSION



## FIGURE 9

Refractory period data for BSR. Panel A shows individual replications. Inset of panel A shows averaged curves at C-T intervals where near-zero slope occurs. Panel B to F show refractory period data (solid line) for rats AG2, AG4, AG6, AG7, and AG918. The dashed line in each panel is the first derivative (slope) of the corresponding refractory period curve.

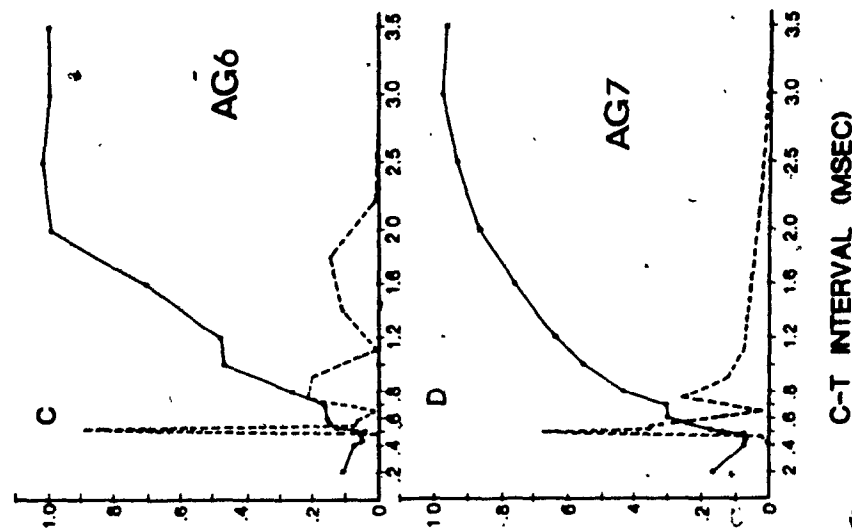
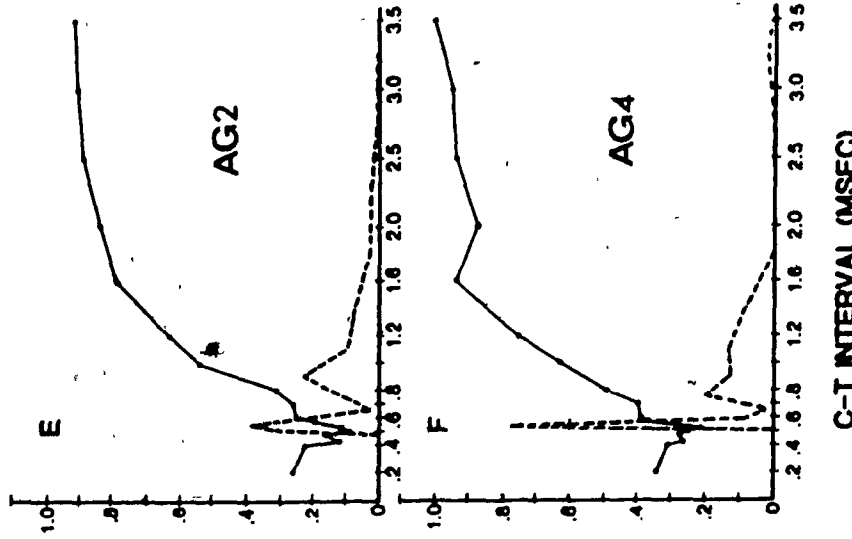
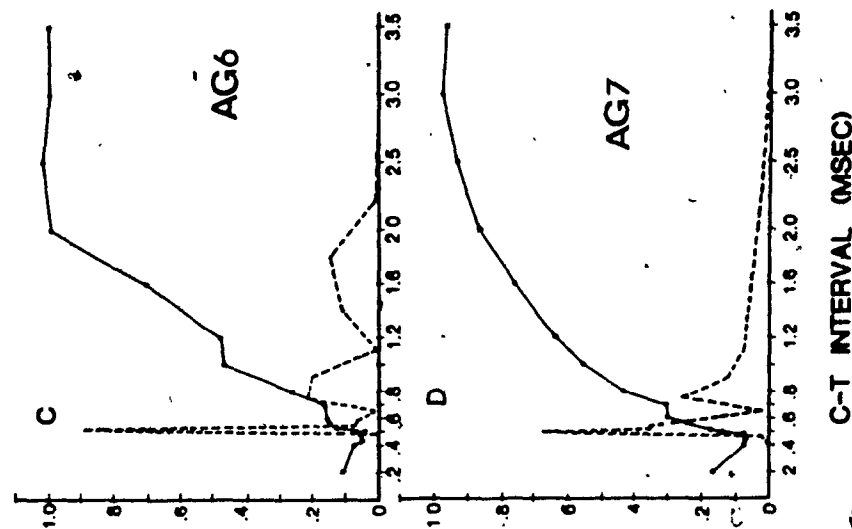
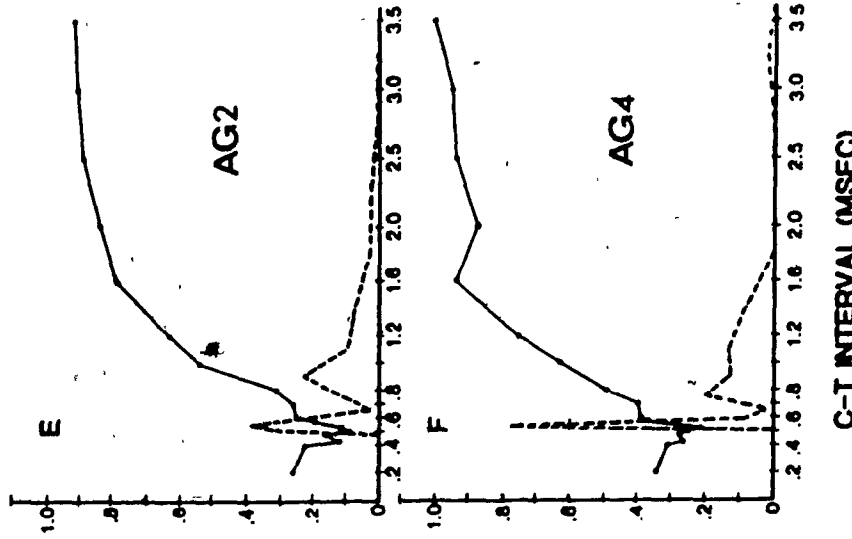
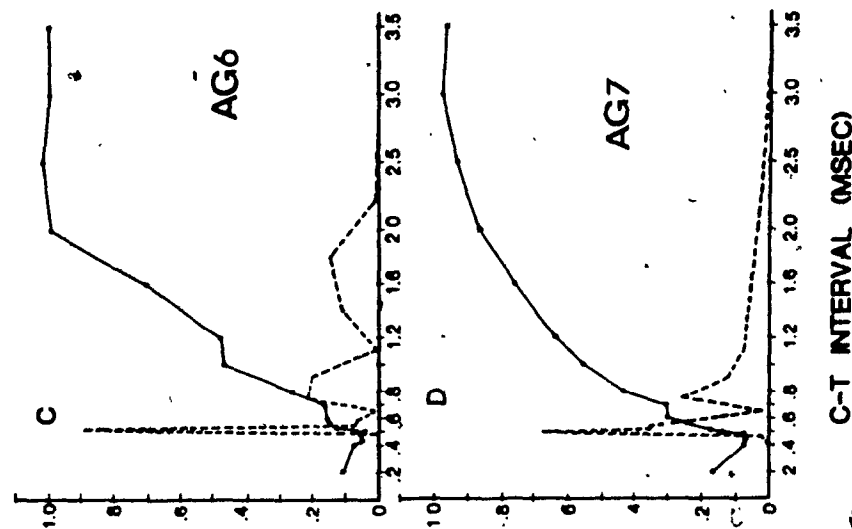
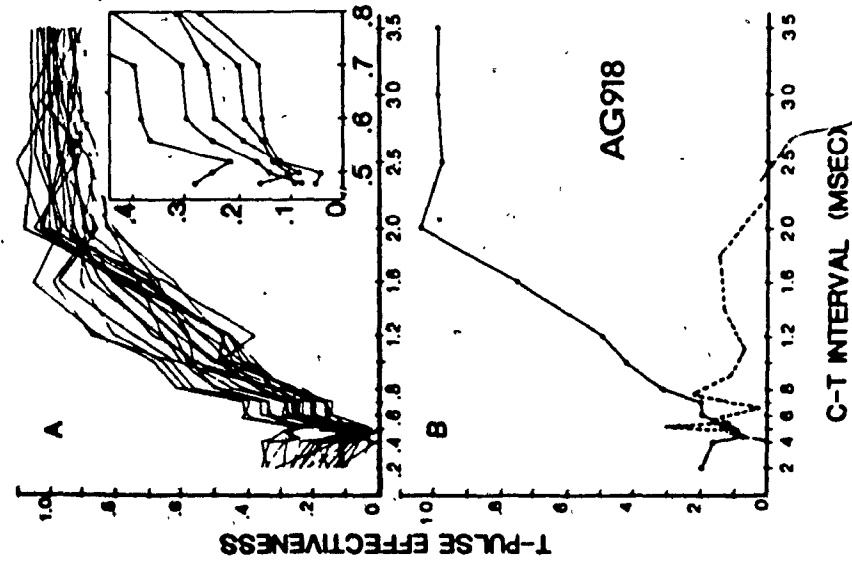
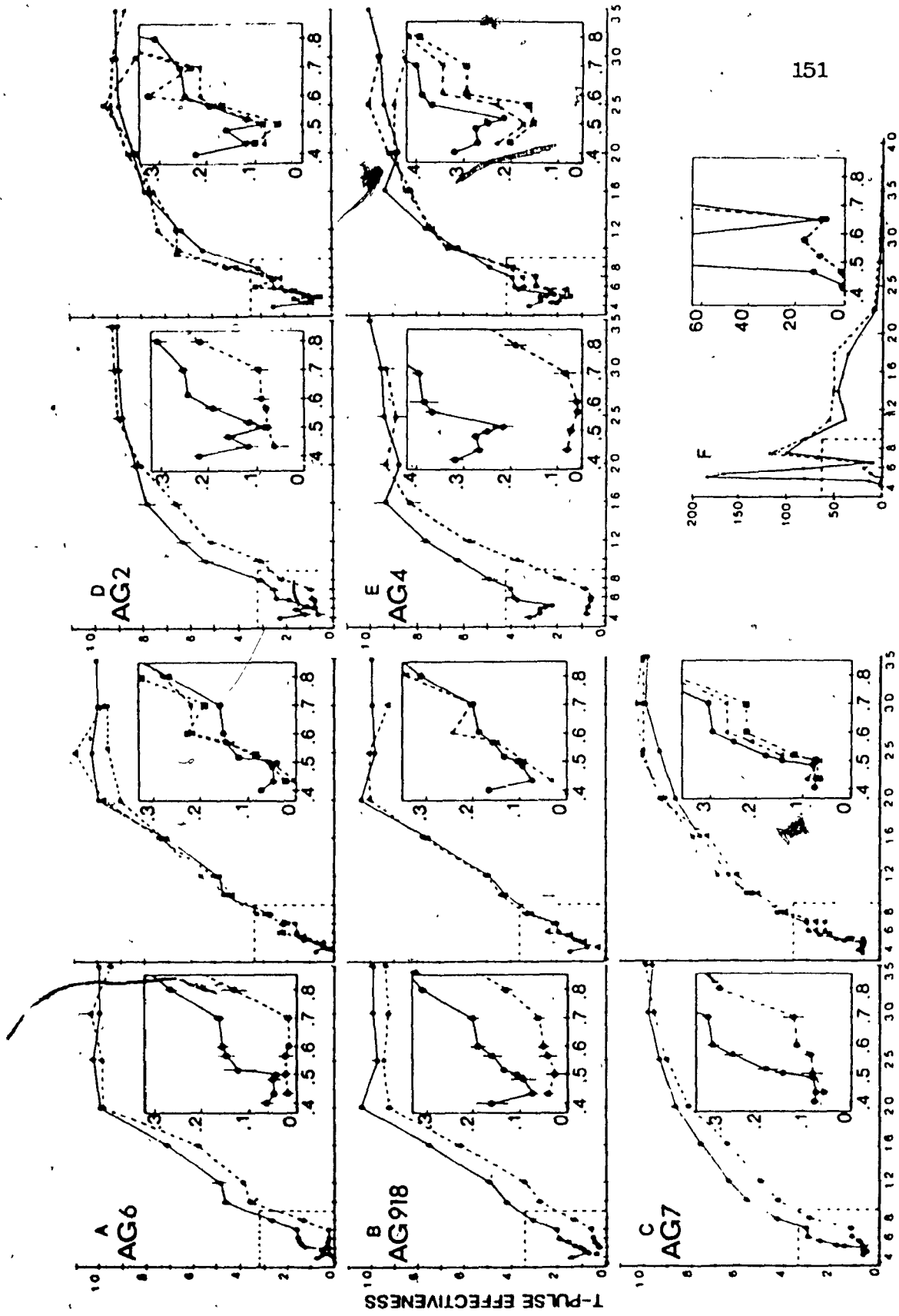


FIGURE 10.

Refractory period curves under various drug conditions. The left panel of frames A to E shows the atropine sulfate data (dashed lines) and the baseline data (solid lines). The right panel of frames A to E shows the baseline data (solid lines), the atropine methyl nitrate data (dashed lines and triangles), and the pimozone data (dashed lines and squares). Again the insets in each panel are expanded versions of C-T intervals between 0.4 and 0.8 msec, where the atropine sulfate effect occurred. Frame F shows the averaged slope as a function of C-T interval under baseline (solid line) and atropine sulfate (dashed line) conditions.



C-T INTERVAL (MSEC)

C-T INTERVAL (MSEC)

TABLE 4

Shortest and longest C-T intervals producing significant increases T-pulse effectiveness for BSR under baseline and atropine sulfate conditions.

Rat:	BASFLINE		ATROPINE SULFATE	
	shortest	longest	shortest	longest
AG2	0.52 msec	2.5 msec	0.8 msec	2.5 msec
AG4	0.56	1.6	0.8	1.6
AG6	0.52	2.0	0.8	2.0
AG7	0.50	2.0	0.8	2.5
AG918	0.52	2.0	0.8	2.0
Mean	0.524	2.02	0.8	2.12
s.e.m.	0.009	0.143	0.0	0.171

TABLE 5

Single pulse frequency thresholds (+/- standard error of measure) under baseline, atropine sulfate, atropine methyl nitrate and pimozide treatment.

Rat.	Baseline	Atropine sulfate	Atropine methyl nitrate	Pimozide
AG2	52.05 Hz +/- 0.74	48.28 Hz +/- 1.16	45.06 Hz +/- 1.29	65.41 Hz +/- 2.06
AG4	52.31 0.98	49.75 0.55	51.20 1.02	63.70 2.51
AG6	67.01 1.33	62.98 1.12	60.67 1.38	74.56 2.71
AG7	55.82 0.72	56.21 1.04	57.04 1.61	65.72 2.16
AG918	66.40 1.00	64.27 0.85	72.83 3.72	not tested
Mean	58.72	56.29	57.36	67.35
s.e.m.	3.33	3.29	4.70	2.44

One possible interpretation of the results of the present experiment is that atropine sulfate blocked the contribution of a component of the MFB first stage reward system which contains mostly cholinergic fibers having refractory periods in the 0.4 to 0.7 msec range. It appears, however, that this interpretation is incorrect: The arguments against such an interpretation will be presented later. The effects of atropine sulfate on the refractory period estimates for BSR appear, nonetheless, to be centrally mediated. This is suggested by the fact that atropine methyl nitrate, which does not readily cross the blood-brain barrier (Imes and Nickerson, 1975), did not affect the refractory period curves.

The present data are also consistent with the idea that dopaminergic neurons do not constitute a significant portion of the first stage system for BSR. Although pimozide treatment produced an overall attenuation of the effectiveness of the stimulation in maintaining BSR, it did not change the rate of increase in the relative effectiveness of the T-pulse as a function of increasing the C-T interval. The lack of any change in the relative effectiveness of the T-pulse in maintaining BSR under pimozide treatment has been reported earlier (Milner, 1976). Hence, although dopamine function was impaired, the rate of increase in the number of first stage BSR fibers that begin to fire to both the C and T pulses as a function of increasing the C-T interval presumably remained unchanged.

Yeomans et al. (1985) have reported that infusions of atropine sulfate into the ventral tegmental area attenuated lateral hypothalamic self-stimulation and suggested that muscarinic receptors in the VTA are an important link in the MFB reward circuitry. The data of Yeomans et al. (1985) also suggest that cholinergic reward-relevant fibers descend through the MFB and synapse on VTA cell bodies. Such an interpretation would be consistent with data suggesting that an important contingent of first stage BSR fibers course through the MFB in the rostral-caudal direction (Shizgal et al., 1980). However, while the VTA does contain moderate concentrations of muscarinic receptors (Rotter et al., 1979) and cholinergic-like terminals (Kimura et al., 1981), it is not at all clear that the cholinergic afferents of the VTA course through the MFB. While fibers originating from the ventral pallidal region of the basal forebrain, which stains intensely for acetylcholinesterase and choline acetyltransferase (Sato et al., 1983), are known to terminate in the VTA (Phillipson, 1979), they do not appear to contain acetylcholine (Grove et al., 1983). Furthermore, there are reasons to doubt that the inhibitory effect of intra-VTA infusions of atropine sulfate on lateral hypothalamic self-stimulation reported by Yeomans et al. (1985) is due to a selective antagonism of muscarinic receptors. When applied locally at concentrations used by Yeomans et al. (1985), atropine sulfate has anesthetic properties (Hoffer, note 4). Hence, it may be that, in the study of Yeomans et al. (1985), atropine



sulfate attenuated lateral hypothalamic self-stimulation by disrupting the conductive properties of the first stage reward fibers, rather than by a pharmacologically specific action on post-synaptic muscarinic receptors.


Since atropine sulfate was administered systemically, the present study is not subject to the same criticisms that have just been levelled against Yeomans' et al. (1985) study. Nonetheless, the absence of MFB cholinergic fibers suggests that the effects of atropine sulfate on the refractory period curves for BSR obtained in the present study cannot be due to the blockade of a cholinergic component of the first stage reward system. There are, however, other anatomical arrangements that can account for the present results. For instance, it may be that MFB first stage reward fibers having refractory periods in the 0.4 to 0.7 msec range, but not those with longer refractory periods, synapse onto cholinergic cell bodies. With such an arrangement the blockade of the output of the cholinergic follower neurons by atropine sulfate would also result in the delayed initial increase in TPF values that was observed in the present study.

Another interpretation of the present data is that atropine sulfate affected the excitability of the first stage reward system by a specific action on voltage-sensitive ionic channels. Ionic channels have in the past been classified in two groups: a) ionic channels that are activated by neurotransmitters that bind to receptor molecules and b) ionic channels that are activated by

changes in the membrane potential (voltage-sensitive). There is, however, increasing evidence indicating that neurotransmitters and their related pharmacological compounds can alter the excitability of neurons by acting on voltage-sensitive ionic channels located either on the cell body or the presynaptic terminal.

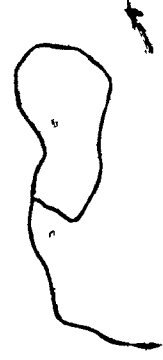
Specifically, it appears that in the central nervous system, there is evidence for an action of acetylcholine on voltage-sensitive potassium channels that results in changes in the firing pattern of the neuron (Halliwell & Adams, 1982; Nicoll, 1982). While it is not clear what action atropine sulfate has on voltage-sensitive channels, it may be that the changes in excitability of reward-relevant fibers suggested by the decreased T-pulse effectiveness between C-T intervals of 0.4 and 0.7 msec is due to a specific action of atropine sulfate on voltage-sensitive channels.

There are numerous reports of a muscarinic involvement in BSR. However, while there are suggestions that the MFB reward circuitry contains a muscarinic link (Coons et al., 1976) most of the earlier reports suggest that muscarinic systems antagonise rather than contribute to the rewarding effect of MFB stimulation (Domino and Olds, 1968; Margules & Stein, 1969; Newman, 1972; Stark and Boyd, 1963). An antagonistic role for muscarinic receptors on reward function was suggested in these studies on the basis of a facilitatory effect of anti-muscarinic compounds on measures of the overall effectiveness of the rewarding stimulation. The present data as well as those of Coons et al.



(1976) do not contradict this conclusion. In both the Coons et al. (1976) and the present study it was the relative effectiveness of the stimulation (T-pulse) that was used to estimate the effects of cholinergic drugs on the MFB reward substrate. Hence it may very well be that the MFB substrate for BSR receives an inhibitory input from a cholinergic system which is independent from the cholinergic system presumably identified in the present study.

Since the main question addressed by the present thesis is whether or not BSR and SIF share a common first stage system, it would be interesting to determine if atropine sulfate produces similar effects on refractory period estimates for SIF. Attempts by the present author to perform this experiment have not been successful. Rats under atropine sulfate treatment invariably choke on their food, presumably because of a decreased secretion of saliva.



## EXPERIMENT 3

## Introduction

It has been suggested that at least a major portion of the first stage fibers for BSR extend along the longitudinal axis of the MFB, between the lateral hypothalamus and the ventral tegmental area (Bielajew and Shizgal, 1982; Shizgal et al., 1980). The anatomical linkage between two MFB sites that support self-stimulation was inferred behaviorally using a collision test. The rationale underlying the behavioral collision test and its application to the elucidation of the anatomical linkage of the first stage BSR fibers has been described earlier (ref. Section A).

The purpose of the present investigation was to test the hypothesis that MFB feeding-relevant fibers, like reward-relevant fibers, extend between the lateral hypothalamus and the ventral tegmental area. If feeding-relevant fibers do extend between the lateral hypothalamus and ventral tegmental area, then the collision effects reported by Bielajew and Shizgal (1982) and Shizgal et al. (1980) for MFB self-stimulation should also be obtained for SIF.

## METHOD

## Animals and surgery

The subjects were male, Long-Evans rats weighing, on average, 500 grams at the time surgery. Under sodium pentobarbital anesthesia (Somnotol; 60 mg/kg) each rat was implanted with two unipolar electrodes. One electrode was aimed at the lateral hypothalamic MFB while the second electrode was aimed at the anterior region of the ventral tegmental area. The electrodes were 254 micrometer stainless steel wires soldered to Amphenol mini-pins and insulated with Formvar except at their square cross section. Prior to surgery, pairs of electrodes were placed in a jig, adjusted so that distance between the two tips was approximately 2.5 mm and cemented together with dental acrylic cement. The electrode assembly was attached to the stereotaxic electrode holder and aligned so that the posterior (VTA) electrode would be 0.5 mm more medial than the anterior (LH) electrode. With the incisor bar on the stereotaxic apparatus adjusted so that the cranium was horizontal, the two electrodes were simultaneously lowered into the brain. The coordinates for the anterior (LH) electrode were: A.P.=2.3 mm behind bregma, Lat.=1.6 mm from midline, D.V.=8.5 mm below dura. With an inter-electrode distance of 2.5 mm, the posterior electrode was expected to be situated 4.8 mm behind bregma, 1.1 mm from the midline and 8.5 mm below dura. Current return was through a stainless steel wire soldered to an Amphenol pin and wrapped around two stainless steel screws imbedded in the cranium. The entire electrode assembly was held together and anchored to the

skull screws with dental acrylic cement.

### Apparatus

The same apparatus as in Experiments 1 and 2 was used.

### Procedure

#### a) Screening and preliminary testing

The screening and preliminary testing procedures for SIF were the same as in Experiment 1, except for the following changes. In order to be included in the experiment an animal had to feed in response to stimulation applied to both its anterior and its posterior electrode. Once the animals were feeding reliably, the current intensity applied to each electrode site was increased to the highest levels that would elicit feeding without producing competing aversive or motoric effects. It was reasoned that increasing the current intensity would enlarge the field of effective excitation and would consequently increase the probability that the two electrodes would activate a common set of fibers.

#### b) Pilot collision test

After the animals exhibited a feeding response with stable single pulse frequency thresholds at each electrode site, a pilot

collision test was conducted. C-pulses were applied to the anterior electrode while T-pulses, delayed by 0.5 or 10.0 msec, were applied to the posterior electrode. The purpose of this procedure was to detect a collision effect with the least amount of work. The C-T intervals of 0.5 and 10.0 msec were chosen because collision effects for BSR have been shown to occur within this range of C-T intervals (Bielajew and Shizgal, 1982; Shizgal et al., 1980). It was reasoned that since the refractory periods for feeding-relevant fibers are similar to those of reward-relevant fibers and since the distance between the two electrodes was similar to that of Bielajew and Shizgal's (1982) study, collision effects were expected to occur at C-T intervals similar to those reported for BSR. Animals showing a collision effect were expected to have a higher pulse frequency threshold at a C-T interval of 0.5 msec than at 10.0 msec, presumably because of the loss of excitation due to collision at short C-T intervals. Animals not showing a collision effect were not tested any further.

### c) Collision test

Animals showing clear differential paired-pulse frequency thresholds during the pilot tests; between the 0.5 and 10.0 msec conditions, were tested under several additional C-T intervals. The collision test proper involved obtaining frequency thresholds under three different conditions: single pulse frequency thresholds at (1) the anterior and (2) the posterior electrode

site and (3) paired pulse frequency thresholds when the C-pulses were applied to the anterior electrode and the T-pulses were applied to the posterior electrode. The testing protocol was similar to that which was used to obtain refractory period estimates. One session consisted of 3 to 4 blocks of paired-pulse frequency threshold determinations and 4 to 5 pairs of single pulse frequency threshold determinations. Each block contained a short, a medium and a long C-T interval (e.g. 0.5, 4.0, 20.0 msec) whereas two single pulse frequency threshold determinations (one with the anterior and one with the posterior electrode) preceded and followed each block of C-T intervals tested. The averaged single pulse frequency thresholds on the anterior electrode and the averaged single pulse frequency thresholds on the posterior electrode were used to estimate the T-pulse effectiveness under the intervening block of paired pulse conditions. The following formula of Shizgal et al. (1982) was used to compute T-pulse effectiveness values.

$$TPE = \frac{(RHz\ spl/RHz\ c-t) - 1}{(RHz\ spl/RHz\ sph)}$$

where TPE= T-pulse effectiveness

RHz spl= the lowest of the two single pulse frequency threshold

RHz sph= the highest of the two single pulse frequency threshold



RHz c-t= the paired pulse frequency threshold at a particular C-T interval

This formula compensates statistically for differences in single pulse frequency threshold between the two stimulation sites. Differences in single pulse frequency threshold between the two electrode sites indicate that under paired pulse conditions the stimulation at one electrode site accounts for more than 50% of the net effect of the stimulation. In other words the stimulation site which requires the lowest pulse frequency (spl) to elicit a constant level of behavior must be more effective than the site which has higher (sph) single pulse frequency threshold. Such between-electrode differences in stimulation effectiveness are compensated for by the above equation.

No less than 4 replications were performed at each of the following C-T intervals: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 10.0 msec. Some rats were tested at additional C-T intervals of 0.2, 1.2, 3.5, 4.0, 20.0 msec. Once the collision data were collected under the A-P electrode configuration (the C-pulse applied to the anterior electrode and the T-pulse applied to the posterior electrode), the configuration was reversed (P-A configuration).

d) BSR collision test

Animals that showed a collision effect for SIF were also

tested for collision in a self-stimulation situation. The procedure used to obtain collision data for SIF was also used for BSR. The collision tests for BSR were conducted at the same current intensities that were used in the collision tests for SIF.

e) Refractory period estimates

Refractory period estimates for SIF and, at times for BSR, were obtained at both the anterior and posterior electrode placements. The procedure used has been described in Experiment 1.

f) Histology

At the completion of the experiment, the animals were anesthetized with chloral hydrate and transcardially perfused with 0.9% saline followed by 10% formalin. Their brains were sliced in 40 micrometer sections and drawings of the coronal slices containing the electrode tracks were made and compared to Paxinos and Watson's (1962) stereotaxic atlas.

RESULTS

a) Collision data

A total of 33 rats were used in the present experiment. Collision data for SIF were obtained for 3 rats while incomplete

collision data were collected for a fourth rat. In rats AG813, AG815 and AG821, complete sets of collision data for SIF were obtained whereas in AG812 collision data were obtained for the A-P electrode configuration only. At the time this report was written, AG812 was still being tested. Two animals (AG813 and AG815) that showed collision effects for SIF, also showed collision effects for BSR. The third animal (AG821) showing SIF collision, exhibited seizures in the self-stimulation situation and therefore could not be tested but, interestingly, demonstrated no such effect when retested for SIF.

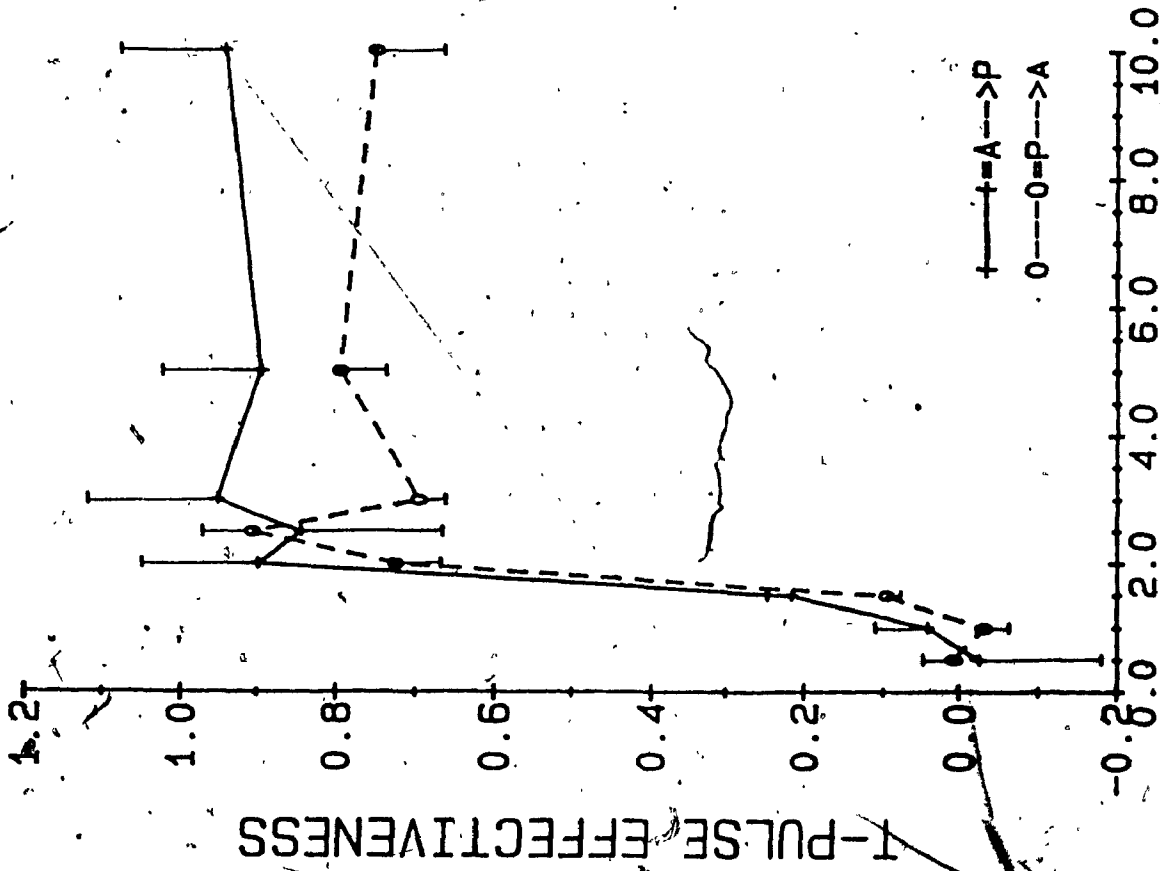
The collision data are presented in Figures 11a to 11d. The left panels in Figures 11a and 11b show the SIF collision data for rats AG813 and AG815 respectively, while the right panel shows the collision data for BSR for the same animals. Since no BSR collision data are available for AG821 and AG812, Figures 11c and 11d have only one panel each. Most of the collision curves share the same basic characteristics; the flat portions of the curve at very short and at longer C-T intervals are joined by a steep rising portion. The basic profile of the collision curves is unchanged by the order of presentation of the C- and T-pulses. However, while the abrupt increase in TPE values occurred in all rats at similar C-T intervals (1.5 to 2.0 msec), the magnitude of the increase in TPE values varied from one rat to another.

The clearest and strongest collision effects, for SIF as well as for BSR, were obtained in AG813. In this animal, abrupt

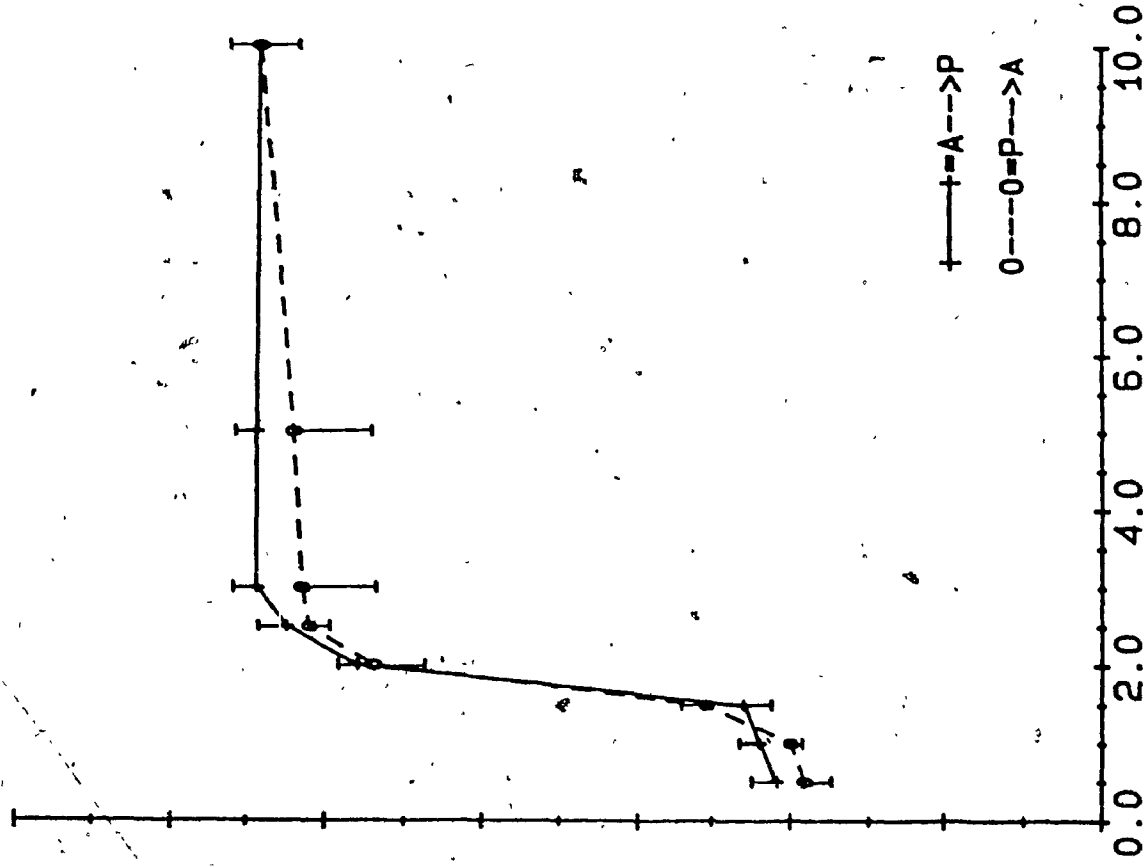
## FIGURE 11a

Collision data for AG813. Left panel shows the collision effect for SIF and the right panel shows the collision effect for BSR. The dashed lines represent the data obtained with the anterior-posterior electrode configuration and the solid lines represent the data obtained with the reversed electrode configuration.

SIF Collision AGB13



BSR Collision AGB13



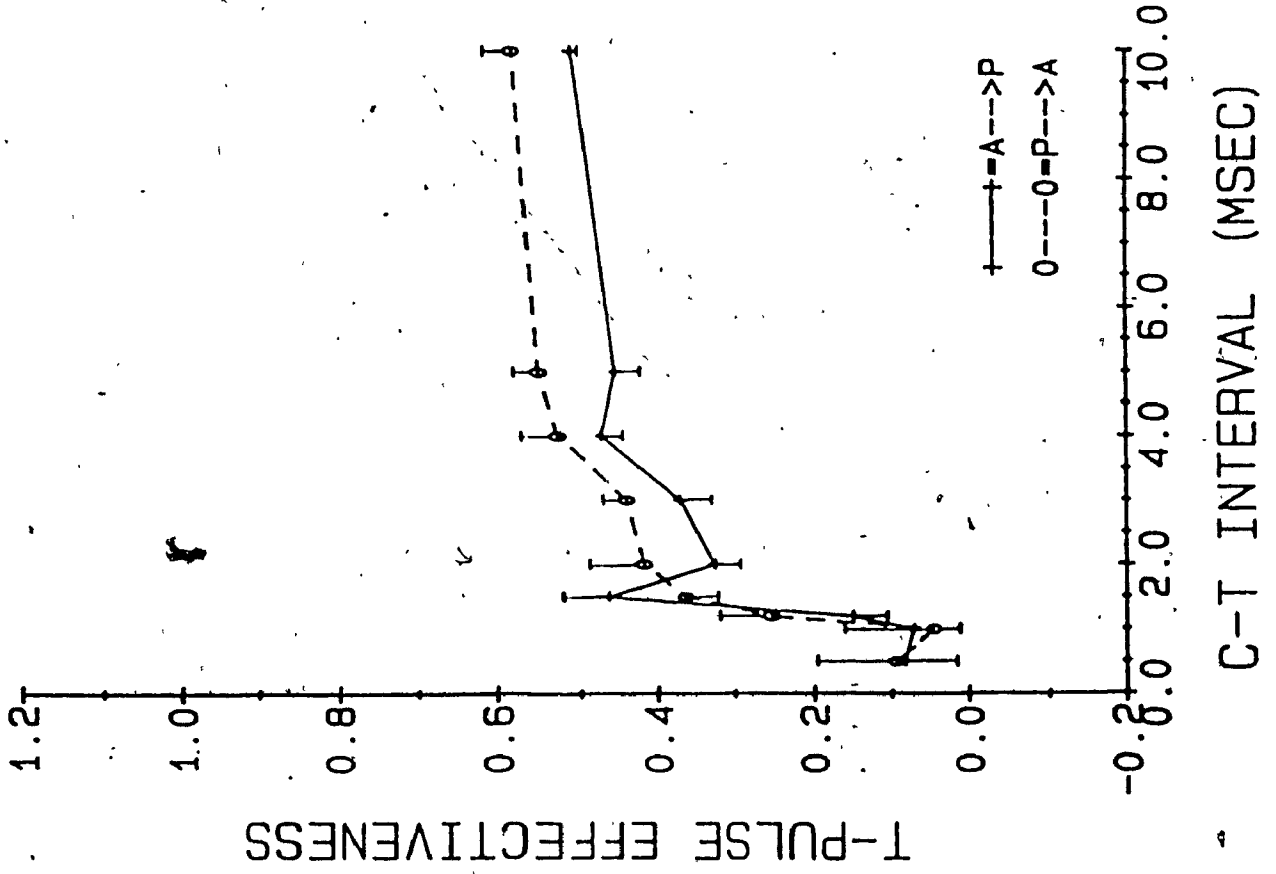
C-T INTERVAL (MSEC)

C-T INTERVAL (MSEC)

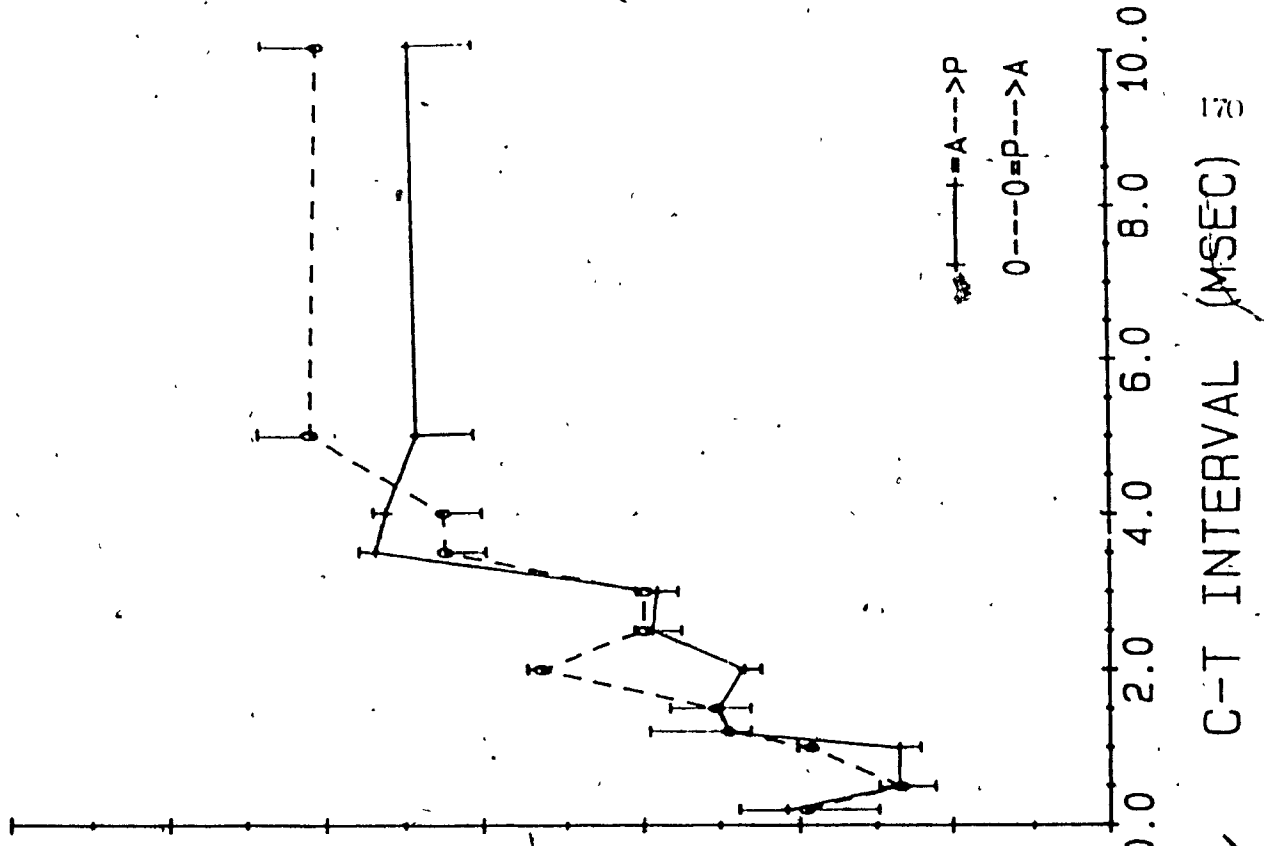
## FIGURE 11b

Collision data for AG815. Left panel shows the collision effect for SIF and the right panel shows the collision effect for BSR. The dashed lines represent the data obtained with the anterior-posterior electrode configuration and the solid lines represent the data obtained with the reversed electrode configuration.

SIF. Collision .AG815



BSR Collision AG815



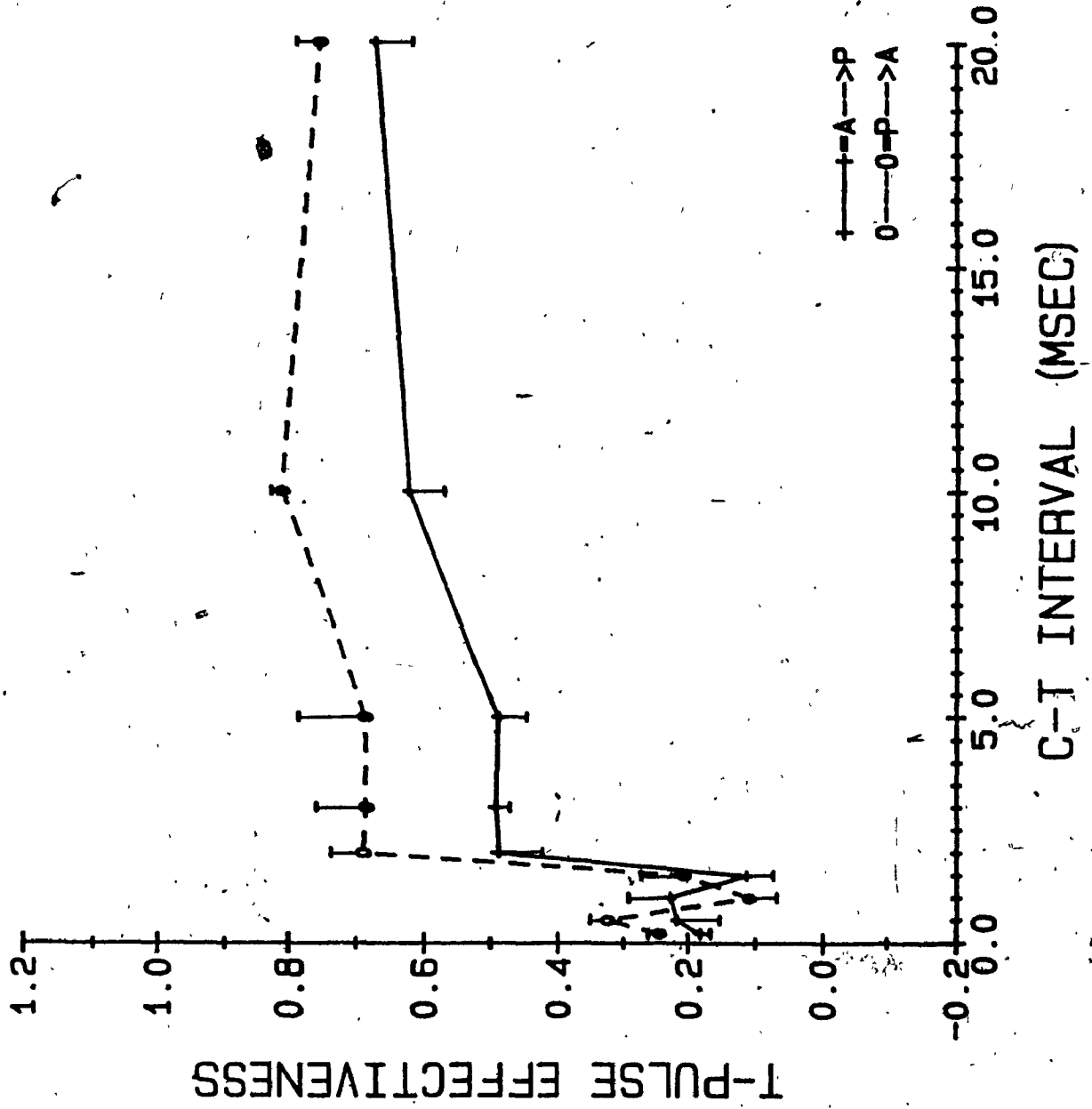
## FIGURE 11c

SIF collision data for AG821. BSR collision data was not obtained for this animal.



# SIF Collision AG821

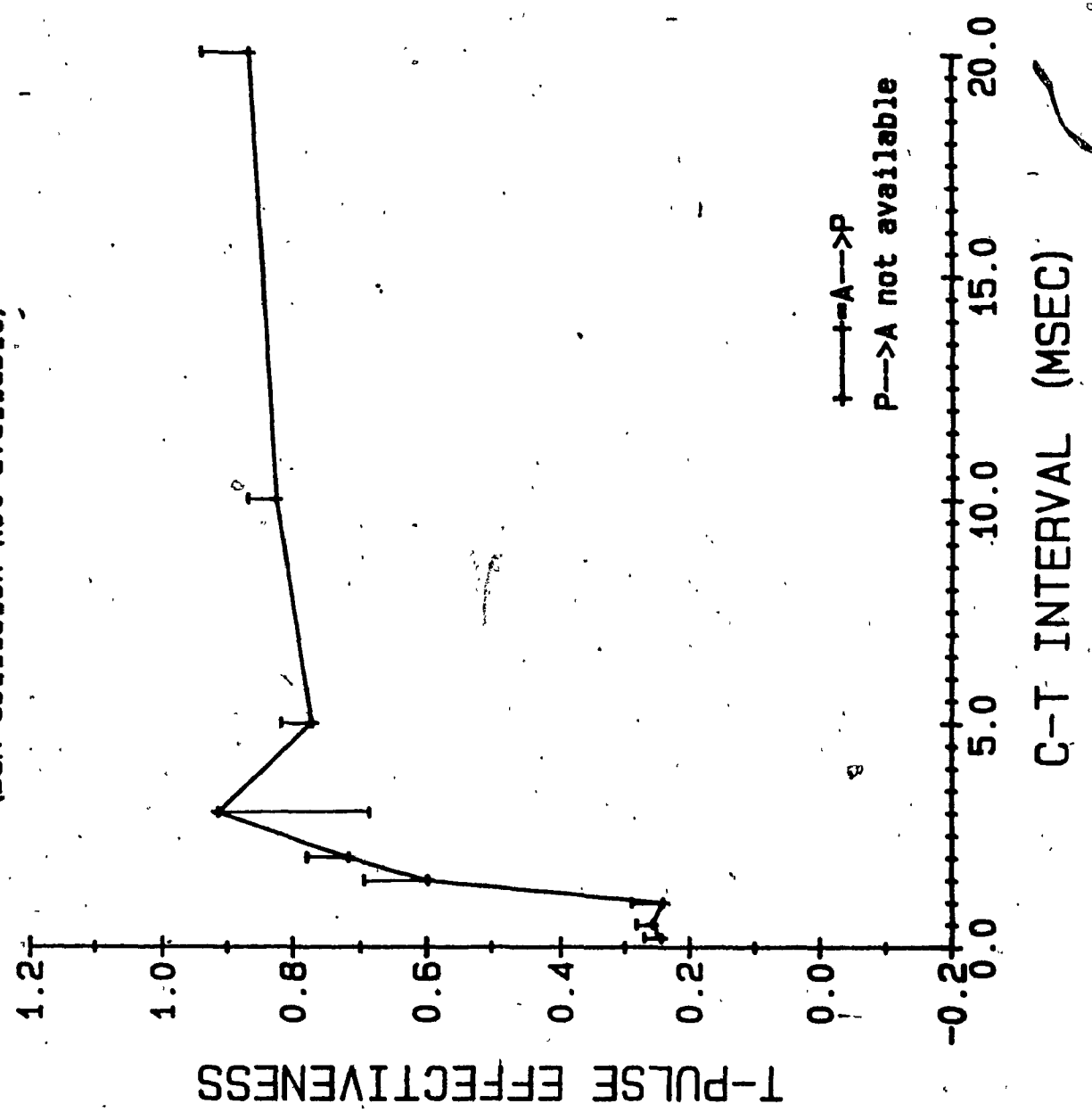
(BSA Collision not available)



## FIGURE 11d

SIF collision data for AG812. BSR collision data was not obtained for this animal and no data for SIF is available for the posterior-anterior electrode configuration.

SIF Collision AG812  
(BSR Collision not available)



C-T INTERVAL (MSEC)

increases in TPE values for SIF and BSR occurred at a C-T interval of 2.0 msec regardless of the electrode configuration tested. In the span of 0.5 msec, the TPE values for SIF and BSR increased by approximately 70 to 90%. Increases in TPE values of a similar magnitude occurred at the same C-T interval in rat AG812, although the collision data in this animal were collected for the A-P electrode configuration only. Smaller increases (40 to 50%) in TPE values for SIF were obtained in rats AG815 and AG821 at C-T intervals of 1.5 and 2.0 msec respectively. The collision data for BSR in AG815 are, however, not as clear. In this animal, increases in TPE values appeared to occur in two phases: an initial increase of approximately 30% at 1.2 msec and a second increase of approximately 40% at 3.5 msec. The SIF collision curve for AG815 does not show a similar two phase increase in TPE values.

#### b) Refractory period data

The refractory period data are presented in Figures 12a to 12d. The left panel in Figures 12a and 12b show the refractory period data for SIF and BSR at the anterior stimulation site, while the right panel shows the corresponding data at the posterior stimulation site. It should be noted that no refractory period data for SIF were obtained at the posterior site of AG815; the feeding behavior elicited at this site gradually became erratic and no reliable measures could be obtained. Refractory period estimates at the anterior

stimulation site of AG812 were quickly obtained to allow at least a tentative estimate of conduction velocity properties of SIF fibers. These estimates will be presented and discussed later.

The refractory period estimates for SIF and BSR obtained in the present experiment are similar to those obtained in Experiment 1. For the most part TPE values began to increase at 0.5 msec and reached asymptote at C-T intervals in the 1.6 to 2.0 msec range. The one notable exception to this trend is the refractory period curve for BSR obtained at the posterior site of AG815. In this case, TPE values levelled off at 1.0 msec. As was seen in Experiment 1, there did not appear to be any differences in the rate of recovery from refractoriness between SIF and BSR and all curves exhibited near-zero slopes between C-T intervals of 0.6 and 0.7 msec. \*

### c) Histology

Figure 13 shows the anterior and posterior electrode placements for 3 of the 4 rats showing collision effects for SIF. All three anterior electrodes were in the perifornical region of the lateral hypothalamic MFB, whereas the posterior electrodes were either in the rostral region of the ventral tegmental area (AG813) or the most caudal region of the MFB (AG815 and AG821). The inter-electrode distance in all three cases was verified by comparing the inter-electrode distance that was set when the electrode pairs were prepared to the number of 40 micrometer

coronal slices that were produced when slicing from the anterior to the posterior electrode. The inter-electrode distance was set at 2.5, 2.2 and 2.5 mm for rats AG813, AG815 and AG821 respectively. During histology, 2.4 (60 slices), 2.04 (51 slices) and 2.32 mm (58 slices) were found separating the anterior and posterior electrodes of rats AG813, AG815 and AG821 respectively. When allowances are made for tissue shrinkage and distortion caused by electrode extraction, fixing and freezing the two measures of inter-electrode distance appear quite consistent. The inter-electrode distance in AG812 was set at 2.6 mm. However there is, at present time, no histological confirmation of this measurement.

d) Estimates of conduction velocity

Estimates of conduction velocity for SIF- and BSR-relevant fibers were obtained using the following equation:

$$\text{Conduction velocity} = \frac{\text{Inter-electrode distance}}{\text{Collision interval} - \text{refractory period}}$$

where inter-electrode distance = the pre-set distance between the anterior and posterior electrodes

collision interval = the longest C-T interval that did not produce an abrupt rise in TPE

values

refractory period= the C-T interval producing the lowest TPE value on the refractory period curve.

The conduction velocity estimates as well as the data from which these estimates are derived are presented in Table 6.

#### DISCUSSION

The collision data for SIF obtained in the present experiment support what was suggested by the mapping study in Experiment 1. The present data suggest that the first stage system for SIF comprises fibers that course along the longitudinal axis of the MFB at least between the lateral hypothalamic area and the rostral pole of the ventral tegmental area. Furthermore the fact that, in two rats, collision effects for SIF as well as for BSR were obtained suggests that the MFB first stage systems for SIF and BSR are one and the same. Another interpretation of the collision data of these two animals, is that SIF and BSR are subserved by distinct first stage system that are tightly interwoven and that both course through the stimulation fields of both electrodes. Although unlikely, the present data cannot unequivocally rule out this possibility.

The estimates of conduction velocity for SIF- and

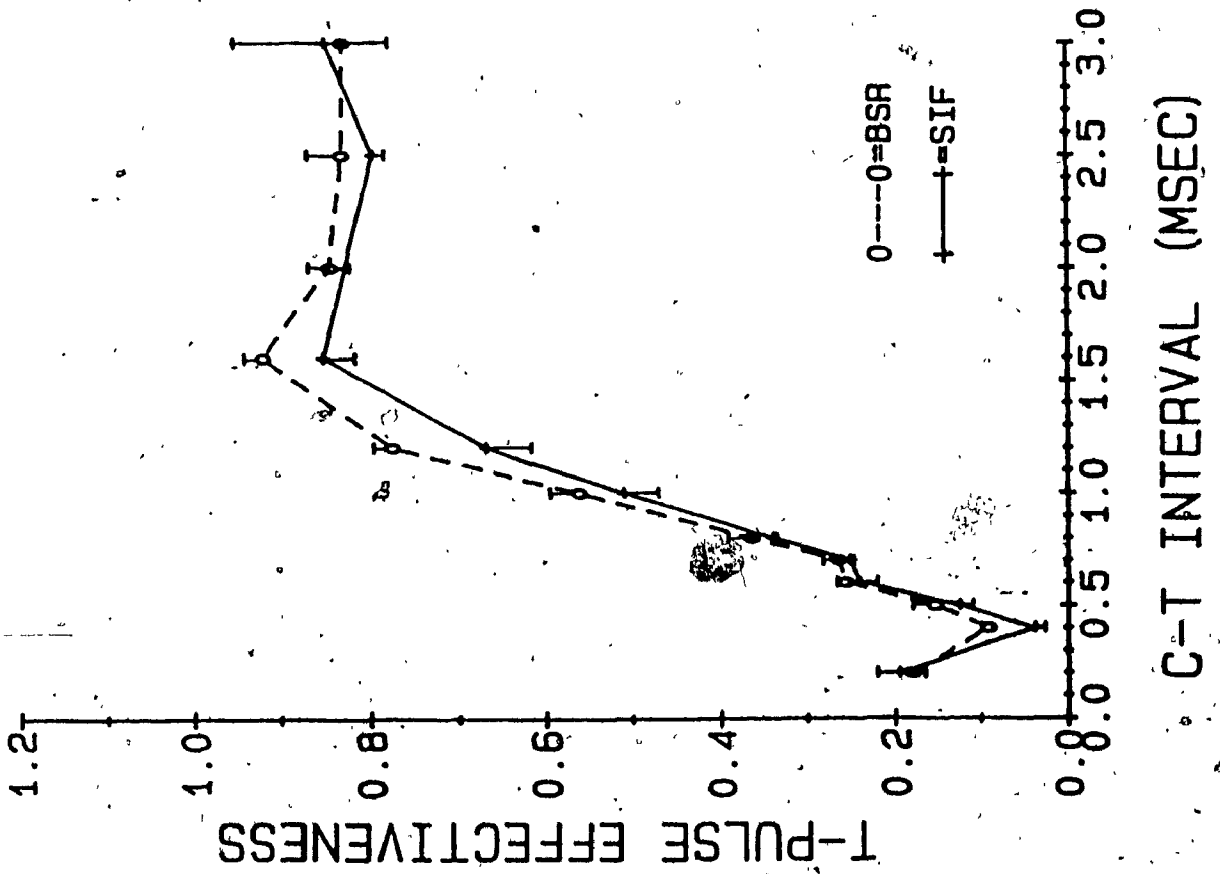
## FIGURE 12a

Refractory period data for AG813. The left panel shows the refractory period data for SIF (solid line) and BSR (dashed line) obtained at the anterior electrode site. The right panel shows the SIF and BSR data obtained at the posterior electrode site.



Refractory period data AG813

Ant. electrode



Refractory period data AG813

Post. electrode

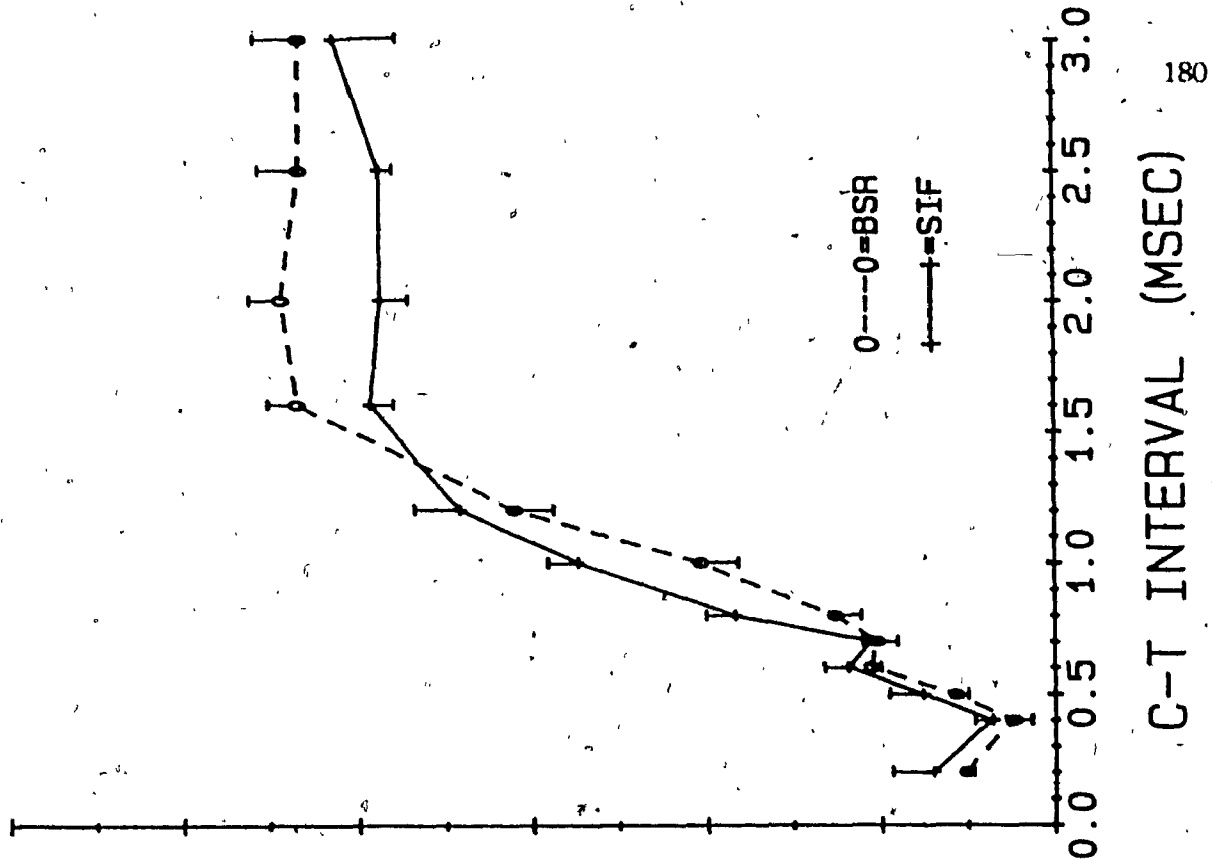


FIGURE 12b

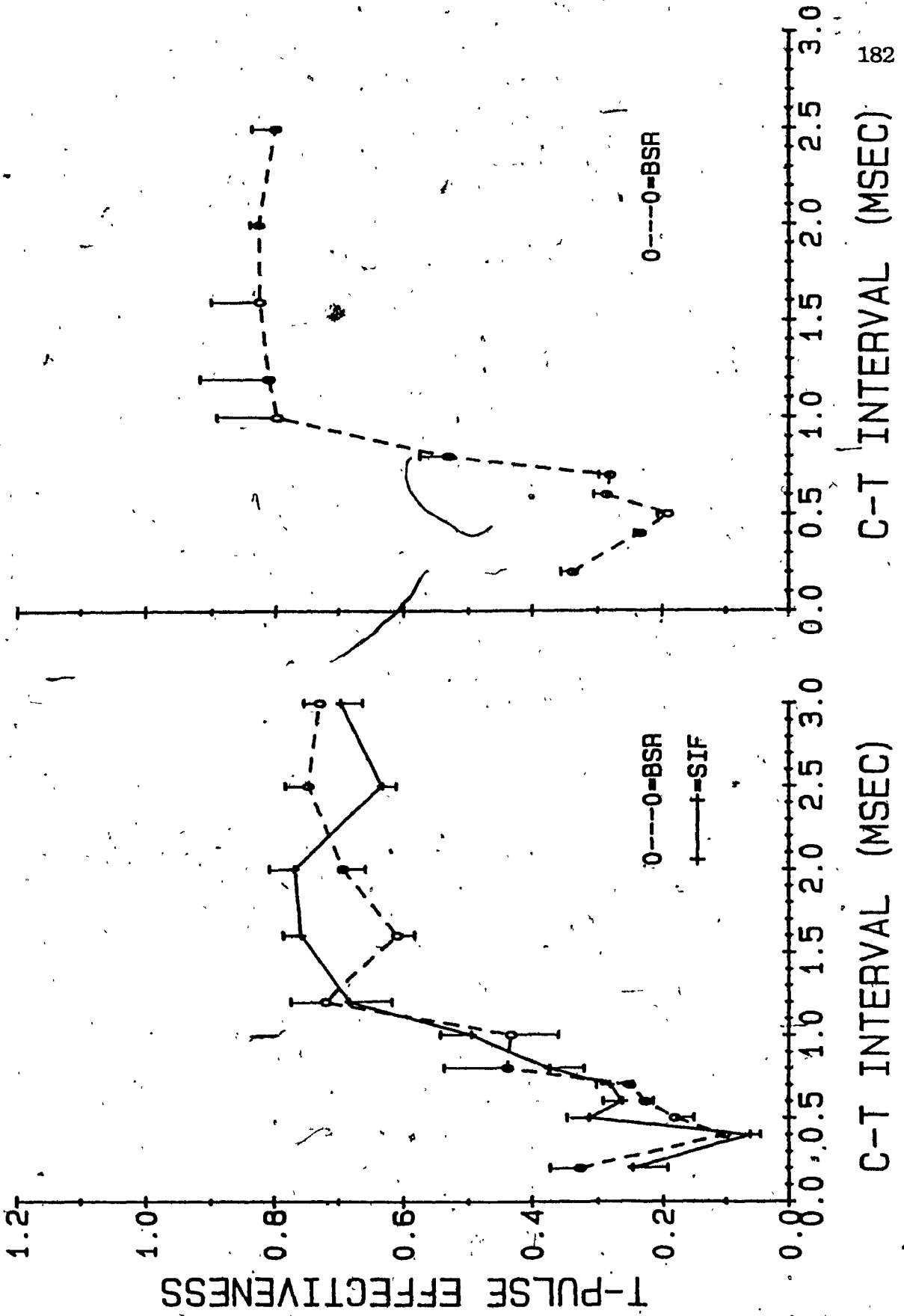
Refractory period data for AGB15. The left panel shows the refractory period data for SIF (solid line) and BSR (dashed line) obtained at the anterior electrode site. The right panel shows only the BSR data obtained at the posterior electrode site. No SIF data are available at the posterior electrode site.

Refractory period data AG815

Ant. electrode

Refractory period data AG815

Post. electrode

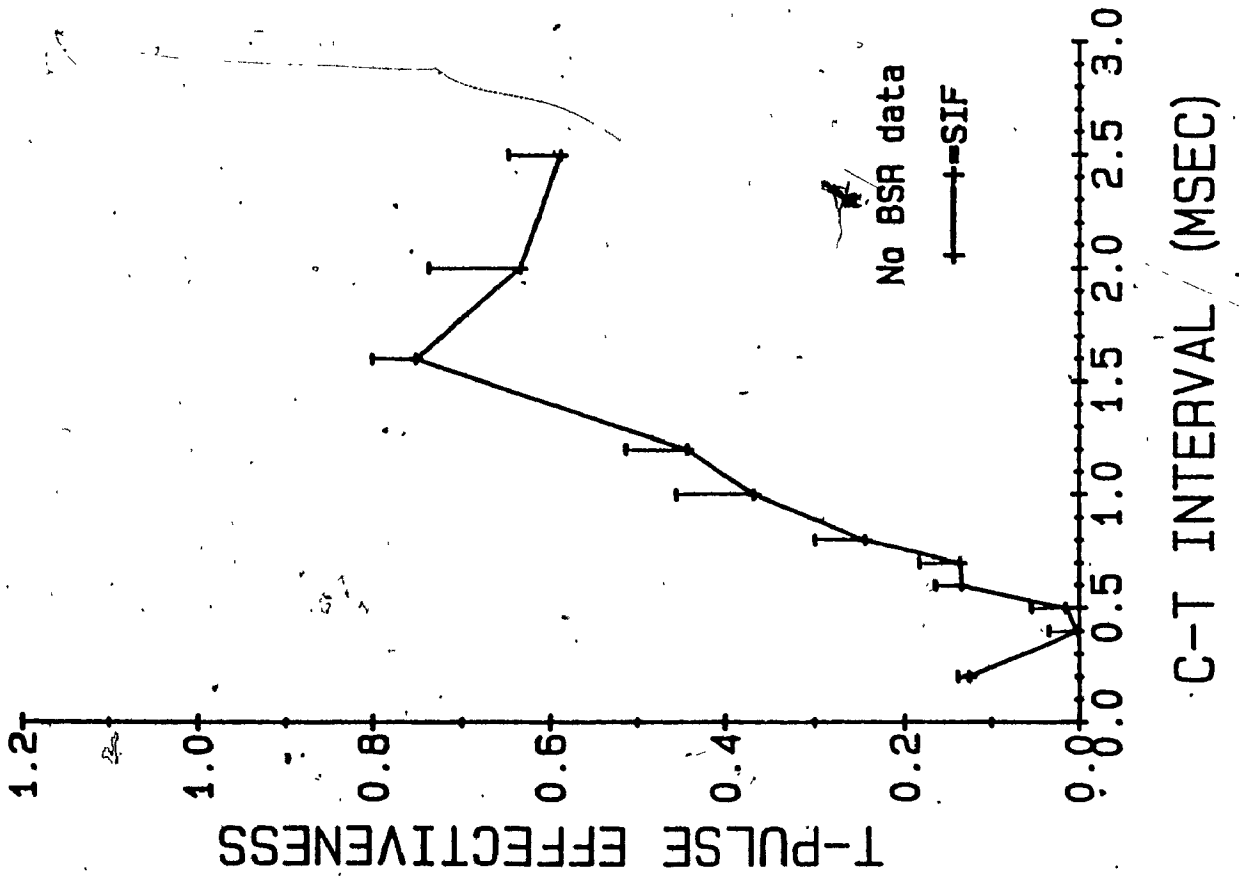


## FIGURE 12c

Refractory period data for AG821. Only the refractory period data for SIF was obtained.

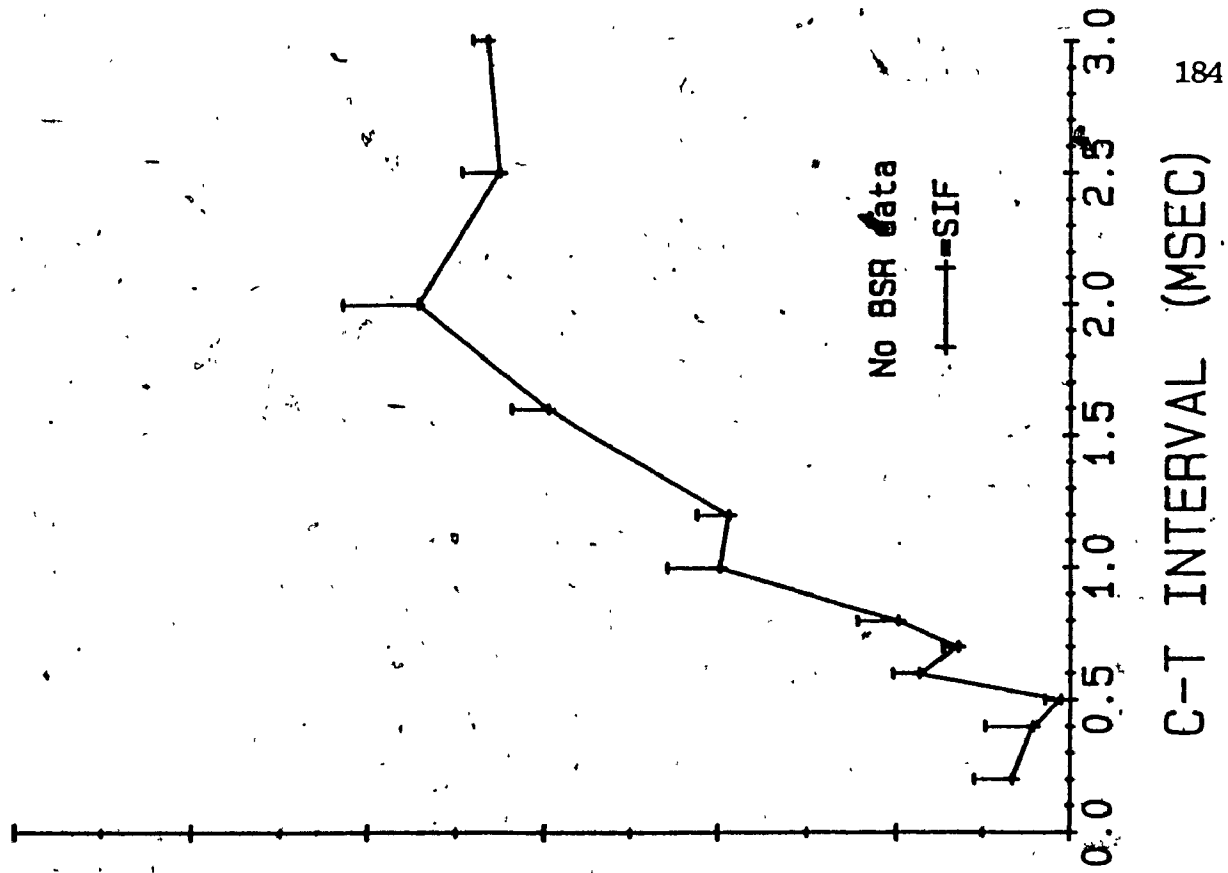
# Refractory period data AG821

Ant. electrode



# Refractory period data AG821

Post. electrode

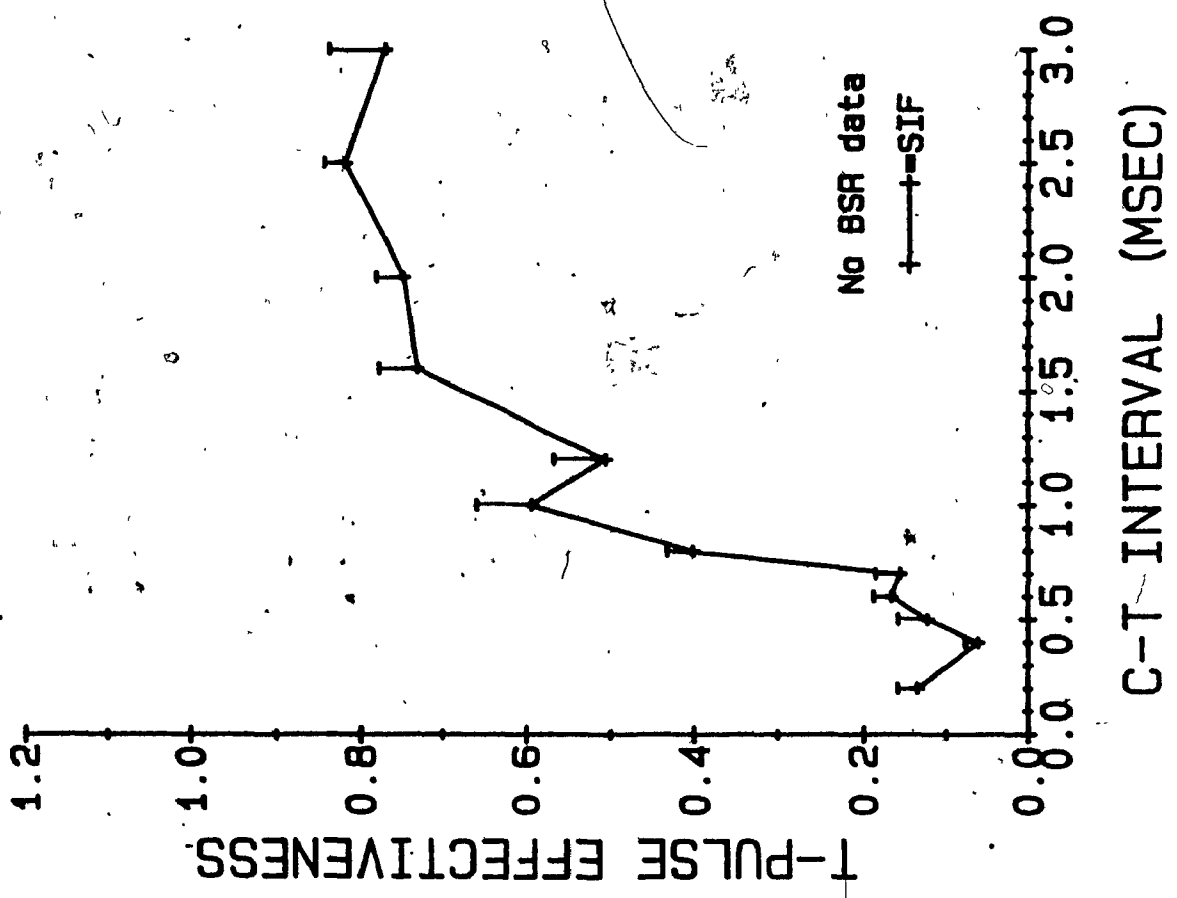


## FIGURE 12d

Refractory period data for AG812. Only the refractory  
period data for SIF at the anterior electrode site is  
available..

# Refractory period data AG812

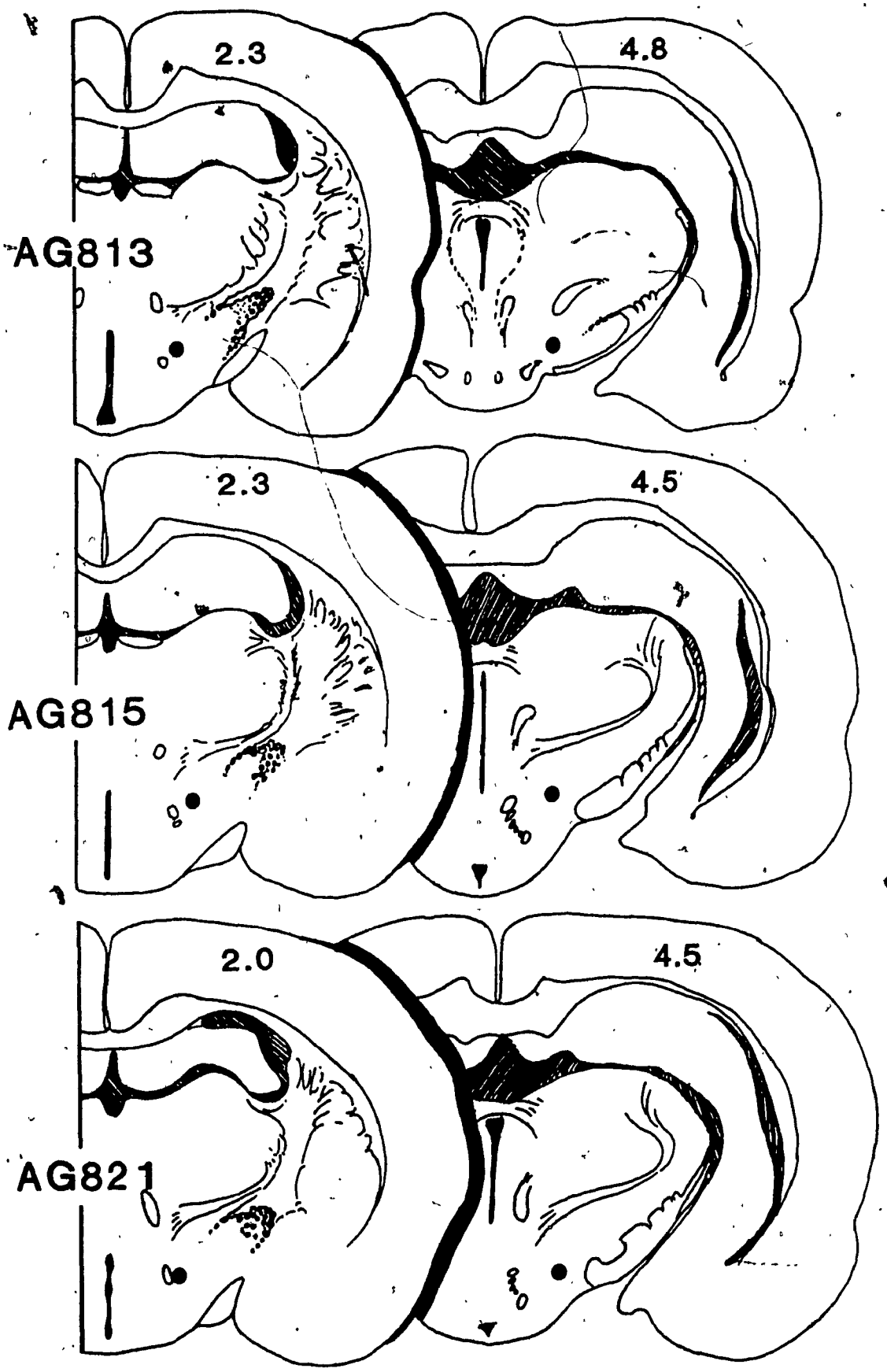
Ant. electrode  
(No post. electrode data)



## FIGURE 13

Histology for AG813, AG815 and AG821. Drawings on the right show anterior electrode positions and left drawings show posterior electrode positions. Numbers on the drawings indicate distance from bregma using the stereotaxic atlas of Paxinos and Watson (1982) as a reference.





AG813

AG815

AG821

ANTERIOR

POSTERIOR

TABLE 6

Anterior (Ant.) and posterior (Post.) electrode current intensity and collision interval, refractory period and conduction velocity estimates for SIF and BSR.

Rat	Ant. current	Post. current	Collision interval	Refractory period	Electrode distance	Conduction velocity
AG813 (SIF)	300uamps	200uamps	1.5 msec	0.4 msec	2.5 mm	2.27 m/sec
AG813 (BSR)	300	200	1.5	0.4	2.5	2.27
AG815 (SIF)	400	600	1.2	0.4	2.2	2.75
AG815 (BSR)	400	600	1.0	0.4	2.2	3.66
AG821 (SIF)	400	250	1.5	0.5	2.5	2.5
AG812 (SIF)	500	400	1.0	0.4	2.6	4.3

Note: Conduction velocity estimate for AG812 is based on partial collision and refractory period data and without histological confirmation of inter-electrode distance.

BSR-relevant fibers obtained in the present study are within the range of conduction velocity estimates (1.0-7.8 m/sec) for BSR-relevant fibers reported by Shizgal et al. (1980) and Bielajew and Shizgal (1982). The range of conduction velocity estimates for SIF-relevant fibers obtained in the present study (2.27 to 4.3 m/sec) are compatible with those of fine myelinated fibers in the 0.5 to 2.0 micrometer diameter range (Swadlow & Waxman, 1976; Waxman & Swadlow, 1977) which have been shown to be present in the lateral hypothalamic IFB (Szabo et al., 1974).

While the present data do suggest that the same SIF-relevant fibers link anterior and posterior sites that support stimulation induced feeding there are certain aspects of the collision data that merit further discussion. First, TPE values in the collision test for SIF (and BSR) in rat AG813 rise from minimal values to maximal values within 0.5 msec. This abrupt increase in TPE values suggests that all the impulses carried by fibers that course through the two stimulation fields, as well as the region of refractoriness that trails behind them, have cleared the downstream electrode before the T-pulse is applied. This is somewhat surprising considering that the estimated rate of recovery from refractoriness of SIF-relevant fiber extends over 1.0 msec. One would expect that the different rates of recovery from refractoriness of SIF-relevant fibers should be reflected in the collision test by more gradual increases in TPE. In this respect the collision data for BSR obtained from AG815 are more readily predicted on the basis of

the refractory period data than is any other collision effect reported in the present study or those reported by others.

One possible interpretation for the abrupt and complete increases of TPE values observed in the present investigation has been put forward by Shizgal et al. (1980) to explain the steep rise in TPE values in their own collision data for BSR. It may be that that only a portion of the feeding-relevant fibers, with a narrow distribution of refractory periods, is common to both stimulation fields. Such an explanation is consistent with the fact that, in three rats, complete blockade by the T-pulse was not achieved, as evidenced by the non-zero TPE values at C-T intervals in the 0.2 to 1.0 msec range. The fact that the T-pulses were effective in this range of C-T intervals suggests that the impulses carried by SIF-relevant fibers that were activated only by the C-pulse, summated spatially with the impulses carried by SIF-relevant fibers that were activated only by the T-pulse. This suggests that only part of the bundle of SIF-relevant fibers was activated by both the C- and T-pulses. This explanation does not appear to explain, however, the SIF collision data for AG813. Although the variability of the data is greater than usual, there does not appear to be any evidence of spatial summation in this animal. The abrupt rise of TPE values from near-zero TPE values in the span of 0.5 msec suggests that the application of T-pulses resulted in complete blockade in most if not all of the fibers activated by the two electrodes. In view of the fact that the current intensities used in AG813 were

low by collision experiment standards, one must also assume that the two electrodes were very closely aligned and that the fibers coursing through both stimulation fields were very homogeneous.

Electrode alignment was, in the present experiment, the determining factor for obtaining collision effects. One way of compensating for electrode misalignment is to increase the current intensity at either one or both electrode sites. By increasing the current spread, the probability of recruiting fibers that are common to both stimulation fields increases accordingly. This procedure, however, is of limited use when testing for connectivity between two feeding sites. Stimulation induced feeding is easily disrupted by competing aversive and motoric effects that invariably emerge when the current intensity is increased. Therefore unless the two electrodes are reasonably well aligned, the probability of obtaining a collision effect for SIF cannot be expected to be dramatically increased by raising the current intensity given the limited upper range of current intensities that can be used to elicit SIF.

Establishing connectivity between two SIF sites is also made difficult by the fact that most animals exhibit strong electrode preferences, to a point where some animals will "refuse" to feed in response to stimulation applied through the less preferred electrode. Under these conditions, single pulse frequency threshold determinations at the less preferred electrode site are not stable and therefore require more

replications to obtain reliable measurements. Since the animals respond reliably at the less preferred electrode site when they are not previously tested at the preferred electrode site, the problem appears to be a contrast effect between the quality of the stimulation experience at the preferred electrode site as opposed to that on the less preferred electrode. This problem was acute in AG815 and in AG812. The strategy employed in the present study to circumvent these difficulties was to obtain several single pulse frequency threshold determinations on the less preferred electrode before the testing session proper and to avoid using the less preferred electrode during the testing session except to obtain paired pulse frequency threshold determinations. Although this procedure decreases the precision of T-pulse effectiveness measures by increasing the within-session variability of T-pulse effectiveness values, it is more than made up by the increased within-trial stability of feeding latency scores.

## GENERAL DISCUSSION

The purpose of the studies described in the present thesis was to further document and compare the electrophysiological and anatomical characteristics of the MFB first stage system for stimulation induced feeding to those of the MFB first stage system for brain stimulation reward. The results of three experiments were presented.

The first experiment showed (a) that a feeding response is obtained by electrical stimulation of MFB sites extending between the anterior region of the lateral hypothalamic area and the ventral tegmental area, (b) that the dorsal-ventral distribution of sites which support SIF and BSR are co-extensive and that the relative sensitivities of each site to the rewarding and motivating effects of the stimulation are closely correlated, (c) that refractory period distributions for SIF and BSR are, in most instances similar and (d) that refractory period functions for SIF and BSR both exhibit a short segment of the ascending portion of the curve over which there is a near-zero increase in TPE values.

The second experiment showed (a) that central cholinergic blockade by systemic atropine sulfate decreased T-pulse effectiveness for BSR at C-T intervals ranging from 0.5 msec to 0.7 msec and (b) that central dopaminergic blockade by systemic

pimozide has no differential effect on T-pulse effectiveness relative to C-pulse effectiveness in the range of C-T intervals tested.

The third experiment showed (a) that the collision effect first reported by Shizgal et al. (1980) between lateral hypothalamic and ventral tegmental BSR sites can also be obtained for SIF at the same stimulation sites, (b) that when a collision effect for SIF is obtained, a collision effect for BSR can also be obtained and (c) that conduction velocity estimates for SIF-relevant fibers are within the range of those of BSR-relevant fibers.

The data of Experiment 2 suggest the involvement in the reward circuitry of a population of cholinergic fibers which receive inputs from first stage BSR fibers which have refractory periods in the 0.4 to 0.7 msec range. The postulation of cholinergic follower neurons which are selectively innervated by a sub-population of first stage reward fibers rests on the assumption that the effect of atropine sulfate on the refractory period functions for BSR reflects a selective blockade of post-synaptic muscarinic receptor-coupled ionic channels. Given the mounting evidence that acetylcholine and cholinergic compounds may affect neuronal function through other, less conventional mechanisms, one must consider the existence of a cholinergic component in the reward circuitry as a tentative explanation of the present data.



Above and beyond the problems of interpreting the effects of cholinergic blockade on the distribution of refractory periods for BSR obtained in the present study, the combined use of pharmacological manipulations and trade-off techniques to elucidate the neurochemical identity of the first stage system for BSR appears to hold some promise for the future. As has been shown previously (Milner, 1976), the relation between paired pulse frequency requirements and C-T interval was insensitive to dopaminergic receptor blockade, suggesting that the technique may be able to dissociate drugs that act at the first stage reward synapse from drugs that act at two or more synapses away from the first stage system.

The straightforward interpretation of the data presented in Experiments 1 and 3 is that the reward- and feeding-relevant signals are most likely propagated by the same MFB first stage fibers. The data of both these experiments are consistent with either one of the anatomical models shown in Figure 2. Figure 2a shows a first stage system common to BSR and SIF which synapses onto a second stage system common to BSR and SIF, whereas Figure 2b depicts a common BSR and SIF first stage system which branches out to synapse on separate BSR and SIF second stage systems. The present data do not give any indication as to which one of these anatomical models is more likely. There is, however, increasing evidence indicating that other components of the reward circuitry are common to the feeding circuitry. Dopamine appears to be part

of both the reward circuitry and the feeding circuitry. While it is not established that ventral tegmental dopaminergic neurons constitute the second stage system of the reward circuitry, the fact that the dopamine receptor blocker, pimozide, attenuates SIF (Jenck et al., note 5) and BSR (Fouriez & Wise, 1976) suggests that both the feeding- and reward-relevant signals either cross a dopaminergic synapse or are modulated by a dopaminergic neuron. Furthermore SIF and BSR both appear to be mediated by endogenous opiate receptors in the ventral tegmental area; intra-VTA infusions of morphine facilitate BSR (Broekkamp et al., 1976) as well as SIF (Jenck et al., 1985). As a whole, these data suggest that the feeding- and reward-relevant signals propagated by the first stage system go on to activate a common circuitry and that the dopaminergic neurons are localized near the efferent projections of at least a portion of the first stage system.

A second, less plausible, interpretation of the data presented in Experiments 1 and 3 is that SIF and BSR are subserved by distinct MFB fiber pathways which are so similar in their membrane characteristics and so tightly interwoven as to preclude a clear dissociation by using the techniques employed in the present investigation. Although unlikely, such characteristics and such an anatomical arrangement for the MFB substrates for BSR and SIF are still possibilities.

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