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A Comparison of the Refractory Periods for Stimulation-Induced Drinking and Stimulation-Induced Feeding

Miriam Beth Noel

A Thesis

in

The Department

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ABSTRACT

A Comparison of the Refractory Periods for Stimulation-Induced Drinking and Stimulation-Induced Feeding

Miriam Beth Noel

The distribution of refractory periods for the behaviorally relevant first-stage neurons for lateral hypothalamic stimulation-induced drinking and feeding were estimated using a paired pulse procedure. The time interval between the first (C) and second (T) pulse of each pulse-pair was varied and the behavioral effectiveness of the T-pulse was evaluated with respect to a single pulse condition. The distribution of refractory periods was inferred by examining the increase in T-pulse effectiveness as a function of the increase in C-T interval. The refractory period ranges were almost identical for stimulation-induced drinking and feeding. The fastest fibers involved in each behavior recovered from refractoriness in under 0.4 msec. The slowest fibers took as long as 2.3 msec to recover. There was a relatively uniform increase in T-pulse effectiveness for C-T intervals ranging from 0.5 to 1.6 msec. Stimulation-induced drinking and feeding appear to be mediated by a common system, or by different systems having nearly identical refractory period ranges. These results are consistent with earlier findings and support conclusions that were based on less sensitive measures.

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Dedication

I dedicate this thesis to my mom and dad whose love for me is unfailing. I can only hope to be so loving in return.

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Since the time of Galen it has been recognized that the body and its functions are controlled by the brain (Robinson, 1976). It has, however, taken centuries before technology advanced enough to enable a proper investigation of the brain. By the nineteenth century electricity was understood sufficiently to use it as a means of stimulating neuronal tissue. In 1870 Fritsch and Hitzig exposed the brain of a dog and were able to induce movements of the limbs by applying electrical stimulation to the cerebral cortex (Robinson, 1976). Other methods of stimulation such as thermal and chemical stimulation were also applied to the brain, in the attempt to induce behavior. Electrical stimulation, however, has been a preferred mode of stimulation, probably because the parameters of electrical stimulation are easily controlled and quantified. It is now well documented that electrical stimulation of the brain can induce a variety of goal directed behaviors; among these are eating, drinking, fighting and copulation.

Work on brain excitation using electrical stimulation

In the 1930's and 1940's, Hess (1932,1957) was able to elicit a behavior which he called a "rage reaction" by applying electrical stimulation to the perifornical region of the cat brain. By systematically stimulating different brain regions, Hess was able to differentiate between two

distinct rage reactions. One reaction Hess termed "sham rage", while the other he called "true rage". "True rage" reactions differed from the "sham rage" reactions in that true rage was always directed towards an appropriate object. An illustration of true rage is seen in the case where a cat receives moderate levels of electrical stimulation of the diencephalon and displays aggressive behavior such as hissing and prowling in the presence of other cats. In the animal exhibiting true rage this behavior is only initiated towards subordinate cats. The most dominant animal is carefully avoided, as it is an inappropriate goal object. In the animal exhibiting sham rage the dominant animal is not recognized as being an inappropriate goal object and is therefore not avoided (Hess, 1932, 1957). Hess described the true rage reaction as being a compilation of emotional, autonomic and somatic behaviors that follow a clearly organised pattern. Hess' work convincingly demonstrated that electrical stimulation could be a useful tool for studying the mechanisms of higher motivated behavior.

Since Hess' work many researchers have investigated stimulation-induced behaviors. The medial forebrain bundle is a particularly interesting site to stimulate as a variety of behaviors can be observed when this region is activated. In 1943 Brugger (Gloor, 1954) demonstrated that animals would increase their food intake in response to electrical stimulation of the lateral hypothalamus. Greer (1955)

observed an increase in water intake in animals receiving electrical stimulation of the same area. Other researchers were able to replicate these results for both stimulation-induced feeding (Delgado & Anand, 1953; Miller, 1957; Hoebel & Teitelbaum, 1962; Margules & Olds, 1962) and stimulation-induced drinking (Andersson & McCann, 1955; Mogenson & Stevenson, 1967). Hypothalamic stimulation has also been shown to induce predatory attack (Wasman & Flynn, 1962; Roberts & Kiess, 1964), gnawing (Miller, 1957; Roberts & Carey, 1965), copulation (Caggiula & Hoebel, 1966) and a number of other species-typical biologically primitive behaviors in a wide range of animal species (Glickman & Schiff, 1967).

In the case of copulation, Caggiula and Hoebel observed that in the absence of the appropriate goal object (the receptive female), the stimulated animal displayed heightened levels of activity and exploration, but no components of copulatory behavior were seen. Futhermore, in animals stimulated in the presence of a receptive female, an organised sequence of movements, commencing with anal sniffing of the female and ending in ejaculation, was executed. If the stimulation was terminated before ejaculation occurred, then the entire sequence of movements prior to ejaculation was re-initiated on the next occurrence of effective stimulation. Thus, in the elicitation of a copulatory response, as in the true rage reaction of Hess'

cats, the behavior exhibited in response to hypothalamic stimulation required the presence of an appropriate goal object and consisted of an organized sequence of preparatory acts. Other hypothalamically induced behaviors are also comprised of orderly sequenced acts having specific goal orientations. In the case of feeding, for instance, rats will learn a maze in order to receive food accompanied by hypothalamic stimulation (Mendelson, 1966) or work to press a lever that controls delivery of hypothalamic stimulation that induces feeding (Coons, 1964). The performance of learned instrumental responses and species-typical sequences of innate acts is typical of the range of goal-directed behaviors that can be induced by electrical stimulation. The traditional view has been that independant circuitry mediates a variety of behaviors induced by hypothalamic stimulation (Glickman & Schiff, 1967; Wise, 1974).

Single or Separate Fiber Systems Mediating Stimulation-Induced Behaviors

It has been observed that animals receiving electrical stimulation of the lateral hypothalamus will display more than one behavior in response to stimulation of the same site with the same stimulation parameters (Valenstein, Cox & Kakolewski, 1968a, 1968b, 1970). Valenstein et al. (1968a) tested animals for stimulation-induced behaviors in cages

containing water, food and a wooden block. Stimulation was delivered on an intermittent schedule for as long as five days. When an animal began to eat, drink, or gnaw in response to stimulation the relevant goal object was removed. Stimulation was continued as before with stimulation parameters remaining unchanged. Valenstein et al. observed that the majority of animals began to exhibit stimulation-induced responding toward one of the two remaining objects within the next day or two. When the initially preferred goal object was returned to the test cage the animals directed about 50% of their responses to the initially preferred goal object and the other 50% of their responses to the second goal object. Due to the fact that stimulation-induced drinkers seemed equally inclined to eat or to gnaw wood, Valenstein et al. questioned the hypothesis that separate fiber pathways mediated responses such as feeding and drinking. They suggested that activation of a single hypothalamic system modifiable by learning, was responsible for several stimulation-induced behaviors (Valenstein, Cox & Kakolewski, 1968a, 1968b, 1970).

The observation that more than one behavior can be seen when the lateral hypothalamus is stimulated can also be explained by the traditional view that separate, distinct anatomically interwoven hypothalamic circuitry is involved in stimulation-induced behaviors (Glickman & Schiff, 1967; Roberts, 1969; Wise, 1974). Wise (1968) observed that while

the thresholds for stimulation-induced behaviors were affected by stimulation experience, both feeding and drinking could be observed without prolonged stimulation experience. Wise administered lateral hypothalamic electrical stimulation to rats in test cages containing both food and water. When an animal began to show either stimulation-induced drinking or stimulation-induced feeding, current thresholds for the induced behavior were obtained. In order to determine whether animals that initially drank in response to stimulation would also eat when stimulated, animals were immediately tested in boxes containing only food. Stimulation was initiated at the current intensity required to observe drinking and was increased until eating was observed. Threshold determinations were then obtained for stimulation-induced eating. Wise observed that all animals that originally drank in response to stimulation ate if stimulated at higher intensities. Similarly, all animals that originally ate in response to stimulation drank when stimulated at higher intensities. Additional stimulation experience, such as was given by Valenstein et al. (1968a), was not necessary in order to observe the second induced behavior. When animals were stimulated over the course of a ten day period, the stimulation intensities required for each induced behavior decreased progressively over the first few days.

These results imply that the circuitry involved in stimulation-induced feeding and stimulation-induced drinking is present from the start of stimulation, and does not need to be formed by extensive stimulation experience. Wise concluded that instead of the occurrence of a second stimulation-induced response being due to a neural reorganization as a result of prolonged stimulation experience, the threshold changes result from the increased sensitivity of the drive systems involved, or a decrease in conflicting responses such as fear and curiosity. This conclusion is consistent with the traditional view of involvement of separate, pre-established fiber systems in stimulation-induced behaviors.

Further evidence for separate systems is provided by Roberts and his colleagues. Roberts (1969) argued that electrical stimulation is a nonspecific input and therefore one should expect to observe nonspecific behavioral activation from this type of stimulation. Roberts, Berquist and Robinson (1969) observed that in the opossum electrical stimulation of the hypothalamus induced grooming, attack, and eating as well as several other behaviors. Using hypothalamic warming as a means of stimulation, Roberts et al. were able to selectively induce grooming, a normal heat dissipation response, from the opossum. From these results it was suggested that functionally and anatomically separate neuronal mechanisms, may well exist.

The fact that electrical stimulation is non-specific makes interpretation difficult. Selective stimulation such as thermal stimulation can conceivably help us to avoid this problem. Unfortunately, thermal stimulation is the only selective form of hypothalamic stimulation which has yet been found. A variety of methods, including pharmacological manipulations, lesion and electrophysiological techniques have thus been employed for the purpose of resolving the question of whether a single system modifiable by learning, or separate distinct yet anatomically interwoven systems are responsible for mediating stimulation-induced behaviors.

Techniques that have been used in an attempt to resolve whether different stimulation-induced behaviors are mediated by a single or by multiple neural systems

Pharmacological manipulations

In an attempt to selectively activate hypothalamic motivational systems, Grossman (1960,1962) reported that application of adrenergic agonists to the lateral hypothalamus induced animals to eat but not drink. Cholinergic agonists applied directly to the same site induced drinking but not eating. Moreover, cholinergic and adrenergic antagonists selectively decreased drinking and eating respectively. Grossman concluded that lateral

hypothalamic feeding and drinking were sensitive to different chemicals.

However, Fisher and Coury (1962) observed that cholinergic stimulation of areas other than the lateral hypothalamus also induced drinking. This observation raised the possibility that chemical stimulation was not acting at the level of the lateral hypothalamus. Routtenberg (1967, 1972) argued that carbachol applied to the lateral hypothalamic area was diffusing to other brain regions via the ventricles. Later, Simpson and Routtenberg (1972) demonstrated that the site of action of carbachol was probably in or near the subfornical region. Although feeding and drinking appeared to be differentiable on chemical grounds, it was not clear that the differentiation occurred at the level of the lateral hypothalamus. Thus, the use of chemical stimulation failed to provide the means for differentiating between the hypothalamic segments of the neural circuitry responsible for feeding and drinking.

Lesions techniques

Following electrolytic lesions to the lateral hypothalamus, some researchers have reported specific deficits for drinking (Montemurro & Stevenson, 1957). Other researchers report that some animals show only aphagia and others only adipsia following ablations of the lateral hypothalamus (Teitelbaum & Stellar, 1954). The locus of lesions for these specific deficits, however, has been

inconsistent. Generally animals demonstrate both adipsia and aphagia following lesions to the lateral hypothalamus (Smith & McCann, 1962; Epstein, 1971). Although lesion studies provide some evidence for a differentiation between the anatomical location of brain regions which when stimulated result in drinking or feeding, no other information about the behaviorally relevant neurons is gleaned through the use of these techniques. Furthermore, since all neurons within the area of the lesion are destroyed or their functions impaired, it is impossible to distinguish between neurons that contribute to feeding or drinking and those that do not.

Since feeding and drinking are different behaviors they must be different at some level of the neural circuitry. To determine if these behaviors differ at the level of the first-stage neurons, techniques other than chemical stimulation and lesions have been required.

Electrophysiological techniques

While it might be preferable to use more selective stimulation (if it could be developed), it is possible, to acquire some information about the physiological properties of medial forebrain bundle motivational systems by more detailed studies of electrical stimulation. For example, Mogenson, Gentil and Stevenson (1971) attempted to differentiate the behaviorally relevant neurons for stimulation-induced drinking and feeding by comparing the

relative effects of different stimulation frequencies in the two behaviors. They found that high frequency stimulation appeared to preferentially induce drinking while low frequency stimulation appeared to preferentially induce feeding. Based on this observation Mogenson et al. concluded that induced feeding and drinking were mediated by different classes of medial forebrain bundle fibers. Although Mogenson et al.'s findings lead us to suspect that there may be some difference between the neurons that mediate induced drinking and those mediating induced feeding, their data do not clearly establish what that difference may be.

One characteristic that can distinguish systems with high and low frequency specificity is the excitability cycle of different classes of neurons. When a neuron is activated an action potential is generated and the neuron is left in a brief state of refractoriness. The duration of this period of refractoriness can be estimated by measuring the minimum amount of time after one action potential before a second action potential can be generated. Conventional unit recording techniques measure the refractory periods of single neurons by using a paired-pulse procedure. The first pulse of the pulse pair is called the conditioning (C) pulse and the second pulse the test (T) pulse. By systematically varying the time interval between the C and T pulses, the minimum C-T interval can be found which allows the T-pulse

to activate the neuron successfully. This minimum period is the refractory period which determines the maximum rate of stimulation that a given neuron or class of neurons can follow.

When large electrodes are used to stimulate the brain, many neurons are stimulated at once. It therefore becomes necessary to generalize from the post-stimulation excitability cycle of a single neuron to the situation where many neurons are activated by the stimulation. The same fundamental approach using trains of pulse pairs rather than single pulse pairs, can be used to characterize the refractory periods of populations of directly activated fibers.

Deutsch (1964) was the first to apply the paired-pulse procedure to behavioral experiments. He wanted to evaluate the refractory periods of neurons mediating brain stimulation reward. Using currents that would activate a multitude of local fibers, Deutsch delivered trains of pulse pairs that would activate each local neuron many times in succession. Deutsch observed that as the time between the C-pulse and T-pulse increased, there was an increase in the rate of an animal's responding for the stimulation. When Deutsch plotted the rate of responding against the C-T interval, he found a sigmoidal relationship. By calculating the initial point of rise of the ogive, Deutsch reasoned that he could estimate when the fastest neurons recovered

from refractoriness from the C-pulse and were able to be activated by the T-pulse. Deutsch further argued that at the point when the sigmoid levelled off, all of the relevant neurons were activated by both the C and the T pulses in each pulse pair. Deutsch was therefore able to obtain an estimate for the range and distribution of the refractory periods for the behaviorally relevant neurons involved in brain stimulation reward. Because the C-T intervals over which response rate continued to increase ranged from 0.5 to 1.1 msec, Deutsch reasoned that a population of directly activated neurons with a range of refractory periods must be activated by the stimulation. Since peripheral neurons having varying diameters had previously been shown to be differentially excitable (Erlanger & Gasser, 1937), it was not unreasonable to expect similar results from central neurons. Thus, Deutsch reasoned that medial forebrain bundle neurons with varying diameters must contribute to brain stimulation reward.

Refractory period estimates can, however, be biased by the choice of parameters used to obtain a behavioral response (Yeomans, 1975). To circumvent this problem, Yeomans (1975) proposed a scaling procedure such that the number of pulse-pairs required to maintain a constant level of behavior is traded off against C-T interval. At short C-T intervals the T-pulse generates fewer action potentials than the C-pulse because most neurons are still refractory

at the time the T-pulse is delivered. In this case, pulses or pulse-pairs must be added to maintain a constant level of behavior. As the C-T interval increases, however, the T-pulse activates more and more action potentials and fewer pulse-pairs are needed to maintain a constant number of action potentials in the circuit and thus, a constant behavioral output. With long enough C-T intervals, all neurons will be activated by both the C and the T pulses. When the T-pulses are completely effective, the number of pulses required to maintain a constant behavioral output will be identical for both the single pulse and pulse-pair conditions. For example, if forty single pulses are needed to maintain a constant behavior, then twenty pulse-pairs will be required. When the effectiveness of the T-pulse is plotted against C-T interval the resulting function is an ogive and is similar in shape to a refractory period curve collected by electrophysiological recording from nerve or fiber bundles (Yeomans, 1975).

Using Deutsch's paired-pulse procedure several investigators have attempted to characterize the distribution of refractory periods for the first-stage neurons mediating stimulation-induced drinking and stimulation-induced feeding. One might expect the distributions to differ if the mechanisms of stimulation-induced feeding and stimulation-induced drinking have different frequency sensitivities. Hu (1973), Rolls (1973),

and Hawkins and Chang (1974) obtained estimates of the refractory periods of neurons mediating these behaviors, however, their estimates were obtained without the benefits of Yeomans' (1975) improvements and such estimates are no longer considered reliable. Halboth and Coons (1973) also estimated the distribution of refractory periods for neurons involved in stimulation-induced feeding. These investigators measured the current required to maintain a constant level of behavior. While threshold measures are better measures than rate measures, current threshold procedures involve variations in testing current that are associated with varying the diameters of effective stimulation. Thus, the relative contribution of different neurons to a given behavior is not held constant. Drawing comparisons between behaviors that have different current thresholds is therefore invalid, since different neurons may be contributing to the two behaviors. Another disadvantage with current thresholds is that it is difficult to gauge the number of neurons being activated as a result of a given increase in current intensity. It is preferable to obtain frequency thresholds with a fixed current intensity. This procedure allows the participating set of neurons to remain fixed. Comparisons between behaviors is further facilitated by the knowledge that a 10% change in stimulation frequency should cause a 10% change in the number of action potentials generated.

To characterize the distribution of refractory periods of the behaviorally relevant neurons for feeding induced by stimulating the lateral hypothalamus, both Hawkins, Roll, Puerto and Yeomans (1983) and Gratton and Wise (1988) have subsequently used Yeomans' (1975) pulse-pair procedure, determining refractory period estimates from frequency thresholds to maintain a constant level of behavior. Hawkins et al. (1983) estimated the range of relevant refractory periods to be between 0.4 and 2.0 msec, while Gratton and Wise (1988) estimated them to be between 0.4 and 2.5 msec. Gratton and Wise (1988) further observed that the rate of recovery of fibers from refractoriness increased steadily between the C-T intervals of 0.4 and 0.6 msec and between the C-T intervals of 0.7 and 2.5 msec, but that there was no increase in T-pulse effectiveness between the C-T intervals of 0.6 and 0.7 msec. They suggested that two non-overlapping sub-populations might contribute to stimulation-induced feeding.

Present Investigation

The present investigation was aimed at describing the refractory period distribution of neurons involved in stimulation-induced drinking using a variant of Yeomans (1975) paired-pulse procedure. It was of primary interest to compare the refractory period distributions thus derived with similar distributions for stimulation-induced feeding.

Refractory period distributions for each behavior from each animal were collected, so that within subject comparisons could be made and between subject variations in electrode placement could be avoided.

METHODS

Animals and surgery

The subjects were male Long Evans rats weighing approximately 400 grams at time of surgery. The animals were individually housed and had ad libitum access to food and water. A 12 hour light cycle was maintained. Each rat was anesthetized with sodium pentobarbitol (60 mg/kg) and implanted with either a fixed or a moveable monopolar electrode aimed at the lateral hypothalamic area (DeGroot plane: A.P.=0.8 mm behind bregma, Lateral=1.2 mm, Dorsal-Ventral=8.2 mm for fixed electrodes and 7.2 mm for moveable electrodes). Electrodes were constructed from stainless steel wire, 254 microns in diameter, and were insulated with baked varnish. The tip of the electrode was cut and polished so that it was hemispherical. Fixed electrodes were made by crimping male Amphenol pins to the electrode. Moveable electrodes were constructed by soldering the electrode to a male Amphenol pin, the top of which had been threaded with a 2-56 die. The moveable electrode was

screwed into a piece of nylon which had been threaded with a 2-56 tap. The nylon served as a holder and anchored the moveable electrode to the skull. A complete turn of the moveable electrode resulted in a 0.454 mm vertical movement of the electrode. An Amphenol pin soldered to a stainless steel wire was attached to four stainless steel screws embedded in the skull--two anterior to bregma and two posterior to lambda--and served as the current return. The entire electrode assembly was held together by dental cement.

Apparatus

A constant current generator controlled the pulse amplitude and a digital pulse generator controlled the temporal parameters of the stimulation (Mundl,1980). Stimulation consisted of rectangular pulses 0.1 msec in width. Current intensity was monitored on an oscilloscope by reading the voltage drop across a 1 kohm resistor that was in series with the rat. The stimulation was delivered through a flexible wire attached to a mercury commutator. This allowed for free movement of the rat.

Test boxes were wooden except for a plexiglass front and measured 15 x 15 x 10 inches. For drinking tests, the box contained four water bottles, one in each corner of the box. The spouts of the water bottles were positioned six inches from the floor of the box and projected an inch into the

box. Water was always fresh. The testing box for feeding was the same as that for the drinking tests except that the floor of the box was covered with 45 mg food pellets and the water bottles were no longer present.

Screening

Each rat was given one week to recover from surgery and then tested for stimulation induced drinking. Initial stimulation began at very low current intensities--25 to 50 uamps, and moderate frequency ranges--20 to 40 hz. Stimulation was given in 30 second trains with 30 second inter-train intervals, and lasted for approximately 30 minutes. Current intensity was raised until the rat displayed forward locomotion and sniffing behavior, or until aversive reactions to the stimulation were exhibited. In the latter case, for animals that had moveable electrodes, the electrode was lowered 0.227 mm; testing at the new electrode position commenced two days later. If the animal had a fixed electrode, the animal was eliminated from the experiment. When forward locomotion and sniffing were seen in response to stimulation, stimulation testing was continued until stimulation-induced drinking was observed or until two weeks had passed, at which point testing ceased and the animal was eliminated from the experiment. When stimulation-induced drinking was observed, the rat was tested over several consecutive sessions until the

stimulation-induced drinking was consistent. This process, termed stabilization, consisted in keeping the current intensity constant and varying the frequency by 0.02 log steps in either a descending or ascending fashion. The animal's behavior was considered stable when the stimulation-induced behavior began and terminated at frequencies that did not differ by more than 0.04 log steps from session to session. Stimulation-induced drinking was considered to have occurred if the animal displayed drinking without interruption for at least four seconds with a latency of no longer than 30 seconds. Once stimulation-induced drinking was stable for a period of at least five days, refractory period testing was initiated.

Once the refractory period testing for stimulation-induced drinking had been completed, animals were screened for stimulation-induced feeding behavior. For each animal, the current intensity was identical to that used in the drinking tests, and the stimulation frequency was varied. Feeding was considered to have occurred when an animal ate two food pellets during stimulation, and when feeding ceased abruptly at the stimulation offset. Feeding was considered to be reliable when the frequency to begin and terminate feeding did not differ by more than 0.04 log units from session to session. As with stimulation-induced drinking, electrical stimulation was given in 30 second trains with 30 second inter-train intervals, and lasted approximately 30

minutes. All animals that drank in response to electrical stimulation also displayed stimulation-induced feeding. Once feeding was considered to be reliable, refractory period testing was initiated.

Refractory period tests

Refractory period tests were conducted first for stimulation-induced drinking, however, the same procedure was carried out for both stimulation-induced drinking and stimulation-induced feeding. A testing session always began by letting the animal satiate for 20 minutes with the test substance. Following this, trains of single pulses were delivered and frequency thresholds were determined. Next, trains of paired-pulses were delivered and the frequency threshold determinations were obtained. The frequency threshold was defined as the minimum pulse frequency at which rats would drink continuously for four seconds, or eat two food pellets with a latency of twenty seconds. Because the latency to meet criterion decreases as a function of increasing the pulse frequency, the pulse frequency determinations involve decreasing the pulse frequency from high values that produced short latency feeding or drinking to low values which induced behaviors at latencies longer than 20 seconds. The frequency threshold was therefore derived from the latency-frequency function by graphical extrapolation.

Eighteen different paired-pulse conditions were tested. The time between the delivery of the first pulse (called the C or conditioning pulse) and the second pulse of each pulse pair (called the T or test pulse), was varied. The C and T pulses in each train of pulses were always of equal amplitude. The time between the C-pulse and the T-pulse in each pulse pair is known as the C-T interval. Identical C-T intervals were used for stimulation-induced drinking and stimulation-induced feeding. Each session consisted in testing six to nine different C-T intervals and three to four single pulse determinations. Three C-T intervals made up a test block. Within each block, one long, one short, and one medium C-T interval was tested. Each block was preceeded by and followed by a single pulse frequency threshold determination. The C-T intervals were randomly presented over sessions and replications. A minimum of two and a maximum of four replications were obtained for each behavior. The frequency threshold of a single pulse determination that preceeded a block and the single pulse determination that followed a block were averaged and this average was used to estimate the relative effectiveness of the paired-pulse condition.

The following C-T intervals were tested: 0.2, 0.3, 0.4, 0.44, 0.5. 0.52, 0.56, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 2.0, 3.0, 4.0, 5.0 msec.

Statistical Analyses

Estimates of the T-pulse effectiveness values were calculated for every C-T interval that was tested for both stimulation-induced feeding and stimulation-induced drinking behaviors. This was done using Yeomans' (1975) formula such that TPE = (RHz SP/RHz C-T) -1. TPE is the T-pulse effectiveness value, RHz SP is the frequency threshold of the single pulses, and RHz C-T is the frequency threshold at the paired-pulse condition. The TPE is equal to zero when the T-pulse is not effective in activating any of the behaviorally relevant directly stimulated neurons in the stimulating field. The TPE is equal to one when the T-pulse is just as effective as the C-pulse in activating these neurons.

The initial and final values for the recovery from refractoriness were obtained by fitting three functions to the refractory period curves. This was done using the derivative-free non-linear regression section of the BMDP statistical analysis program. The initial portion of the curve, attributed to the exponential decay of local potential summation, is fit with an exponential function. The middle or rising portion of the curve is fit with a straight line, and a horizontal line fits the portion of the curve that is approaching an asymptote. Initial values for the y-intercept, the time constant for the exponential function, the beginning and the end of recovery from

refractoriness for the linear portion of the curve, and the asymptotic value for the T-pulse effectiveness, are roughly estimated and these estimates used as the starting parameters. The final values for the beginning and end of recovery from refractoriness are determined by minimizing the sum of least square residuals for these parameters, and then using the x co-ordinates of the end points of this best fit segment as parameters representing the values for the beginning and end of recovery.

Histology

At the completion of the experiment each animal was anesthetized with chloral hydrate and perfused intracardially with a 0.9% saline solution followed by a 10% formalin solution. The brains were immediately removed and stored in a solution of 10% formalin for a minimum of four days. Following this, brains were frozen and sliced in 40 micron sections with the aid of a microtome. Every third slice was mounted on gelatin coated slides and stained with thioneine. Histological localization of the placement of the electrode tips were recorded and reconstruction was made based on the stereotaxic atlas of Pellegrino, Pellegrino and Cushman (1979).

Results

Similar refractory period curves were obtained for stimulation-induced drinking and stimulation-induced feeding. The similarity is most clearly evident when the refractory period curves are plotted on the same graph (figures 1 to 4). For the purpose of comparing the shortest and longest refractory periods for stimulation-induced drinking and feeding, the data were fit with three functions (figures 5 to 12). Estimates of the shortest and longest values of recovery from refractoriness were obtained by determining the sum of least squares residuals for the beginning and end of recovery from refractoriness for the linear portion of the refractory period curves (figures 5 to 12). The estimated average of the initial recovery from refractoriness was 0.405 msec for stimulation-induced feeding and 0.540 msec for stimulation-induced drinking. The average of the estimated points of final recovery from refractoriness was 1.657 msec for stimulation-induced feeding, and for stimulation-induced drinking was 1.628 msec (Table 1). The unfitted curves are presented alongside the fit curves, as they allow for the observation of any unusual patterns that may occur in the refractory period curves and

that would remain undetected by the fitted analysis (figures 5 to 12).

The estimated value for the initial recovery from refractoriness appeared consistently larger for the stimulation-induced drinking curves than for the stimulation-induced feeding curves (figures 5 to 12; table 1). However, a two tailed t-test for dependant samples showed this result to be insignificant at the probability level of Ø.Ø5 (df=3). There were no significant differences found for either the initial point of recovery or the final recovery from refractoriness between the stimulation-induced drinking and stimulation-induced feeding curves.

The estimate for the final recovery from refractoriness for stimulation-induced feeding is much longer for animal L9 than it is for the other animals. Similarly the value of the final recovery from refractoriness for stimulation-induced drinking is much longer for animal W4 than it is for the other animals (figures 9 to 12,table 1). These estimates result in some skewing of the average values for the end of recovery for stimulation-induced feeding and stimulation-induced drinking.

A t-test was performed to test for differences between the average single pulse frequency thresholds (table 2) for stimulation-induced feeding and for stimulation-induced drinking. Results of the test were insignificant at the alpha level of Ø.Ø5. No test was done for stimulation

intensity as the same current levels were used for each behavior within each animal (table 2).

Upon examination of the stained brain slices the electrode tips for each animal were determined to be within the medial forebrain bundle (figure 13). Animals L9 and W4 had electrode tips that were at the ventral portion of the zona incerta and the dorsal edge of the lateral hypothalamic area. The electrode tip of animal A2 was relatively dorsal in the lateral hypothalamic area while the electrode tip for animal L1 was more in the central region of the lateral hypothalamic area.

Figure 1. The refractory period curves for stimulation-induced drinking and for stimulation-induced feeding are plotted on the same figure for animal A2. The dotted line connecting the squares is the stimulation-induced feeding curve and the solid line connecting the circles is the stimulation-induced drinking curve.

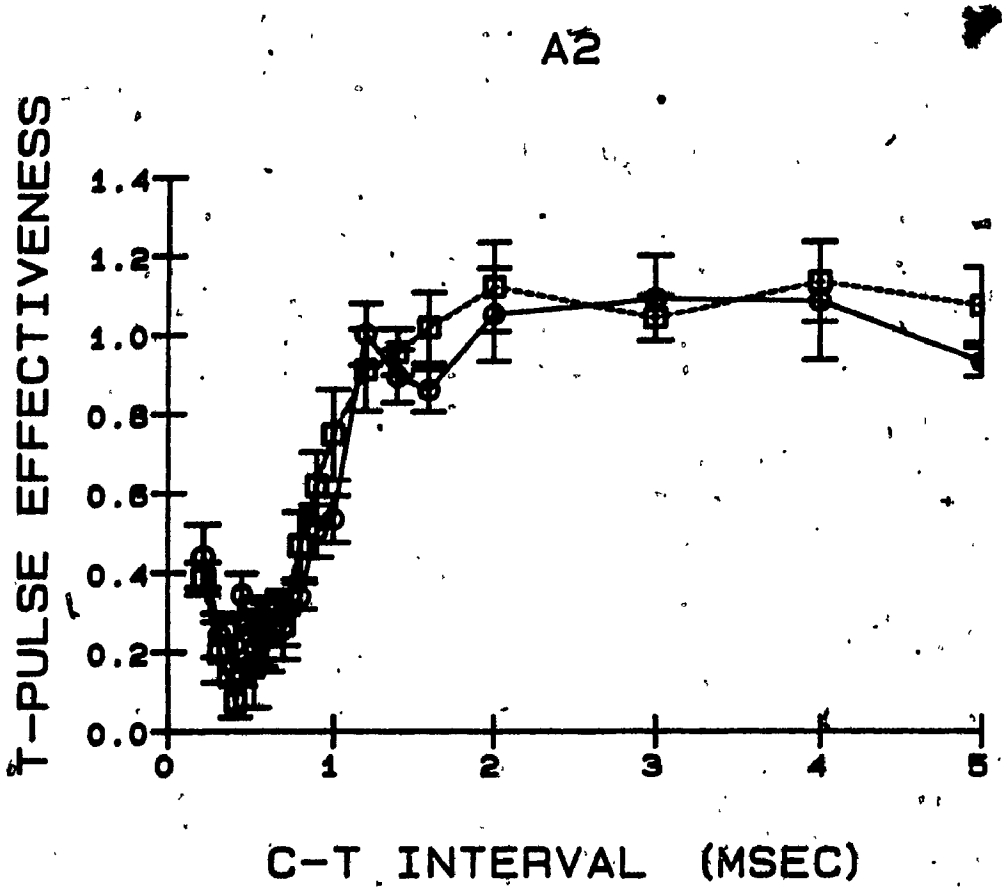
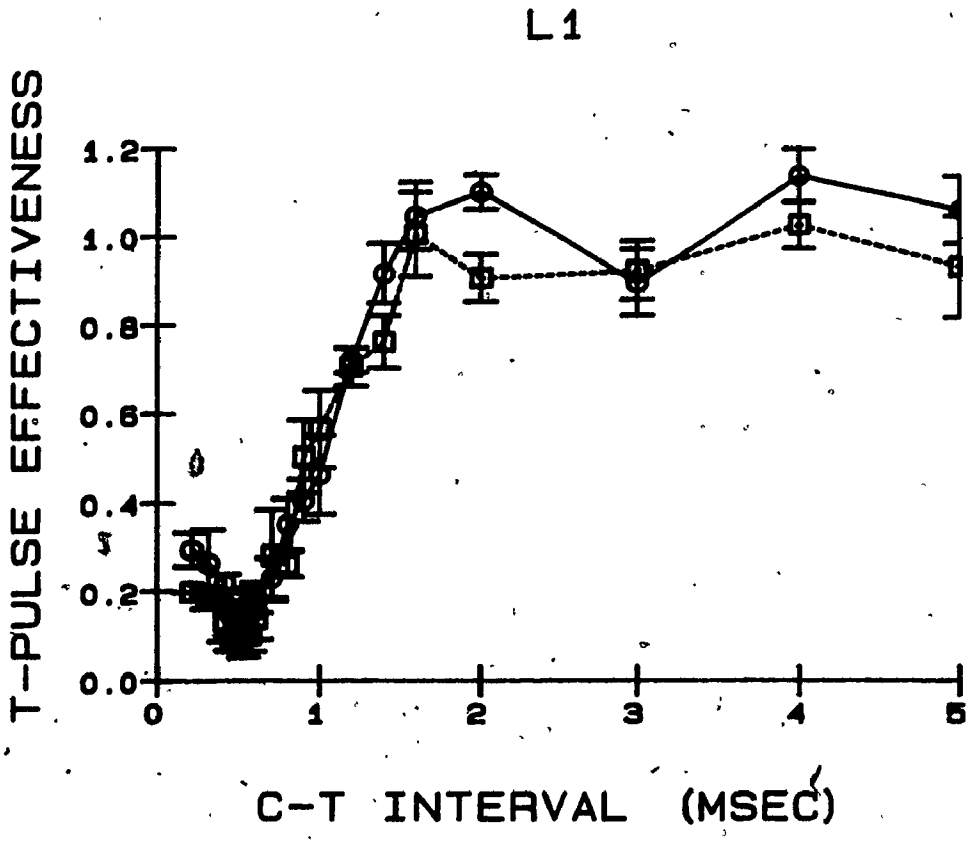

A2
T-PULSE EFFECTIVENESS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 2. The refractory period curves for stimulation-induced drinking and for stimulation-induced feeding are plotted on the same figure for animal L1. The dotted line connecting the squares is the stimulation-induced feeding curve and the solid line connecting the circles is the stimulation-induced drinking curve.


L1
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

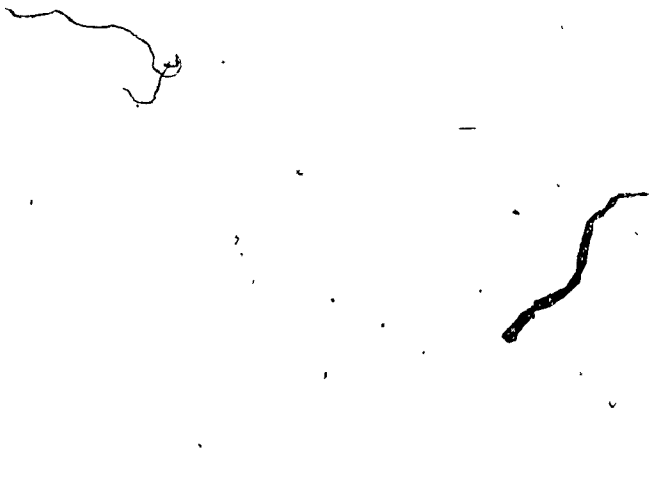


Figure 3. The refractory period curves for stimulation-induced drinking and for stimulation-induced feeding are plotted on the same figure for animal L9. The dotted line connecting the squares is the stimulation-induced feeding curve and the solid line connecting the circles is the stimulation-induced drinking curve.

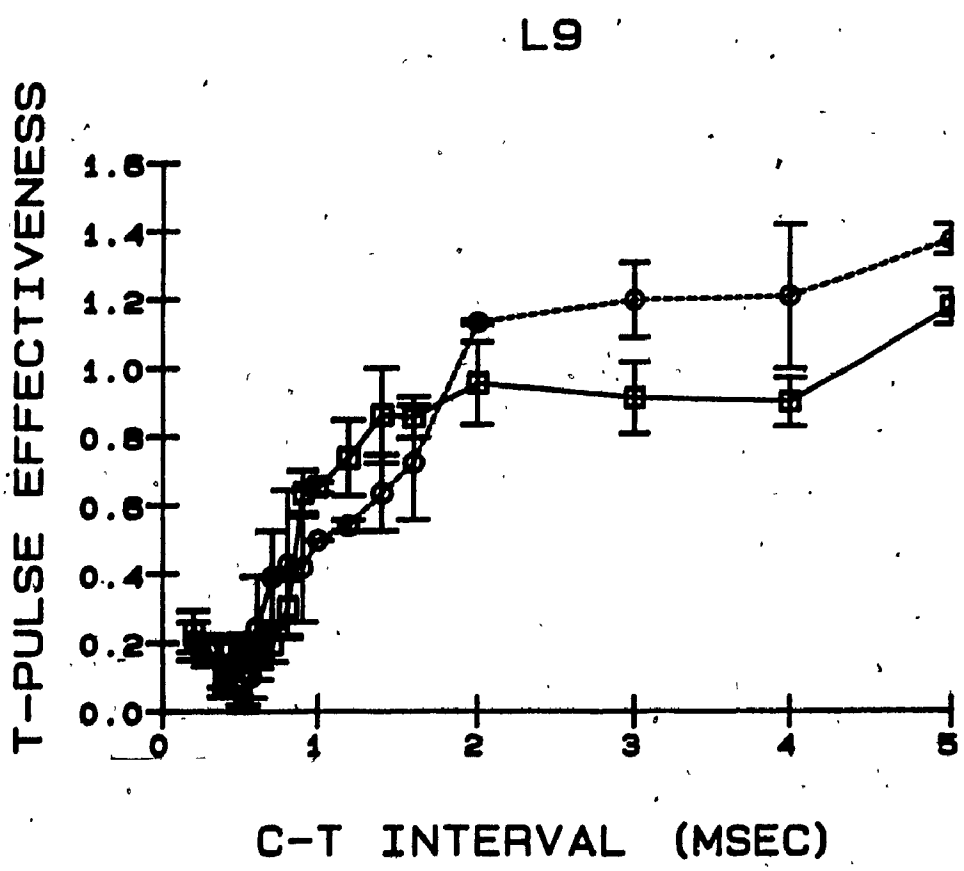

L9
T-PULSE EFFECTIVENESS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 4. The refractory period curves for stimulation-induced drinking and for stimulation-induced feeding are plotted on the same figure for animal W4. The dotted line connecting the squares is the stimulation-induced feeding curve and the solid line connecting the circles is the stimulation-induced drinking curve.

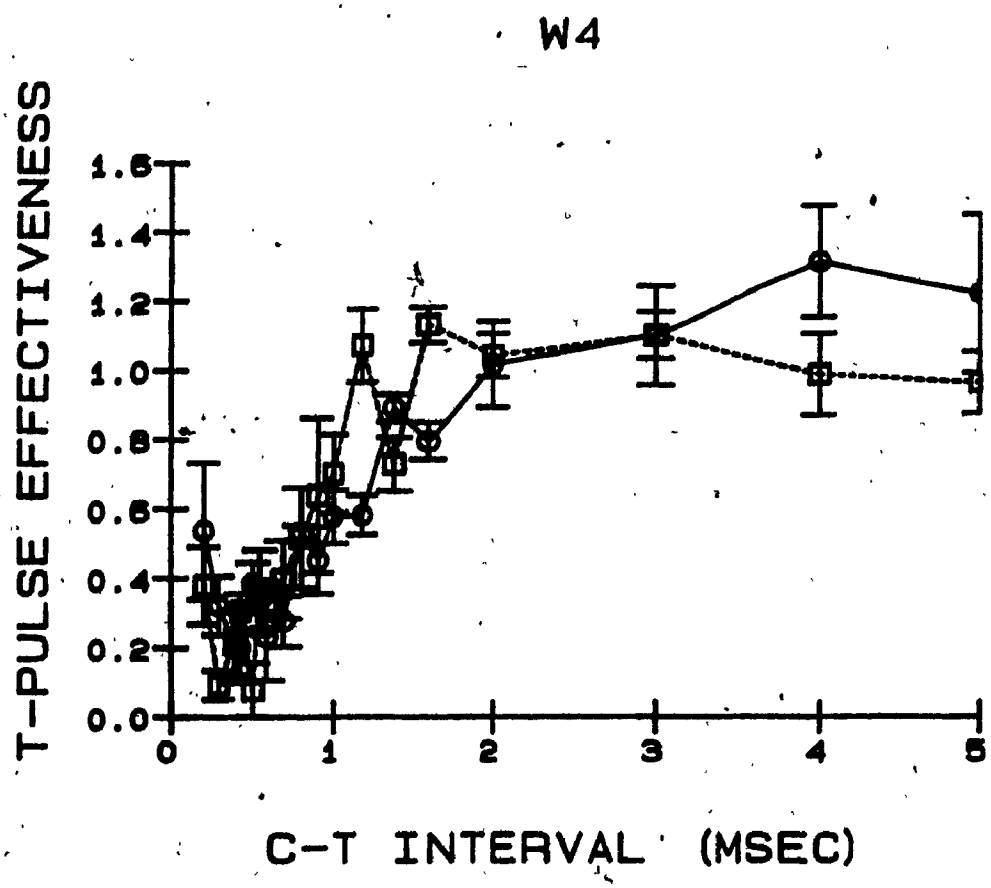

W4
T-PULSE EFFECTIVENESS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 5. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced feeding (SIF) for animal A2.

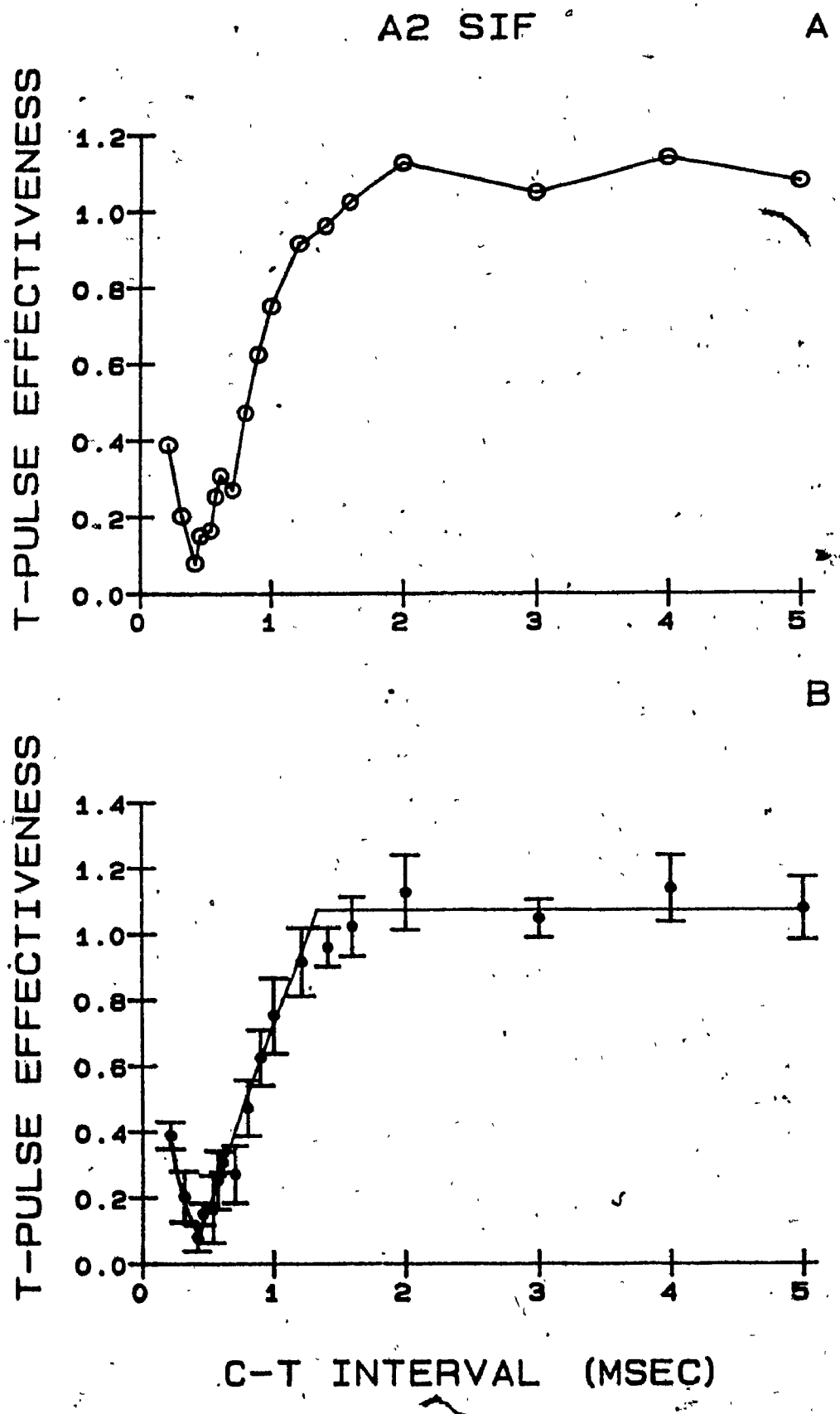

A2 SIF
A
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 6. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced drinking (SID) for animal A2.

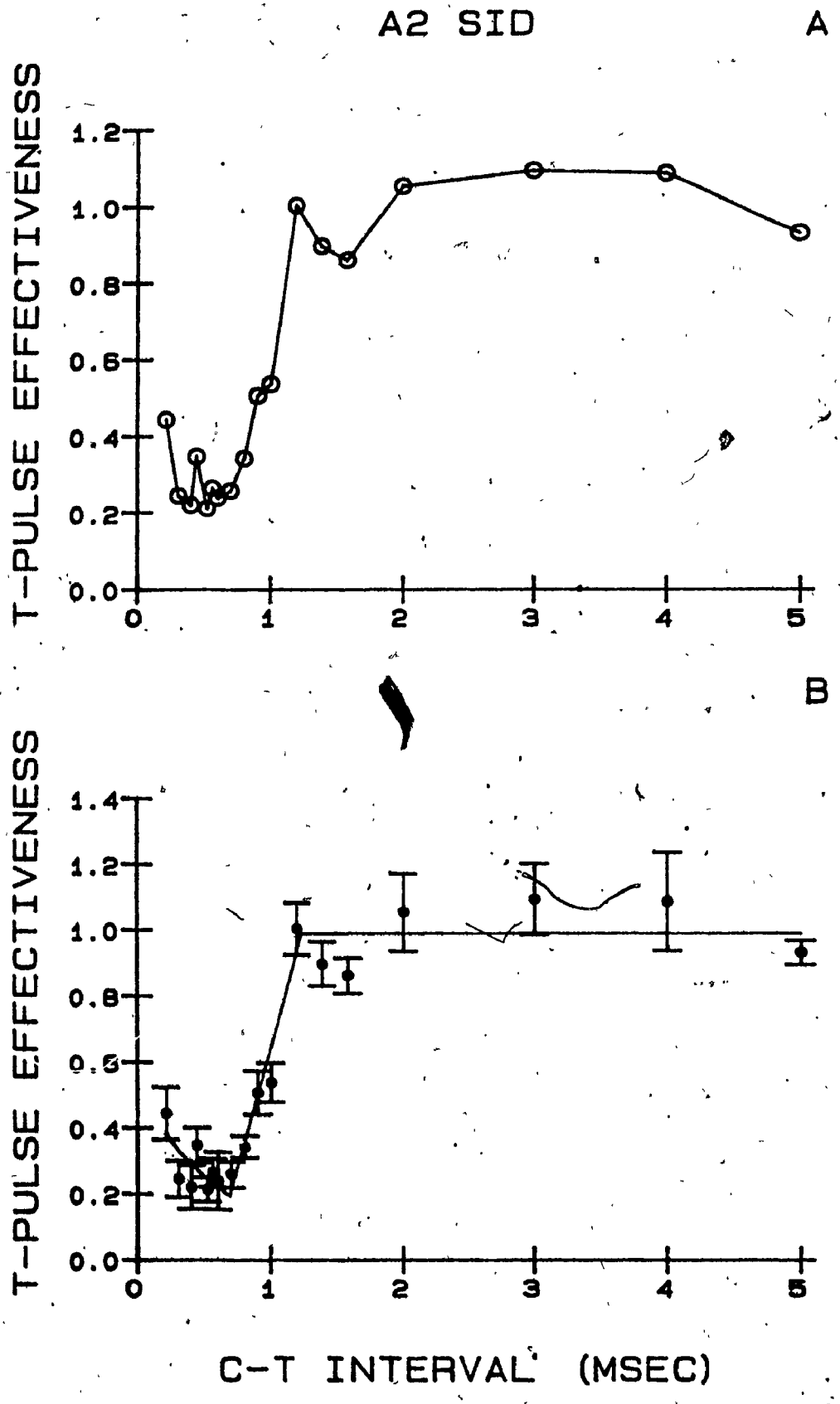

A2 SID
A
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 7. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced feeding (SIF) for animal L1.

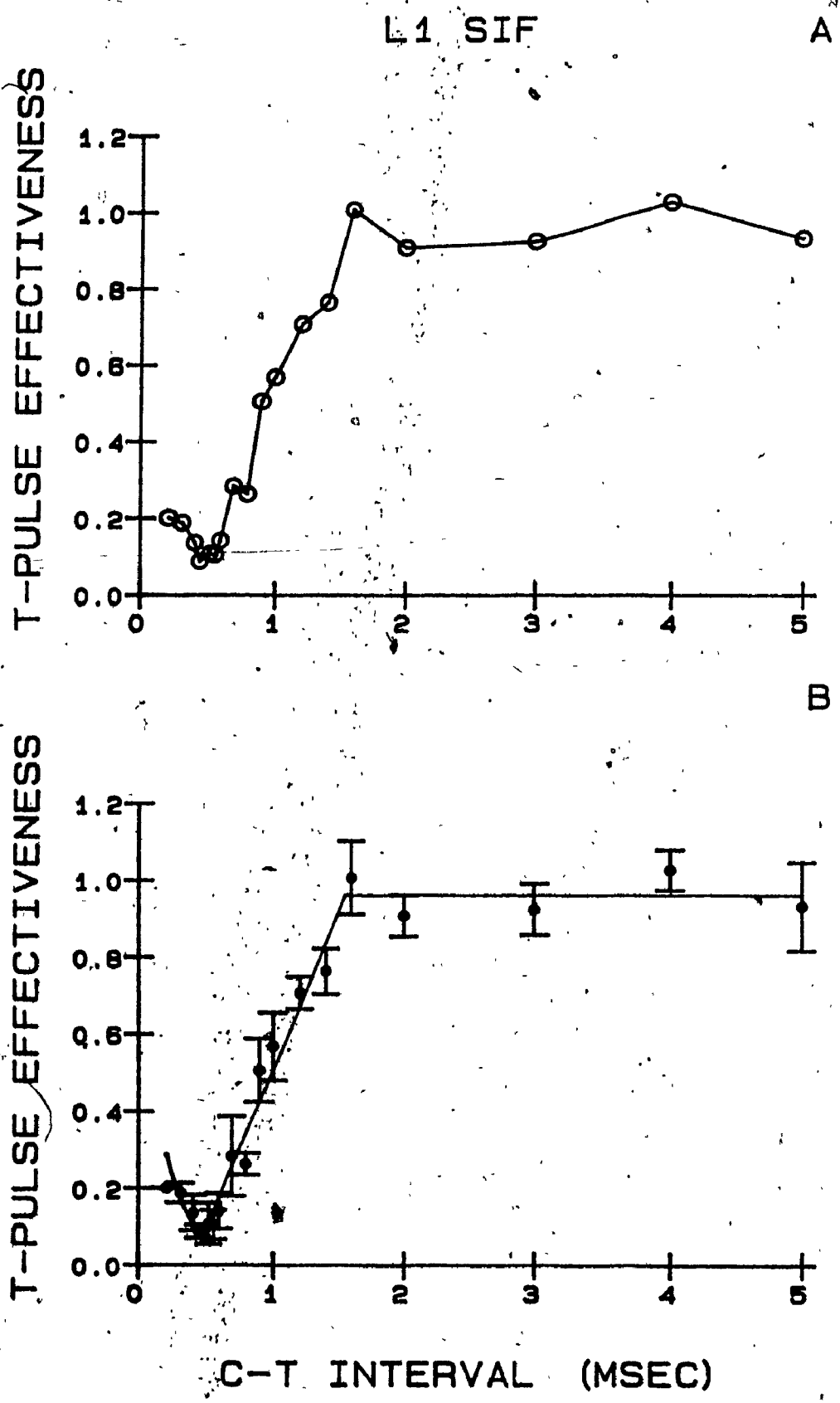

L1 SIF
A
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
C-T INTERVAL (MSEC)

Figure 8. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced drinking (SID) for animal L1.

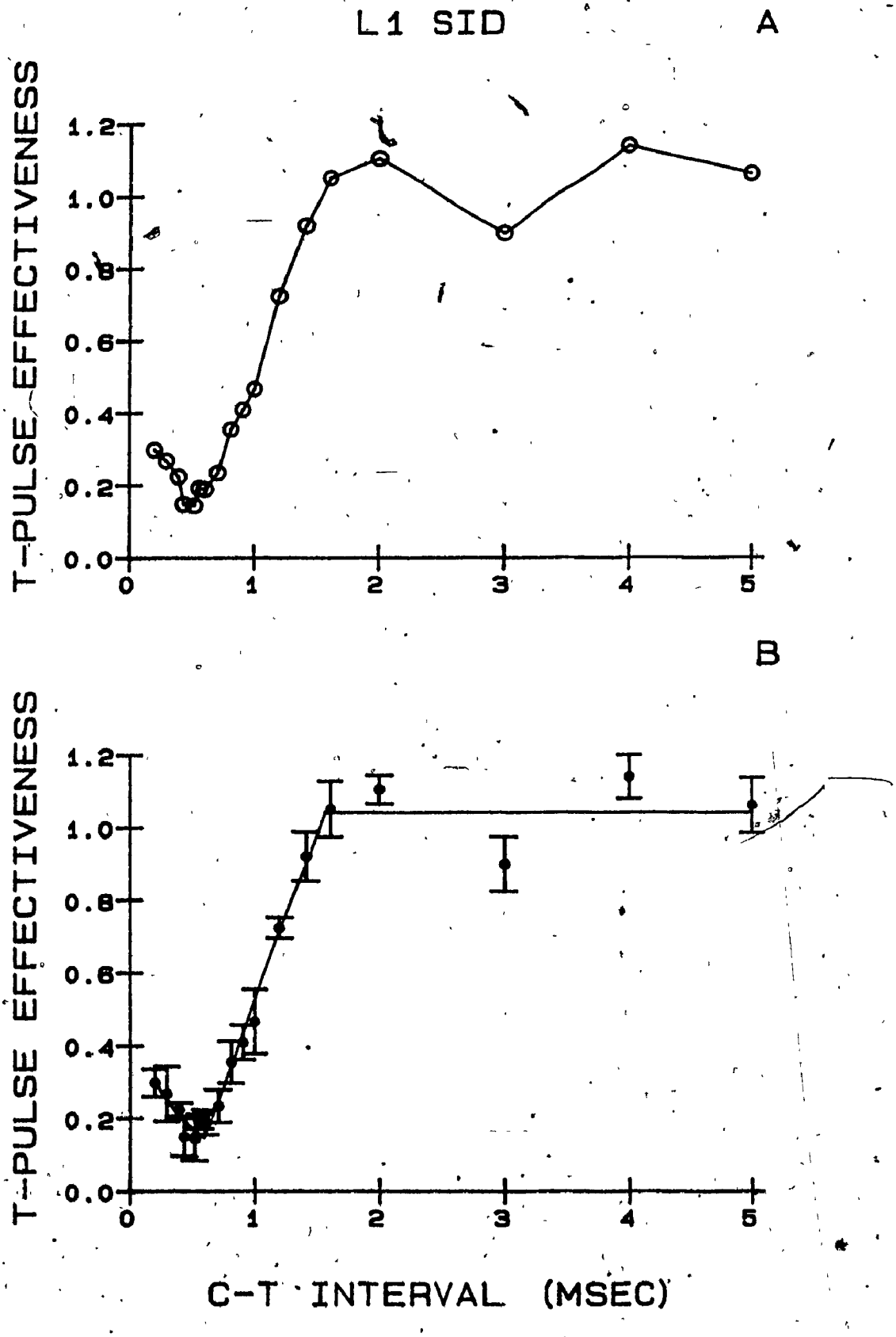

L1 SID
A
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
C-T INTERVAL (MSEC)

Figure 9. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced feeding (SIF) for animal L9.

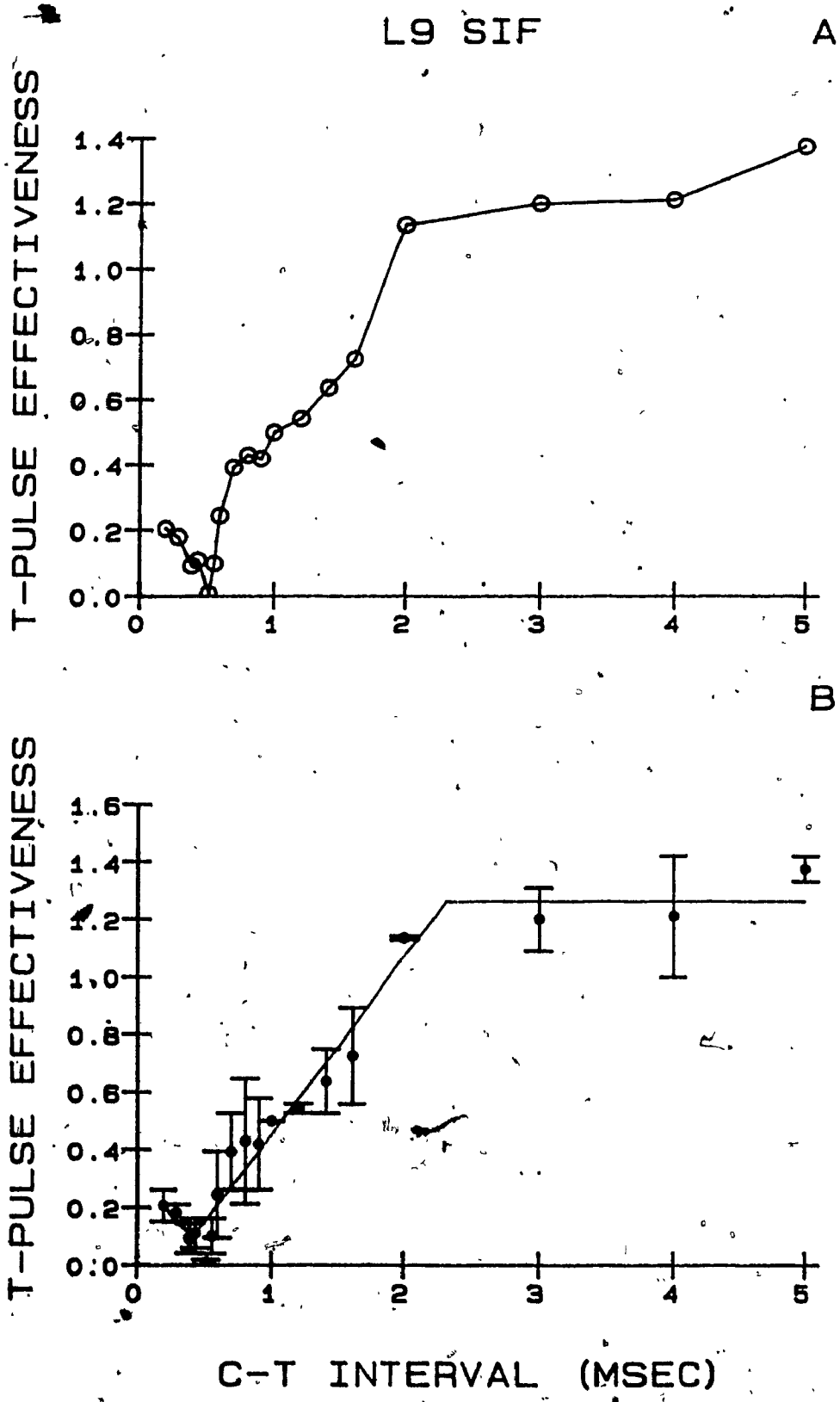

L9 SIF
A
T-PULSE EFFECTIVENESS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 10. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced drinking (SID) for animal L9.

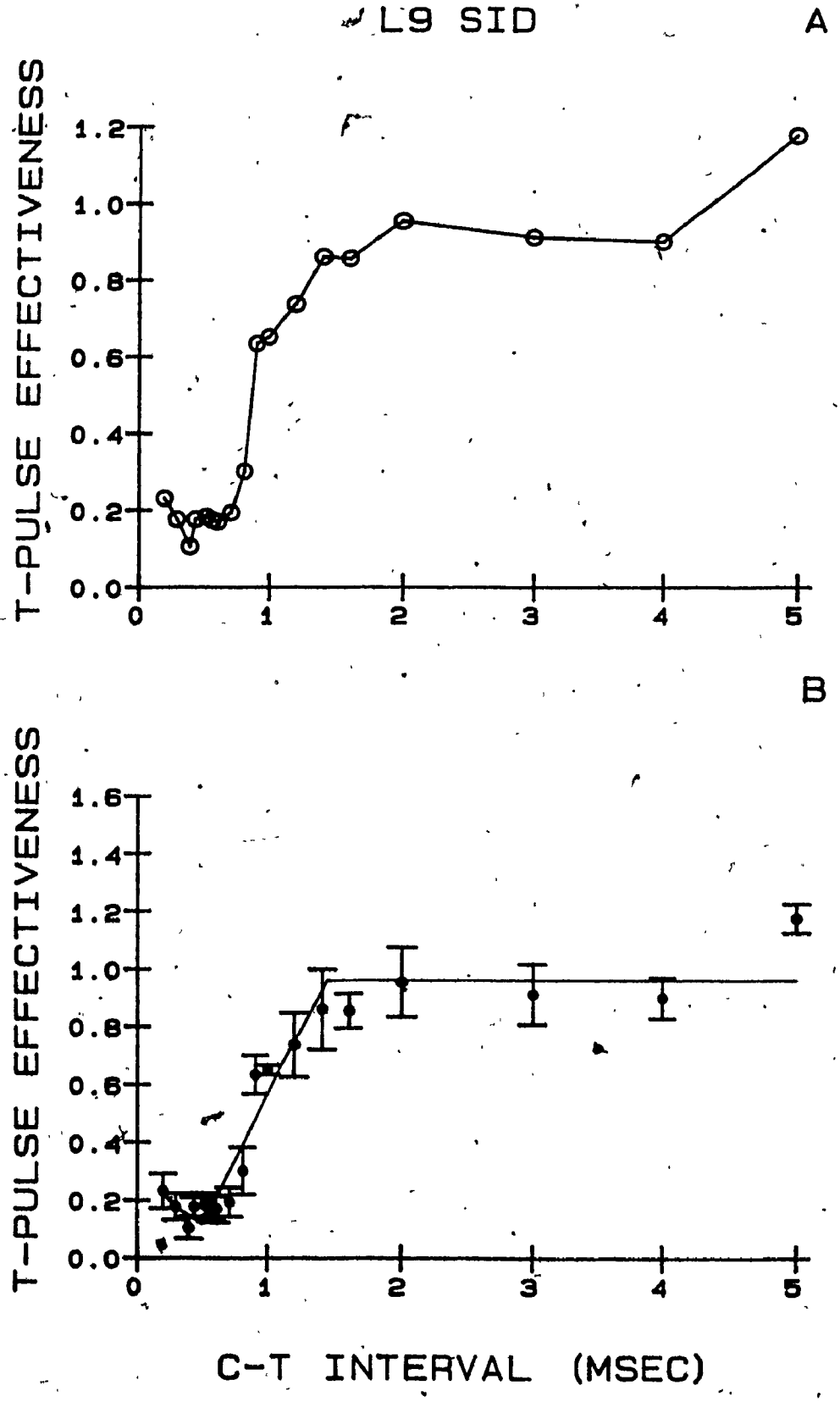

L9 SID
A
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 11. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced feeding (SIF) for animal W4.

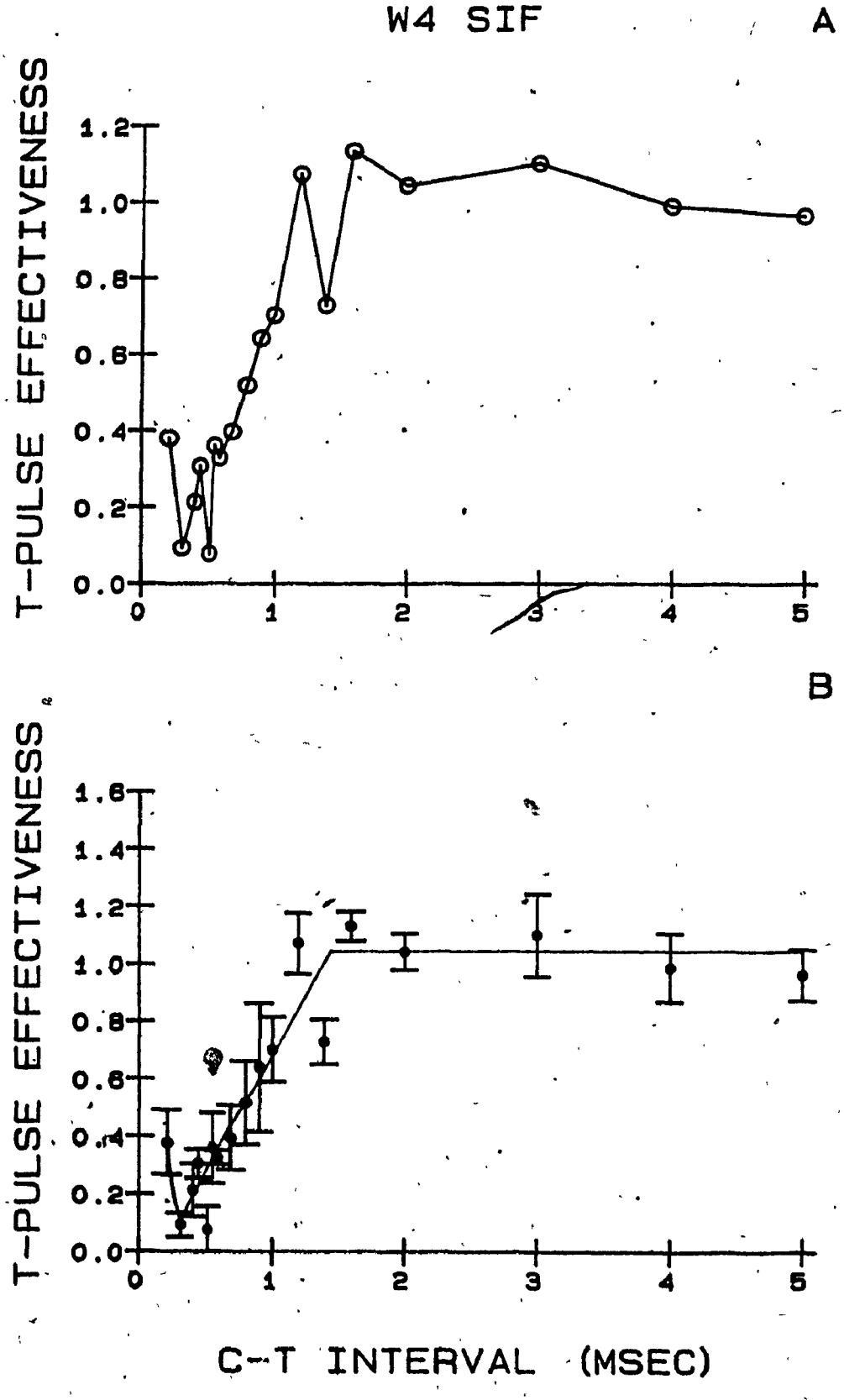

W4 SIF
A
T-PULSE EFFECTIVENESS
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Figure 12. The unfitted refractory period curves (A) are plotted on the same page as the fitted refractory period curves (B) for stimulation-induced drinking (SID) for animal W4.

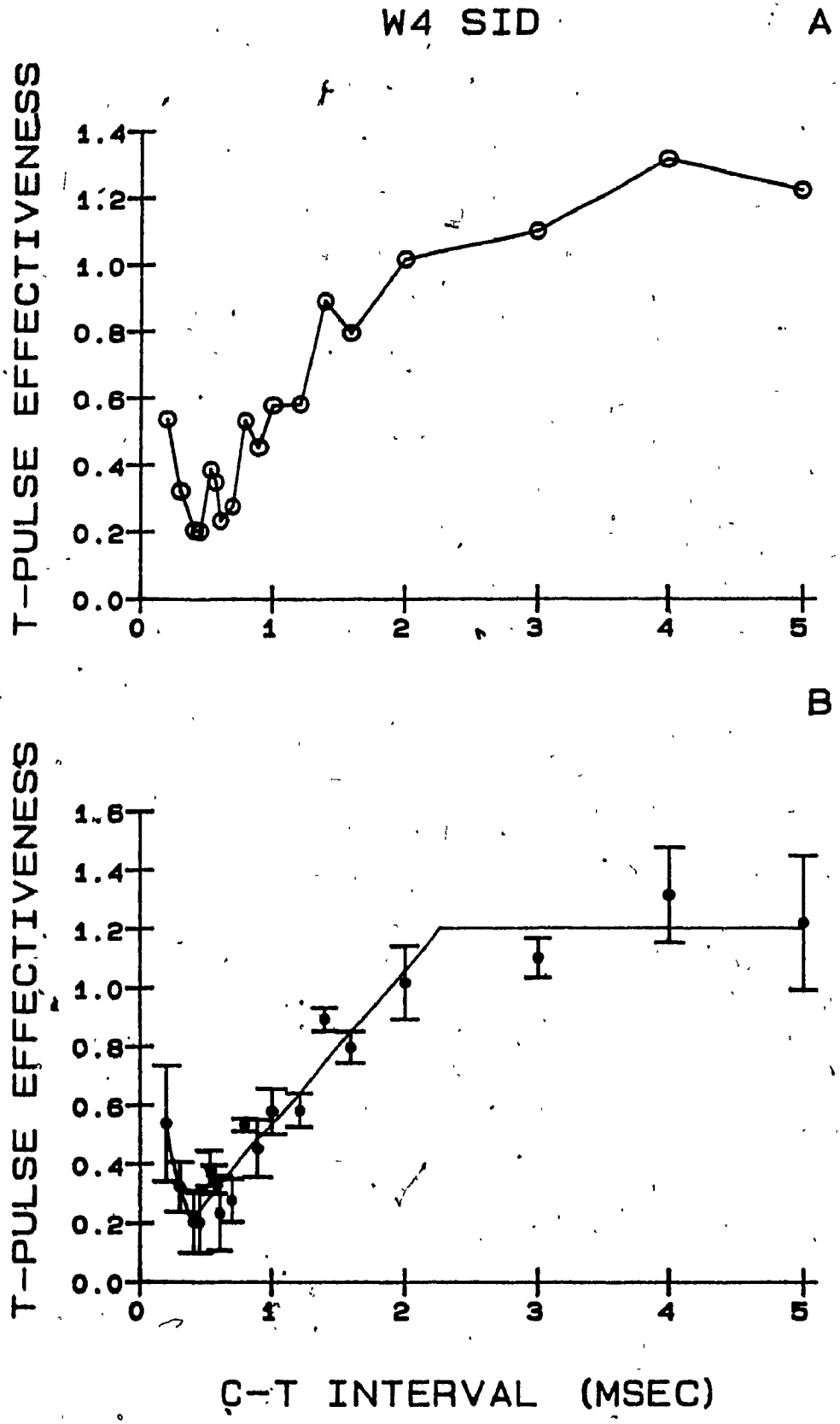

W4 SID
A
T-PULSE EFFECTIVENESS
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
B
T-PULSE EFFECTIVENESS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
C-T INTERVAL (MSEC)

Table 1

The values for the initial and final recovery from refractoriness for stimulation-induced feeding (SIF) and for stimulation-induced drinking (SID).

	SIF		SID	
Rat	initial	final	initial	final
	(msec)		(msec)	
L1	0.479	1.552	0.592	1.555
L9	0.431	2.309	0.499	1.443
A2	0.410	1.318	0.689	1.234
W4	0.301	1.450	0.381	2.278
Mean	0.405	1.657	0.540	1.628
s.e.m.	0.033	0.193	0.058	0.195

Figure 13. Histological localization of electrode tips aimed at the medial forebrain bundle at the level of the lateral hypothalamus. Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The numbers in the left column indicate the individual animals, while the numbers in the right column represent the corresponding distance posterior to bregma for each brain slice.

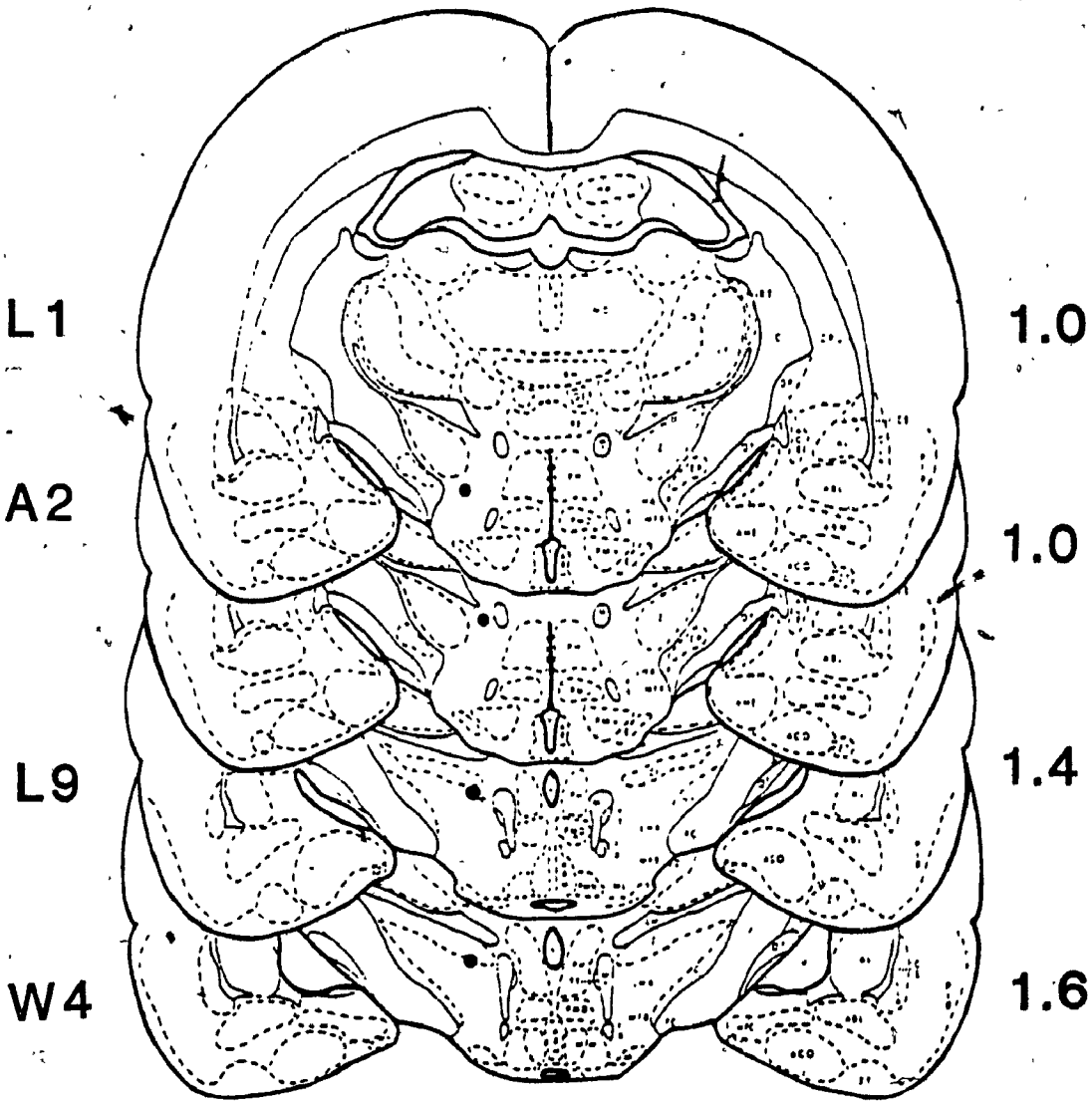

L1
1.0
A2
1.0
L9
1.4
W4
1.6

Table 2

Stimulation intensity (I) and single pulse frequency (SP) requirements for SIF and SID (+/- s.e.m.).

Rat	I	SP(SIF)	SP(SID)
	microamps	Hz	
L1	175	57.6	60.4
L9	250	43.5	52.6
A2	150	67.7	42.6
W4	200	56.6	43.5
Mean	193.8	56.35	49.78
s.e.m.	21.35	4.30	3.62

The values for the single pulse requirements for individual animals were obtained by averaging the single pulse threshold values over all testing days within each behavior.

Discussion

The Range of Refractory Periods for Stimulation-Induced Drinking and Stimulation induced Feeding

Hu (1973) estimated the refractory periods of neurons mediating stimulation-induced feeding and characterized them with the single value estimate of 0.9 msec. Rolls (1973) characterized the refractory periods of neurons involved in both stimulation-induced feeding and stimulation-induced drinking with a narrow range of values (0.5 to 0.7 msec). These estimates would each imply that the population of first-stage neurons involved in stimulation-induced drinking and stimulation-induced feeding are very homogeneous. However, the restricted range of values suggested by the results of Hu (1973) and Rolls (1973) can be attributed, at least in part, to the procedures these investigators used; in each case a small number of C-T intervals was tested. Hawkins, Roll, Puerto and Yeomans (1983) and Gratton and Wise (1988) tested a greater number of C-T intervals, and found that the refractory periods of neurons contributing to stimulation-induced feeding have a much greater range than was previously thought (from 0.4 to 2.0 msec). With a fine grained analysis in which numerous C-T intervals were tested, the present study confirms that some of the behaviorally relevant neurons for stimulation-induced

feeding recover from refractoriness as early as 0.4 or 0.5 msec and that other neurons appear to require as much as four to five times as long to recover. A similarly broad range of refractory periods appears to characterize neurons mediating stimulation-induced drinking. Thus the fibers subserving stimulation-induced drinking and stimulation-induced feeding are not as homogeneous as was once thought.

Comparison of Refractory Periods for Stimulation-Induced Feeding and Stimulation-Induced Drinking

Mogenson, Gentil and Stevenson (1971) reported that different frequencies of stimulation had differential effectiveness for stimulation-induced drinking and stimulation-induced feeding. Induced feeding was the predominant response with low frequencies (20 and 40 hz), and induced drinking was the predominant response when high frequency stimulation was used (100 and 200 hz). These findings were interpreted to suggest that different classes of neurons were involved in stimulation-induced feeding and stimulation-induced drinking. If different classes of neurons having different sensitivities to stimulation at different frequencies, mediate stimulation-induced drinking and stimulation-induced feeding, then one might expect to observe a difference between the characteristics of the first stage neurons subserving these behaviors.

The characteristic of the individual neuron that is thought to be directly related to its ability to follow increases in the frequency of the stimulation is the neuron's refractory period. However, on the basis of the present results, it must be suggested either that the same system subserves both stimulation-induced drinking and stimulation-induced feeding, or that separate systems with nearly identical refractory period ranges and thus nearly identical ability to follow increases in the frequency of the stimulation, mediate these behaviors. These results confirm the conclusion drawn by Rolls (1973), such that the refractory period ranges for stimulation-induced drinking and stimulation-induced feeding appear to be very similar.

Candidate Fibers

It is of interest to compare the refractory period estimates for the substrates of stimulation-induced drinking and stimulation-induced feeding to the known refractory periods of the catecholaminergic and cholinergic systems that have been implicated in these behaviors.

The catecholamine systems

Dopaminergic and noradrenergic systems have each been implicated in various aspects of drinking and feeding. Grossman (1960, 1962) demonstrated that centrally administered noradrenergic agonists increase feeding and Leibowitz (1975) has been able to obtain both drinking and feeding from sated rats injected with noradrenaline.

Blockade of dopamine receptors with haloperidol or spiroperidol reduces deprivation-induced drinking (Fitzsimmons,1976; Leibowitz,1981) and administration of the dopamine antagonist pimozide reduces water intake in non-deprived rats (Neilson & Lyon, 1973). Pimozide has also been shown to attenuate stimulation-induced feeding (Jenck, Gratton & Wise, 1986). Futhermore, it is well documented that destruction of the dopamine nigrostriatal bundle results in both adipsia and aphagia (Montemurro & Stevenson,1957; Teitelbaum & Epstein, 1962; Epstein, 1971; Marshall, Richardson & Teitelbaum, 1974). As both dopaminergic and noradrenergic fibers pass through the lateral hypothalamus, these fibers have been considered as candidates for the directly activated system or systems subserving stimulation-induced drinking and stimulation-induced feeding.

Dopaminergic and noradrenergic neurons were once thought to have refractory periods that were too long to account for any portion of the refractory period curves obtained from stimulation-induced drinking and stimulation-induced feeding. Wang.(1981) estimated dopaminergic neurons in the ventral tegmental area to have refractory periods of about 2.5 msec, and the refractory periods of noradrenergic neurons have been estimated to be about 2.0 msec (Faiers & Mogenson, 1976). Swadlow (1982), however, has shown that

the methods used by these investigators result in overestimates of refractory period values.

Using an improved method Yeomans, Maidment and Bunney (1988) have found that the refractory periods of dopaminergic neurons in the ventral tegmental area can be as short as 1.2 msec. Still, it remains unlikely that direct dopamine activation contributes to the rapidly rising portion of the refractory period curves; most recovery occurs before 1.2 msec. While it now seems possible that direct activation of dopamine fibers makes some contribution to stimulation-induced drinking and stimulation-induced feeding, it is clear that some faster system or systems comprise the major portion of the first stage mechanism of the two behaviors.

Given that noradrenergic fibers have similar characteristics to the dopamine fibers, it is possible that noradrenergic neurons can also be activated somewhat earlier than 2.0 msec after the C-pulse, and therefore may also make some contribution to stimulation-induced drinking and stimulation-induced feeding. Again, however, fibers with faster refractory periods than the noradrenergic fibers must make the major contribution to these behaviors. Taken together, the evidence suggests the possibility of some partial contribution of dopaminergic and noradrenergic fibers in stimulation-induced feeding and stimulation-induced drinking, but it is unlikely that either of these

systems carries a major portion of the directly activated signal subserving the two behaviors.

The cholinergic system

The cholinergic system has also been implicated in drinking. Central injections of cholinergic drugs facilitate drinking in sated rats (Grossman, 1960,1962; Fisher & Coury, 1962). There is also evidence suggesting cholinergic involvement in feeding with rats. This evidence has come primarily from work done with the feeding response that can be induced with electrical stimulation of the lateral hypothalamus. Cholinergic agonists decrease thresholds for stimulation-induced feeding, while antagonists block the effect; cholinergic drugs which do not cross the blood-brain barrier have no effect (Stark, Totty, Turk & Henderson, 1968), thus demonstrating that the effect is specific to central cholinergic neurons.

Since cholinergic fibers pass through the lateral hypothalamus (Shute & Lewis, 1967; Cuello & Sofronium, 1984; Butcher & Woolf, 1986; Woolf, Eckenstein & Butcher, 1984), cholinergic neurons can be considered as candidates for the directly activated substrate involved in stimulation-induced drinking and stimulation-induced feeding. The absolute refractory periods of putative cholinergic neurons have been estimated to be as short as 0.9 msec, and as long as 5.0 msec (Lamour et al, 1984; Aston-Jones et al, 1984, 1985).

The fastest refractory periods of the cholinergic neurons appear to be short enough to contribute to the quickly rising portion of the refractory period curves obtained for stimulation-induced drinking and stimulation-induced feeding; however, they seem too slow to account for the initial rising portion of the curve.

The Possibility of Sub-populations

Gratton and Wise (1988) stimulated the lateral hypothalamus and obtained refractory period curves for stimulation-induced feeding. These researchers observed that the T-pulse effectiveness increased between the C-T intervals of 0.4 and 0.6 msec and 0.7 and 2.5 msec but no increase was observed between the C-T intervals of 0.6 and 0.7 msec. The suggested interpretation of these data was that while fibers with refractory periods between 0.4 and 0.6 msec and between 0.7 and 2.5 msec contributed to stimulation-induced feeding, no fibers with refractory periods between 0.6 and 0.7 msec did so. The lack of fibers with refractory periods between 0.6 and 0.7 msec suggested two distinct, non-overlapping sub-populations of fibers. Gratton and Wise suggested that the "plateau" in the refractory period curves reflected the refractory period range separating the two sub-populations.

The plateau reported by Gratton and Wise was not clearly evident in the refractory period curves observed in the

present study. For three of the animals the refractory period curves for stimulation-induced feeding suggest a plateau, however, these plateaus were not seen consistently between the C-T intervals of 0.6 and 0.7 msec. Moreover, the error-bars associated with the points bounding each plateau were large suggesting that plateaus may be unreliable. There was no tendency towards any near-zero increments in T-pulse effectiveness in any of the stimulation-induced drinking refractory period curves.

The refractory period curves observed in the present study suggest that there may well be more than two sub-populations of neurons involved in the mediation of stimulation-induced drinking and feeding. As well as the sub-populations proposed by Gratton and Wise, there may also be a third sub-population that contains neurons having refractory periods between 0.6 and 0.7 msec; such fibers would span the gap between the sub-populations proposed by Gratton and Wise, and perhaps overlap with each. Fibers with such refractory periods would be required to account for the continuous recovery of the refractory period curves observed in the present study.

The placement of electrode tips could conceivably account for the difference in refractory period curves observed by Gratton and Wise and those observed by the present author. Gratton and Wise's electrode placements were predominantly in the ventral and posterior portion of

the lateral hypothalamus, whereas the electrode tips of all animals in the present study were in the dorsal portion of the medial forebrain bundle; two of the electrode tips were at the ventral edge of the zona incerta. The results of Rompre and Miliaressis (1987) illustrate that the shape of refractory period curves can vary with small changes in electrode placement. Rompre and Miliaressis observed a consistent plateau in refractory period curves when the mesencephalic brain region was stimulated. When the electrode was lowered to a more ventral site, a plateau was not observed. A subsequent ventral move of the electrode resulted in the recurrence of a plateau at the same C-T intervals as before. Given that the fibers contributing to the recovery from refractoriness can be sensitive to small changes in electrode placement, it is not surprising that the plateau observed by Gratton and Wise (1988) was not seen in the refractory period curves observed by the present investigator.

Suggestions for Future Research

Gratton and Wise (1988) observed stimulation-induced feeding when the ventral tegmental area was electrically stimulated. These authors also observed that some fibers contributing to stimulation-induced feeding are common to both the lateral hypothalamic area and the ventral tegmental area. Durivage and Miliaressis (1986) observed similar

results investigating stimulation-induced exploratory behavior. Stimulation-induced exploration was seen when either the lateral hypothalamus or the ventral tegmental area was electrically stimulated. Furthermore, fibers contributing to the behavior of stimulation-induced exploration were common to both sites. Given these results it would not be unreasonable to expect to observe stimulation-induced drinking when electrical stimulation is delivered to the ventral tegmental area. It may also be true that fibers contributing to stimulation-induced drinking are common to both the lateral hypothalamus and the ventral tegmental area. A more thorough mapping of the brain for stimulation-induced drinking is required, as well as more detailed characteristics of the first stage neurons involved in mediating this behavior.

To elucidate the neurotransmitters that are involved in the mediation of drinking and feeding, more pharmacological work needs to done in conjunction with behavioral testing. It is of particular importance to evaluate different forms of a single behavior, such as free feeding, deprivation-induced feeding and stimulation-induced feeding, under identical pharmacological manipulations. By carrying out such experiments it is possible not only to evaluate the effect of pharmacological manipulations on a given behavior, but also to compare the same behavior when the organism's internal state is varied. It is entirely possible that very

different results will be observed when the same behavior is examined under varying internal conditions.

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