Gimino, Frederick A.

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by

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Abstract

THE SITE SPECIFIC EFFECTS OF MORPHINE AND AMPHETAMINE ON INTRACRANIAL SELF-STIMULATION BEHAVIORAL INTERACTIONS BETWEEN THE LOCUS COERULEUS AND OTHER CENTRAL REWARD LOCI

by

Frederick A. Gimino

Advisor: Professor Solomon S. Steiner

Rats were implanted with pairs of stimulating electrodes aimed at various intracranial self-stimulation (ICSS) loci. After subjects demonstrated stabilized rates of lever pressing, they were administered a daily injection consisting of either low doses of morphine, the opiate antagonist naloxone, or morphine plus naloxone. Subjects received these drugs under two ICSS conditions. In one condition electrical stimulation was delivered solely to one ICSS area, while in a second condition the pulse pairs were "split" between both ICSS sites.

Response rates supported by stimulating the medial forebrain bundle (MFB), the locus coeruleus (LC), and the fields of Forel (FF) were increased when subjects were injected with morphine. Morphine injections had no significant effects on ICSS rates maintained by cru cerebri (CC) stimulation.

Naloxone decreased ICSS rates from MFB sites at all doses while this agent affected placements ventral to the mammillothalamic tract only at high doses.

The "split" pulse condition produced an interaction. Response rates in this condition were always greater in magnitude than the sum of ICSS rates obtained when each site was stimulated alone. Animals with electrodes in the LC and either the fields of Forel or the cru cerebri displayed directional interactions. These interactions were always greater in magnitude when the LC was stimulated first. Animals with implants in the locus coeruleus and the medial forebrain bundle displayed non-directional interactions.

Morphine produced a significant rate facilitation. This effect was not contingent on the order of site activation in the non-directional interactions. The directional interactions demonstrated a significantly greater effect when the locus coeruleus was stimulated first.

The d-isomer of amphetamine produced a significantly greater rate facilitations than did the l-isomer. The d-isomer was also equally effective in facilitating rates regardless of the order of site stimulation in the non-directional, but not the directional interaction. The l-isomer had no effect on interactions between the LC and the CC.

Clonidine decreased ICSS rates systematically from the LC and MFB, but not the FF or CC electrode sites. This drug also decreased rates in the non-directional interaction condition. Clonidine blocked morphine's rate facilitatory effects only when the LC was activated first.

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Table of Contents

List of Tables

List of Figures

Numerous studies have been reported in the literature (Deutsch, 1964; Gallistel, 1964; Olds, 1973; Olds and Milner, 1954; Olds and Olds, 1963; Olds and Travis, 1960; Routenberg and Malsbury, 1969) which have demonstrated that an animal will emit responses to obtain electrical stimulation of the brain. A long debated notion in this literature has been the question as to whether or not there exist discrete neuroanatomical loci which are responsible for the mediation of intracranial self-stimulation (ICSS) behavior. This initial question has been further extended to include the search for the existence of an integrated ICSS reward system composed of multiply connected neuroanatomical loci. It has been hypothesized by some authors (Keene et al., 1978, Thomas et al., 1979) that such a putative system may differentially code both the rewarding and aversive properties of an affective stimulus.

There have been several main research techniques employed in this search for a common neural substrate of reward. These approaches have revolved around the central premise that a systematic neuroanatomical map could be developed and correlated with ongoing ICSS behavior (Olds and Olds, 1963). One approach to this problem was by either pharmacologically or electrophysiologically manipulating the neurochemical substrate underlying a putative central reward system, e.g., catecholamines (German and Bowden, 1972). A

second approach employed the electrophysiological or neurological destruction of one ICSS area and then observing the effects of the manipulation on ICSS response rates (Farber et al., 1976; Stein and Wise, 1971). The last method employed for delineating ICSS systems has been by delivering either simultaneous or near simultaneous electrical brain stimulation to pairs of discrete ICSS loci. This last technique allows for the behavioral description (by observing ICSS response rate changes) of neurophysiological summations and interactions between ICSS areas (Ackerman et al., 1976; Bodnar et al., 1979; German and Holloway, 1973; Albino and Lucas, 1962). In the present study, a combination of both pharmacological interventions and behavioral/neurophysiological interactions will be employed in order to further delineate the existence of an integrated ICSS network.

Previous studies investigating interactions between pairs of intracranial self-stimulation sites were able to demonstrate this phenomena in the diencephalon, mesencephalon, metencephalon, and telecephalon. Albino and Lucas (1962) first demonstrated that when two discrete ICSS sites were stimulated simultaneously the response rates were greater than the sum of the ICSS rates elicited by the same current intensities within each site alone. Ungeleider and Coons (1970) were able to demonstrate interactions by using

a more discrete technique which employed monophasic pulse pairs (C-T monophasic pulse pair technique).

The C-T technique was first elucidated by Sherrington (1917) in peripheral nerves, and later by Deutsch (1968) in behavioral preparations. This procedure employs negative going monophasic pulse pairs which are varied in time to produce alterations in responsivity.

The first pulse in a pair (Diagram 1) (C-conditioning pulse) is parametrically followed in time by a second (T-test pulse). This stimulation usually consists of a fixed train length in which pairs of C and T pulses are cycled. The time between successive C pulses is called the C-C interval, while the time between C and T pulse is called the C-T interval. Variations in the duration of this C-T interval will produce a concomitant change in an animal's ICSS responding. An animal will typically emit low rates of responding at short C-T intervals (0.0 - 1.2 msec.) while exhibiting optimally high responding rates at longer intervals (1.5 msec. - 5.0 msec.) (C-T monophasic pulse pair function).

The C-T technique provides a valuable research tool by allowing for changes in ICSS response rates (C-T monophasic pulse pair function) without altering the ICSS current level for a given animal. One of the major difficulties in multiple site stimulation experiments is the possibility that stimulation at one electrode site affects the

properties of stimulation at the second electrode site by non-neuronal means, such as by passive current spread from one site's electrode to the second site's electrode. Vallenstein (1967) proposed that one way of overcoming the difficulty of passive current spread was by interdigitating the pulses delivered to either site so that current was never delivered to one site at the same time as the other. Thus, the use of the C-T technique will also insure that the delivery of these pulses will be free of current spread within an individual subject's C-T function.

Steiner (1977) has also noted that this technique produces less variability across experimental sessions than does more conventional kinds of stimulation (60 cycle biphasic sine wave). A second advantage is reported by Coons et al. (1976) who found that pulse-pairs were very sensitive to drug manipulations. In contrast to Stein et al. (1962), this laboratory clearly illustrated that imiprimine does affect self-stimulation behavior when response rates are controlled by C-T trains as opposed to more conventional sinusoidal parameters.

The present study employed the C-T monophasic pulse pairs technique as the dependent measure of reward since it was both sensitive to drug manipulations and to changes in the rewarding properties of brain stimulation while remaining a methodologically sound procedure. Thus, this technique provided the means for avoiding the problems

typically associated with more conventional methods such as the rate-intensity function (Hodos and Vallenstein, 1962; Pliskoff, Wright and Hawkins, 1964; Steiner and Stokely, 1973).

C-T Interactions: A Review

Several (Erlander and Gasser, 1937; Lloyd, 1937; Sherrington, 1906) studies have employed the monophasic pulse-pair technique to determine the refractory characteristics of peripheral nerves. With the C-T technique, the time between impulse pairs can be shortened to some critical value after which the second (T-pulse) becomes ineffective in eliciting a response. Some authors have described such a refractory period using a behavioral preparation in which the dependent measure was lever pressing behavior for ICS (Coons et al., 1976; Rolls, 1976; Deutsch, 1964; Smith and Coons, 1976). This relation between the C and T pulses is not response dependent, nor is it contingent on whether or not the pulses are anodal or cathodal in nature (Bodnar et al., 1978).

Ungeleider and Coons (1970) and Coons et al. (1976) illustrated that when these monophasic pulse-pairs were "split" between bilateral MFB placements within an animal, the current thresholds necessary to elicit ICSS became equivalent across C-T intervals. Thus, splitting the C and T pulses between the ipsilateral and contralateral sides of the MFB eliminated the behavioral "refractory" period normally seen at between .5 to 1.2 msec. interpulse intervals. This data suggested the possibility of behaviorally measuring the temporal and spatial summative effects of ICS pulses delivered to CNS neural loci.

German and Holloway (1973) continued the use of this C-T "split" pulse technique by delivering simultaneous or near simultaneous stimulation to the posterior MFB and the hypothalamus of animals. These researchers discovered the existence of an asymmetrical interaction effect in these areas when the C pulse was delivered to the preoptic hypothalamus and the T pulse to the posterior MFB. Thus, response rates when the C-pulse was delivered to the posterior MFB first were much lower than when the reverse condition was true. These researchers postulated that the behavioral interactions which were evidenced when C-T pulses were "split" between sites were being produced by neurophysiological summations which were occurring concomitantly with the delivery of these pulses. Furthermore, they hypothesized that the rate enhancements displayed by these animals were being caused by the directional propagation of pulses between these underlying neural loci. In fact, they further argued that the structures responsible for this summation were located more distally from the preoptic area than from the posterior MFB. This "directionality hypothesis" assumed that the differential response rates between loci were being caused by the simultaneous arrival of stimulation at a summation point that was at a greater distance from the preoptic hypothalamus than from the posterior hypothalamus.

Bodnar et al. (1979) demonstrated the LC-HYP interactions depended on the locus of the hypothalamic electrode. Rates elicited in the interaction condition when the C and T pulse were "split" between loci were significantly greater than the sum of the response rates obtained when the C pulse was delivered to each site alone. These effects were not due to non-specific factors such as current spread since, as this author suggests, the influence of .1 msec. duration square wave cathodal pulses dissipate entirely by the end of each pulse delivery. Therefore, the C and T pulses delivered at one site could not affect the second site stimulated even at the shortest C-T interval of .5 msec. Furthermore, current spread does not explain the order effect of sequentially altering the site stimulated first. Thus, the existence of symmetrical and asymmetrical interactions, such as those seen between the LC and the far-lateral (DFLH) hypothalamus versus the medial forebrain bundle, also argues for the specificity of the ICSS interaction effect. Bodnar's study demonstrated that the ICSS rates of an animal depended upon the order of site stimulation when the stimulating electrodes were localized in the DFLH and the LC while stimulation order did not matter when the electrodes were localized in the MFB and the LC. According to the "directionality" hypothesis then, the summation point for these placements must be more distant from the LC than from the DFLH. This is because only when

the LC is stimulated first could pulses arrive simultaneously at the DFLH or some third site to produce a facilitation in LC-DFLH animals. The LC-MFB animals probably had placements which were summating at a point which was equidistant from the LC and MFB and thereby producing equivalent rates independent of site stimulation order.

Since ICSS rates are higher when the C pulse is played to LC first and to the DFLH second, then an ascending ICSS network is defined by the "directionality" hypothesis. This is because it would take a longer period of time for a pulse to ascend from the LC to the DFLH and/or forebrain areas, and thus allows a second pulse delivered to the DFLH later in time to have greater summative properties due to its simultaneous or near simultaneous arrival at the post-synaptic membrane of the DFLH (or other CNS summation locus). Another possible explanation for the "split" pulse interactions between the LC-MFB and the LC-DFLH areas might be due to the qualitative differences in the types of neurons existing in these two areas and their inter-neuronal connectivity.

Szabo (1972) and Millhouse (1969) both demonstrated, using electrophysiological and neuroanatomical techniques respectively, that long descending fibers of the medial forebrain bundle terminate in path neurons of the lateral hypothalamus and preoptic area. The dendrites of these

neurons are spatially organized perpendicularly to the long axonal fibers in these regions. These path neurons bifurcate near their cell bodies and project to both rostrally and caudally located structures. These cells then give off many collaterals which synapse or branch by other path neurons in the vicinity. Thus, a series sagittally oriented reverberating circuit is created synaptically and transynaptically between neurons throughout the lateral preoptic zone (Millhouse, 1969). Szabo (1972) studied this system using the C-T technique and obtained results which suggest that negative feedback inhibition was reflected in the C-T "excitability" curves, and that these findings supported a role for path neurons in mediating hypothalamic/MFB ICSS. Bodnar (1979) suggested that the differential distribution of these path neurons between the LC-MFB and the LC-DFLH networks might account for their differential behavioral responsivity. He argued that the number of reverberating circuits in the LC-MFB system was greater than obtained in the LC-DFLH system. The speculation was that both the LC and the MFB stimulated the reverberating loops in either direction of stimulation, e.g., delivered first to the LC, while only the LC stimulated path neurons in the LC-DFLH condition. Thus, activation of the reverberating path neurons would allow a pulse delivered to a neural site to remain neurophysiologically "active" for a longer temporal

duration, and thereby increasing the probability of a summative post-synaptic interaction.

A "neurotransmitter half-life" hypothesis has also been conjectured by a few researchers (Bodnar et al., 1979; Ungeleider and Coons, 1976). This hypothesis was developed on the basis of neurochemical kinetics. It suggests that the differential responsivity of neural networks in the C-T interaction experiments may be due to different neurotransmitter "half-lives." If one were to postulate a "long" versus "short" living neurotransmitter in each of the ICSS systems, then differential interaction between loci having two long-acting as opposed to two short-acting transmitters would be predicted. The long-acting transmitter could keep the neuronal membrane activated for a greater temporal duration, and thus increase the probability of temporal and spatial summative effects from the second system.

Bodnar continued to examine CNS reward networks by employing the C-T "split" pulse technique to examine other pairs of neural loci. He extended this analysis to include the substantia nigra (SN), the mid-ventral periaqueductal gray (MV) with those hypothalamic placement within the MFB or outside the MFB (non-MBF) (Bodnar et al., 1982).

These studies indicated that in SN-MFB animals displayed asymmetrical interactions. Thus, when the MFB was stimulated first, the response rates were greater than in

the reverse condition. Conversely, in the SN-non-MFB animals, lower response rates occurred when the SN was stimulated first than if the non-MFB were stimulated prior to the SN. The behavioral interaction between MV-MFB ICSS sites was greater when the MV was stimulated first. Therefore, MV-MFB interactions were asymmetrical in nature. However, non-MFB placements exhibited greater response rates when the non-MFB was stimulated first and then followed by stimulation of the MV.

The results of this study suggested a descending ICSS system if response rates were greater when the C pulse was delivered to the rostral site first. Conversely, if the ICSS response rates were greater when the C pulse was delivered to the caudal site first, then an ascending system is suggested between that pair of ICS sites. Thus, according to the "directionality" hypothesis, the MV-MFB and SN-non-MFB comprised a system in which rates were higher when the caudal site was stimulated first, and therefore defines an ascending system. The MV-non-MFB and SN-MFB placements showed greater rates when the rostral site received the C pulse first. Therefore, the MV-non-MFB and SN-MFB sites were descending systems as defined by their "directionality."

It is easy to deduce the behavior of each system on the basis of the number of long-acting versus short-acting neurotransmitters. For example, systems with only one long-

acting transmitter would demonstrate an asymmetrical interaction since summation on the post-synaptic membrane would only occur when the short-acting transmitter was released prior to the release of the short-acting transmitter, but not in the reverse condition. Some data supporting the sensitivity of the C-T technique in detecting the effects of psychoactive agents was collected by Ungeleider and Coons (1970). These authors successfully discriminated the heterosynaptic and homosynaptic effects of imiprimine and di-isopropyl-flourophosphate which both influence transmitter neurochemical temporal activity.

ICSS Networks

Several studies have employed simultaneous and near simultaneous stimulation of two ICSS sites. The combination of sites examined include:

1) Bilateral Hypothalamus (Coons et al., 1976; German and Holloway, 1973; Ungeleider and Coons, 1970).
2) Amygdala and Hypothalamus (Szabo et al., 1970).
3) Substantia Nigra and Hypothalamus (Ackerman et al., 1973).
4) Mid-Brain Ventral Tegmentum and Hypothalamus (Shizgal et al., 1980).
5) Mid-Ventral Central Gray and Hypothalamus (Albino and Lucas, 1962; Bielajew and Jordan, 1981; Bodnar, 1979).

6) Locus Coeruleus and dorsal Far Lateral Hypothalamus (Bodnar et al., 1979).

7) Locus Coeruleus and Medial Forebrain Bundle (Bodnar et al., 1979).

8) Lateral Hypothalamus and Ventral Periaqueductal Gray (Bielajew et al., 1980).

Bodnar found symmetrical interactions between the LC and MFB. He demonstrated asymmetrical interactions between the LC and DFLH (Bodnar et al., 1979). Bielajew et al. (1980) found interactions which were symmetrical LH-PAG interactions, while LH-Ventral tegmental placements have been reported to be symmetrical and asymmetrical in different animals (Yeomans et al., 1979). These authors employed the trade-off function method of C-T and C-C analysis developed by Yeomans and others in his group (Yeomans, 1973; Yeomans and Davis; 1975; Yeomans et al., 1979).

Two ICSS Network Hypotheses

The data of Bodnar et al. (1979) supported the existence of two discrete ICSS systems emanating separately from the nucleus locus coeruleus in the pons. One system coursed between the LC and the medial forebrain bundle and was characterized by symmetrical "split" pulse C-T interactions. The second system was defined between the LC and dorsal far lateral hypothalamic area (DFLH) and was

characterized by asymmetrical interactions. Additional support for these systems comes from different but converging lines of evidence. There is pharmacological support for this notion in that the d- but not the l-isomer of amphetamine enhances both the LC and the MFB at low doses (Ellman et al., 1975; Ellman, Ackerman, Bodnar, Jackler & Steiner, 1976; Phillips, Brooke, and Fibiger, 1975; Phillips and Fibiger, 1975; Stephen and Herberg, 1975).

There has been electrophysiological support for this model from the work of Gallistel and Rolls respective laboratories in which they delineated unit recordings from the brainstem loci including the midbrain LC and its ascending dorsal noradrenergic bundle projections (Gallistel, Rolls and Greene, 1969; Mathews and Gallistel, 1975; Rolls, 1971A, 1971B, 1971C). These authors demonstrated the direct activation of hypothalamic units by stimulation of the LC. The refractory characteristics of these cellular recordings (.8 - 1.1 msec.) were similar to those obtained from behavioral experiments for these same hypothalamic areas. Such intervals also correspond with the onset of peak ICSS response rates as the C-T intervals are extended beyond this point (Bodnar et al., 1978; Gallistel, 1969; German and Holloway, 1972; Rolls, 1971C; Smith and Coons, 1970; Szabo, 1974; Yeomans, 1975).

Histofluorescent studies have verified the existence of a major ascending noradrenergic pathway between the

posterior hindbrain and the telecephalon in the rat (Dahlstrom and Fuxe, 1964; Ungerstedt, 1971). The dorsal noradrenergic bundle (DBN) which emanates from the LC innervates the hypothalamus/MFB and ascends to structurally connect the septum, hippocampus and cortices. The ventral noradrenergic bundle (VNB) emanates from nuclei posterior to the LC and innervates the hypothalamic area primarily. The work of Farber (1975) examined the behavioral implications of these ascending NE bundles. Farber (1975) reported that lesions of the LC abolished or reduced ICSS rates from the posterior hypothalamus, but did not affect or increased rates from the MFB. Thus, this study established the behavioral connectivity between the LC-DNB and medial forebrain bundle as well as those between the VNB and MFB/DFLH. The implications of this study was that ICSS behavior maintained in the DFLH depended primarily on the LC remaining intact, while MFB/ICSS was subserved by systems other than or in addition to those controlled by the LC-DNB projection systems.

Another line of evidence supporting the specificity of these ICSS networks comes from the work of Nelson at al. (1977) who, in further examining the site-specific effects of opiates such as morphine, delineated the responsivity of ICSS loci using the C-T technique to characterize drug effects. This author delineated the site-specific or morphine and d- and l-amphetamine, and showed that opiate

responsivity of a site could be predicted by the effects of administering d- and l-amphetamine at a particular neural locus. Nelson's work described MFB placements which yielded facilitations under morphine sites which were medial to the MFB and ventral within the MFB showed depressions under morphine. Sites which demonstrated morphine facilitations were also responsive to a greater degree to the d- rather than the l-isomer of amphetamine. Thus, the LC and the MFB showed both morphine facilitations and a d-isomer pattern of responsivity than did non-MFB placements. This effect seemed to be predicted by the degree of noradrenergic innervation by the DNB. The work of Jackler et al. (1979) also supports this morphine site-specificity as has the work of other laboratories (Adams, Lorens and Mitchell, 1972; Bozarth and Reid, 1977; Bush, Bush, Miller and Reid, 1976; Glick and Rappaport, 1974; Holtzman, 1976; Lorens, 1972, 1976; Lorens and Mitchell, 1973; Marcus and Kornetsky, 1974; Pert and Hulsebus, 1975; Schaeffer and Holtzman, 1977; Wacquier and Niemegeers, 1976).

Tempel (1982) extended the work of Farber (1977) and Nelson (1977) by combining the electrolytic lesion technique, the C-T monophasic pulse pair technique with opiate site specificity. This researcher hoped to elucidate the pharmacological properties of the LC-MFB, with those of the DFLH system by using both morphine and the d- and l- "screening" technique to "neurochemically" mark the

behavioral changes in ICSS after LC lesions. Tempel found that LC lesions reduced ICSS rates in animals with H2 fields of Forel (FF), Zona Incerta (ZI), Intenal Capsule (IC), Mammilothalamic Tract (MF) and thalamic nuclei placements. Subjects with electrodes localized in the MFB or perifornical areas were not affected by LC lesions. Morphine produced response facilitations in the H2FF, ZI, mammilothalamic and thalamic nuclei sites, but did not affect Ss with internal capsule or cru cerebri placements. However, the MFB and PF groups of animals in this study did not show differences in their morphine induced ICSS rate changes when the pre-lesion results were compared to those obtained in the post-lesion condition. Thus, morphine produced facilitations from the medial forebrain bundle and peri-fornical areas were not altered by LC lesions, while animals with H2 fields of Forel, zona incerta, mammilothalamic and thalamic electrode placements response to morphine was by demonstrating a return to pre-lesion control rates. These findings support the existence of separate ICSS networks and further suggests a differential responsivity of the neurochemical substrate underlying these networks to the opiates.

ICSS Networks: Neurochemical Substrate

The previously cited studies in the present thesis have suggested two primary candidates for the mediation of ICSS

in the proposed networks emanating from the nucleus locus coeruleus. It has been suggested that ICSS systems projecting the MFB and DFLH from this cell body are most probably mediated by either catecholamine (NE or DA) and/or by the endogenous opiates, e.g., the enkephalinergic pathways. This hypothesis has been based on the single site and "split" pulse data and the results obtained with both the amphetamine isomers and the narcotic analgesics. To date, the localization of the enkephalinergic systems in the CNS have indicated that these projections overlap with those of the catecholamines. Many nuclei and fiber tracts previously identified as being innervated by catecholamines have themselves been identified as containing concentrations of enkephalins. For example, the adrenal medulla and the superior cervial ganglion are both rich in enkephalins, epinepherine, and norepinephrine (Alumets et al., 1978). The nucleus locus coeruleus and the NE A2 cell body are both rich in enkephalinergic fibers (Pollard et al., 1977). The LC has also been demonstrated to be rich in opiate receptors by the technique of autoradiography. These enkephalinergic sites are also responsive to microiontophoretic applications of opiate agonists (Aghajanian, 1978) in the LC. It seems probably that some interaction between CA and enkephalinergic systems is occurring at levels of the nucleus locus coeruleus. The most pronouncedly innervated enkephalinergic areas are in the Basal Ganglia. In

particular, the globus pallidus and the nigro-striatal system in general are richly innervated with enkephalins (Hong et al., 1977). There are numerous enkephalinergic cell bodies and fibers located in the hypothalamic area, in particular the median eminence (Sar et al., 1978). Morphine and opiates in general may affect the median eminence and the hypothalamic-hypophyseal portal system. Enkephalins are also densely localized in the area postrema of the hypothalamus in the rat. This area, involved in emesis in other organisms, is also richly innervated by norepinephrine. However, the medial forebrain bundle, the DFLH, and the cru cerebri contain only low densities of opiate receptors as described in autoradiographic techniques (Pert, Kuhar, and Snyder, 1976).

It is also well known that the opiate analgesics directly affect catecholamines in the CNS. For example, naloxone, a powerful opiate antagonist, blocks many of the effects of biogenic amines and catecholamines altering drugs (Ahtee, 1979; Kuschinsky, 1977; Kornetsky et al., 1980). Thus, morphine induced catecholamine releasing effects and indoleamine effects are directly antagonized by small doses of naloxone HCL. Furthermore, there is an increase in whole brain NE levels after chronic morphine administrations, while Gunne et al. (1976) has demonstrated that the methyl ester of alphamethyl-para-tyrosine (AMPT), a competitive inhibitor of tyrosine hydroxylase, precipitated withdrawal

in morphine dependent mice with concomitant whole brain decreases in NE but not dopamine.

The relationship between NE and endogenous opiate systems is also exemplified in the abstinence literature. Drugs which reduce NE levels given prior to withdrawal reduce the morphine abstinence syndrome (MAS). Pharmacological agents which increase the availability of NE at the synapse given prior to withdrawal, increase the MAS (Glick et al., 1976; Glick and Goldfarb, 1977; Aketra and Brody, 1968; Glick and Charap, 1973). In fact, Glick et al. (1974) illustrated that when an animal is allowed ICSS during morphine administration, they are protected to some degree from the MAS. Glick, Zimmerberg, and Charap (1973) found that alpha-methyl-para-tyrosine (AMPT) following passive morphine administration, that there was an amelioration of MAS induced weight loss. There has also been data to suggest that ICSS in the MFB or LC can substitute for opiate administration during withdrawal from chronic morphine use (Steiner et al., 1979, 1980, 1981; Gardener et al., 1980; Glick and Charap, 1973). In fact, the administration of chlonidine, an alpha-2 agonist/antagonist, can amerliorate the MAS during withdrawal in both animals and humans (Gold et al., 1982; Resnick and Washington, 1981).

Gunne et al. (1974) has also found that a depletion of DA also occurs at dopaminergic terminals following chronic

morphine administrations. However, this depletion disappears over time. A more important finding is that there is no significant effect on dopamine (DA) levels in morphine abstinent rats. However, nigro-striatal lesions abolish the MAS while MFB lesions are ineffective in affecting the MAS. Similarly, DA agonists, like d-amphetamine which stimulates striatal neurons, and apomorphine which directly activates DA receptors, exacerbates the MAS. Conversely, DA receptor blockers like haloperidol antagonize the hypersensitivity of the MAS withdrawn rat. Thus, the DA system also seems to be involved in the MAS.

To summarize, there are many lines of converging evidence which support the existence of two discrete neurophysiologically and neuroanatomically delineated ICSS networks. These networks are selectively reactive to drugs which modify either catecholamine or endogenous opiod levels. The exact neurochemical constitution of this system is as yet to be defined completely with traditional neurochemical and physiological techniques. In fact, the reductionist philosophy of contemporary behavioral physiology would lead to new speculation on the neurochemical elements which compose the catecholamines and enkephalins. For example, recent studies have formulated several types of multiple neurotransmitter receptors which might exist in the CA and enkephalinergic systems under

question (Snyder, 1980). Thus, there exist alpha-1 and alpha-2, beta-1 and beta-2 noradrenergic receptors, or the Mu, Delta and Kappa receptors of the opiate variety.

One behavioral approach for studying these ICSS systems would be to combine the following findings into a systematic study: 1) the relationship between ICSS sites and opiate responsivity; 2) the results of the d- and l-amphetamine "screen" studies; and 3) those findings obtained with the C-T "split" pulse procedure delineating ICSS networks. A series of experiments could then be designed to examine specific neuroanatomical, neurochemical and behavioral predictions based on these findings. For example, one could incorporate these findings to test the belief in the existence of at least a two channel ICSS network which the contained catecholaminergic (NE, DA) and enkephalinergic components. Such experiments could then serve to parcel out the influences of these neurochemical systems on the basis of the effects of opiate and catecholaminergic specific drugs on ICSS behavior maintained by interacting each of these networks.

For example, one set of experiments on ICSS reactivity to the opiates could be based on identifying the type of interactions obtained between two sites which had been labelled as being d-sensitive when stimulated in isolation. These results could be contrasted with those obtained from the interaction of two l-sensitive, or a combination of d-

and l-sensitive sites. Furthermore, these interactions obtained under both the opiates and the d- and l-isomer of amphetamine should in part depend on the type of interactions obtained under control conditions. Thus, the symmetry or asymmetry of an interaction produced in the "split pulse" condition could function as a "behavioral marker" for different classes of drugs and their responsivity at a particular pairs of ICS site. In a similar fashion, the response of a single site to drug administrations can serve as a "marker" for predicting that sites directionality in the "split pulse" condition.

The d- and l-Amphetamined Differentiation of ICSS

One pharmacological tool for determining the underlying neurochemical specificity of ICSS loci is based on the differential responsiveness of neural tissue to the effects of d- and l- amphetamine (Coyle and Snyder, 1969). The Johns Hopkins University group (Coyle and Snyder, 1969; Snyder, Taylor, Coyle and Meyerhoff, 1970; Taylor and Snyder, 1970; Snyder, Taylor, Coyle and Horn, 1972) demonstrated that the d- and l-amphetamine isomers have differential effects on the uptake of DA and NE in the CNS. The l-isomer was approximately ten times less potent that the d-isomer in blocking catecholamine uptake into NE neurons, but was equally as efficient in the blockade of uptake into striatal dopaminergic neurons.

Phillips and fibiger (1973), using Snyder's biochemical data as a basis, proceeded to show that the two amphetamine steroisomers were equally effective in increasing ICSS rates from the substantia nigra, pars compacta (SNC) while being four to eight times as potent as the l-isomer in enhancing the LH rates. Numerous studies followed this initial work in an attempt to further differentiate different ICSS sites with respect to their underlying neurochemical specificity (liebman and Butcher, 1974; Kojima et al., cited in Stein, Belluzzi, Ritter and Wise, 1974; Carey, Goodall, and Lorens, 1975; Ellman, Ackerman, Steiner, Bodnar, Jackler, and Ellman, 1977). However, the original biochemical data has recently come under question since other investigators failed to replicate the basic finding (Ferris et al., 1972; Harris et al., 1973; Horn et al., 1974; Svensson, 1971; Von Voigtlander, 1973; Chiueh, 1974; Holmes and Peterson, 1976). Steiner's laboratory, however, contests that this behavioral data is in fact consistent and dependable in discriminating NE from DA ICSS sites.

Steiner's laboratory (Ellman, Ackerman, Bodnar, Jackler, and Steiner, 1975; Ackerman, Steiner, Bodnar, Jackler, and Ellman, 1977) has examined various ICSS loci using the d- and l-amphetamine screening technique. When d-amphetamine was administered to animals with placements in the locus coeruleus, the dorso-lateral portion of the periaqueductal gray (Lindvall and Bjorklund's (1974)

periventricular NE system), and the dorsal noradrenergic bundle an increase in ICSS response rates was evidenced across all subjects. Injections of l-amphetamine produced no similar effects. Animals with electrodes implanted in the mid-ventral periaqueductal gray and midline pontine periventricular sites exhibited equal increments in ICSS rates following both d- and l-amphetamine injections. These mid-ventral placements were hypothesized as being innervated by either a doral extension of the dopaminergic A-10 area or as a caudal extension of the A-11 nuclear group (Lindvall and Bjorklund, 1974; Lindvall et al., 1973).

Thus, numerous authors have successfully used this d- and l-isomer screening technique to distinguish between NE mediated ICSS sites and DA controlled systems (Ellman et al., 1975; Phillips and Fibiger, 1973; Herberg, Stevens and Franklin, 1976).

Neurophysiological and Neurochemical Differentiation of the Locus Coeruleus, the Medial Forebrain Bundle, and the Dorsal Far Lateral Hypothalamus

In the present study, a series of experiments were proposed in order to differentiate the relationship between the two ICSS networks emanating from the nucleus locus coeruleus. These two putative systems were composed of the following pairs of loci: 1) the locus coeruleus and the medial forebrain bundle and 2) the locus coeruleus and the dorsal far lateral hypothalamic area (fields of Forel, the

cru cerebri). These systems will be differentiated on the basis of their responsivity to two types of ICS stimulation and to the drugs of abuse, i.e. the opiates, the amphetamines. The two types of electrical stimulation consisted of: 1) a condition in which C-T ICS was delivered to a single site in isolation; 2) a condition in which the C- and T-pulses will be "split" between the single sites. The drugs of abuse will be administered to animals under both conditions of ICS stimulation.

Neurochemical Differentiation of Morphine's Site-Specific Effects

An ancillary set of experiments were also performed in the present thesis in order to parcel out the endogenous neurochemical substrate underlying morphine's rate facilatory and depressive effects on ICSS. These studies specifically employed opiate and catecholaminergic agonists and antagonists. In particular, naloxone was chosen as the opiate antagonist while clonidine was chosen as the specific alpha-2 agonist. A brief description of these agents will be given below in order to substantiate their selection.

The electrode sites to be employed in all the above experiments will be chosen to represent combinations of low and high density NE, DA, and endogenous opiod levels. Thus, the LC and CC will represent placements which are both high in endogenous opiate levels, but demonstrate differential concentrations of the catecholamines dopamine and

norepinephrine. The fields of Forel and the medial forebrain bundle placements in contrast exhibit a low density of opiate receptors (Pert et al., 1976).

Clonidine is a potent alpha-2 noradrenergic agonist/antagonist which has been successfully employed in the treatment of the morphine abstinence syndrome in humans (Gold, 1980). this drug is believed to selectively activate the alpha-2 autoreceptors on the locus coeruleus and thereby decrease noradrenergic activity of this and other NE sites (Herberg et al., 1976; Guynet and Cabot, 1981; Gold and Potash, 1981). This drug is postulated to produce this decrement in firing rates via a presynatic negative feedback autoreceptor mechanism. Thus, this agent could be employed to investigate the role of norepinephrine in the maintenance of ICSS behavior from noradrenergic loci such as the locus coeruleus.

Naloxone has been shown to be effective in reversing both the morphine abstinence syndrome and stress induced analgesia (Bodnar et al., 1978) at doses as low as 1.0 mg/kg. It has also been demonstrated to reverse the facilatory effects of morphine, d-amphetamine, ethanol, and chlorodiazipoxide on ICSS responding (Esposito et al., 1980, Liebman and Siegal, 1977; Lorens and Sannati, 1978; Holtzman, 1977). However, the effects of this drug alone on ICSS has been less than consistent. For example, Belluzzi and Stein (1977) reported that .5 mg/kg of naloxone

significantly depressed ICSS responding from the ventral tegmental and periqueductal electrode sites, while Van de Koy et al., 1977, found only a non-site specific response suppression caudate and hypothalamic ICSS sites at a dose of 40 mg/kg.

GENERAL METHOD

The general methods section to follow outlines those subjects apparatus and procedures which are common to all sets of experiments. This section also outlines those procedures, etc., which differs between paradigms. The first experiment deals with the question of whether or not morphine and amphetamine can differentially affect the two ICSS networks emanating from the locus coeruleus. This experiment shared a common subject pool throughout the two ICS stimulation conditions, e.g., within vs. between, as well as under the various drug administrations. The second and third set of experiments dealt with the differentiation of morphine's site-specific effects on ICSS by opiate and catecholaminergic agonist/antagonist. A flow chart illustrating the relationship between the first main experiment and the two ancillary ones are depicted in diagram 3.

Experiment I

Subjects

Forty-six male albino Sprague-Dawley rats (Holtzman Co.) weighing between 300-550 grams prior to surgery were housed in individual plexiglass cages with a sawdust substrate. All Ss had ad libitum access to food and water throughout the course of the experiment. Subjects were maintained on a 12 hour reverse light-dark schedule. The

light periods extended from 0700 to 1900 hours while in the remaining period animals were in darkness.

Surgery

All subjects were anesthetized with sodium pentobarbital (Nembutal-Abbot Co.) and were sterotaxically (Kopf Co.) implanted with two pairs of chronic bipolar electrodes (plastic products: MS 303/1) and one indifferent skull electrode. The ICSS sites were chosen to represent differential concentrations of opiate receptor binding, amphetamine isomer responsivity and the symmetry or asymmetry of interaction with the LC. The sites included the following combinations: medial forebrain bundle (MFB) cru cerebri (CC), fields of Forel (FF) and the locus coeruleus (LC). The stainless steel electrodes were entirely insulated except at the tip. These tips were separated by a distance of .24 mm.

All tips were implanted ipsilaterally, placed in the left hemisphere, and were aligned in the medio-lateral plane (perpendicular to the midsaggital suture). The indifferent electrode was formed from a bipolar electrode which was unwound, stripped of insulation and wound around two skull screws. The electrode assembly and skull screws were attached to the skull by dental acrylic.

All sterotaxic coordinates were calculated on the skull coordinates of bregma and lambda. Konig and Klippel (1963)

was the rat atlas employed to determine the coordinates for each HYP placement. The coordinates for the MFB were 1.0 mm anterior to the midpoint between the bregma and lambda points, 1.5 mm lateral to the midsaggital suture, and 8.8 mm from the surface of the skull. The coordinates for the FF were: the midpoint between bregma and lambda points, 1.2 mm lateral to the midsaggital suture, 8.3 mm ventral from the surface of the skull. The coordinates for the cru cerebri were: the midpoint between the bregma and lamda points, 2.4 mm lateral to the midsaggital suture, 8.5 mm ventral to the surface of the skull. The coordinates for the LC were: .3 mm caudal to the lambda line, 1.0 mm lateral to the midline, and 7.1 mm below the surface of the skull. (Pelligrino and Cushman, 1972)

The incisor bar was set at 5 mm to prevent the rupture of the transverse sinus beneath the lambda suture.

Apparatus

The stimulation was delivered to each subject in a Plastic Products A100 standard operant chamber. The lever was a BRS/LVE retractable lever which when depressed closed a microswitch which triggered a Scientific Prototype RY205 electromechanical cradle relay. The relay contacts operated the stimulation unit which delivered the trains of electrical C-T monophasic pulse pairs to the rat. The operant chambers were enclosed in soundproof acoustically

tiled enclosure cabinets. These cabinets contained a plexiglass viewing window on their doors. A Granger Turbo S100 fan served as both a masking noise and a ventilation system for the chamber.

Data acquisition and operant contingency management was programmed on a combination of Scientific Prototype electromechanical equipment and a Digital Corp. DEC PDP 8 Lab E computer system. Data transfer to an IBM 360/1130 asynchronous processing system was accomplished via an Anderson Jacobson 242 ADAC acoustic couple modem and a Teletype model 43 teleprinter. The operating system for the IBM network was the interactive VMS/WILBUR language.

The brain stimulation was delivered to an animal by a constant current multichannel electrical stimulator designed specifically for this experiment. This stimulator was virtually totally isolated from ground potentials by use of mechanical (relay ladle-bucket system), transformer (isolation transformer), and optical (4N26 opto-isolators) isolation in combination. This system is displayed in Diagram 2. All stimulation parameters were continuously monitored by observing the current drop across a 100 ohm resistor in series with the rat on a differential input oscilloscope (Hewlett-Packard 1200B).

The system to be further detailed meets the above requirements, and provides the additional advantages of a line A.C. power source, compatibility with either TTL or

CMOS logic systems, and a guarantee that no net current will accumulate between pulses delivered to the subject. (See Diagram 2).

The system shown in Diagram 2 provides energy from the A.C. line and complete mechanical isolation. A large storage capacitor was charged from the A.C. power line. Negative and positive current magnitude was provided by separate current controls. Duration and time of occurrence was controlled by the logic system driving the optoisolators.

The logic system was powered by a transformer, rectifier and filter. Each optoisolator LED was driven by a transistor through a current limiting resistor and powered by a 12 V.D.C. regulator. Logic input was current sunk through an input diode. The inputs were directly compatible with the low output state of our TTL and CMOS logic. Both output transistors could not be turned on simultaneously, by a logic error, since each logic input clamped the alternate input off. The logic inputs, therefore, work on a "first-in" basis.

Basically, the output consisted of biphasic square current pulses in the range from 10 microamps to 1 milliamp. Rise and fall time was under 5 microseconds and duration was unlimited. All pattern information is generated by external control logic. (For a complete review of this system see Steiner et al., 1981).

Histology

At the end of the experiment all animals were sacrificed with an overdose of sodium pentobarbital and perfused with .9 percent saline and 10 percent formalin solutions. These brains were then removed and placed in 10 percent Formaline or a 10 percent sucrose and formalin solution for at least two days. Brains were then cut into 40 micron sections using the frozen section method. This tissue was mounted on slides and histologically prepared according tot he method of Kluver and Barrera (1953).

Five animals were also prepared using autoradiographic sections subsequent to being injected with tritiated 2-Deoxy-glucose according to the technique of Sokoloff (1977). These sections were prepared for both the LC and HYP placements in these animals (see figure 5). All electrode placements were localized by two raters not familiar with the animals behavioral data using the atlas of Konig and Klippel (1963) and Palkovitz and jacobwitz (1974).

Within-Site C-T Function

Procedure

Forty-six albino Sprague-Dawley rats were divided into three groups. After surgery and a week recovery period, animals were shaped for a maximum of 15 daily sessions of each electrode site at a variety of current intensities (150-500 ua), C-T intervals (0-15 msecs.) and C-C intervals (10-30 msec.). Each electrode site in each animal which

sustained ICSS behavior was tested under the following stimulus parameters. Stimulation consisted of 0.1 msec. pulses delivered for a 700 msec train duration. For each electrode site, that pole of the bipolar electrode which elicited the higher response rates in preliminary testing served as the cathode and a cortical screw as the anode. The C-C interval was fixed at either 20 or 30 msecs.; at such C-C intervals, summation between succeeding C pulses is relatively small, yet reliable ICSS behavior can still be elicited. For each electrode site, an intensity was chosen which yielded consistently high response rates at a C-T interval of 5.0 msec., but only operant responding (usually less than 10 responses per minute) if the T pulses were omitted. Any animal deemed a non-responder at both sites was eliminated from the study.

Each day, every animal bar pressed for stimulation delivered to one of its electrode sites during a 72-min. session, which was divided into nine 6-min. periods with a 1 min. time out between each interval. ICSS data from the first two minutes of each 6-min. period was discarded and the mean response rate over the last four minutes of each 6-min. period constituted the dependent variable. The C-T intervals (0.5, 0.8, 1.2, 1.5, 2.5, 5.0 and a condition in which the T pulse was omitted) was changed during each 1-min. time out, and presented in each of nine days in a Latin Square design. A C-T interval response rate function

averaged over the 9 day paradigm, was determined for ICSS sites in each animal.

The threshold for each animal was determined by using a modified version of the psychophysical method of limits. During the threshold session, an animal was tested on only one site for that given day. The initial current used was that current at which the animal was shaped on, during its first testing sessions. The C-T interval was fixed at 5.0 msec. while the C-C interval was maintained at 30 msec. The train was kept constant at 700 msec. The subject was then presented with 6 minute periods during which the lever was extended into the cage and a house-light located to the right of the lever (GE 1812 28V) was illuminated. During this period the animal was presented with its shaping current intensity at a C-T 5 msec., and the responses on the retractable lever were recorded for each of the 6-minute intervals. After the 6-minute interval was completed, the bar was automatically retracted from the cage and the mean of the first two and last four minutes was calculated by the experimenter. If responding in the last four minute interval was lower than 10 responses per minute, then the E raised the current 50 microamps and the 6-minute testing was repeated subsequent to the 1-minute time -out. If responding was greater then 10 responses per minute during the four minute interval, the current intensity was decreased 50 microamps. This procedure was continued in

ascending and descending fashion until a current intensity was obtained which produced at least 10 responses per minute at that interval, and produced less than 10 responses per minute when the current was reduced by 50 microamps. Thus, threshold was operationally defined when the latter procedure was accomplished in two ascending/descending series successively. All animals were then tested at the threshold current with the C-T interval fixed at 0.0. If an animal's response rate at a C-T of 0.0 (T-pulse is omitted) was less than operant level (10 responses/min.), then the threshold current was confirmed for subsequent use in the paradigm.

All subjects were tested daily at each site until they were stable. Stabilization consisted of 5 days of testing at a C-T interval of 5.0 msecs., a C-C interval of 30 msecs., and a train duration of 700 msecs. Some animals were also tested at a C-C of 15 msecs. and of 60 msecs. for a parametric analysis of the trade-off function between the C-C pulse pair interval and current intensity. This analysis was done as suggested by the work of Yeomans, Gallistel and Shizgal (1981) who indicated that such a procedure was necessary in order to accurately determine the neural characteristics of an ICSS system, e.g., refractory period, time constant, by allowing the separation of local potential from axonal properties. During this early phase

of stabilization the current intensity employed was the value obtained during the shaping.

After this five day period, animals had their threshold determined at each site according to a modified method limit (described earlier in this section). Subjects were then tested at their threshold currents at a C-C of 30 msec., C-T intervals ranging from .5 to 5.0 msecs. and a no T-pulse condition, and at a train duration of 700 msecs. The 7 C-T intervals were changed during seven 6 minute periods and a 1 minute time-out (see above). This procedure was repeated at each site for a least 9 days of "baseline" testing. After the ninth day, the mean response rates and the standard error of the mean (SEM) for each animal at each C-T interval was determined over the course of the last 4 experimental sessions. These values were then compared to the value obtained on day 10. If a subject emitted rates on day 10 which were within one SEM of his last four day means at each interval then that animal was defined as being stable. If an animal failed to reach this stability criteria in the first 10 days of baseline testing, then the procedure was repeated on the following day using the previous four day moving average as the criterion period. Each subject continued to be tested on baseline until they met the stabilization criteria at each site. The sequence of which site was tested first on a given baseline day session was determined by use of counterbalanced design.

After stabilization, each animal was maintained in their baseline condition for another 3 to 9 day period in order to insure that steady-state control rates of responding of within one SEM would continue prior to the initiation of drug administrations.

Drug Administrations

Drug administrations during the within site C-T response rate function determination consisted of the following agents: .9% physiological normal saline, 1.25 and 2.5 milligram per kilogram (MPK) morphine sulphate (Penick Corp.). and 1 MPK naloxone hydrochloride (Endo Co.).

Each animal in each of the three ICSS groups (LC-MFB, LC-FF, LC-CC) was administered 6 daily injections of .9% saline followed by 6 days of morphine sulphate injections (1.25 or 2.5 MPK). Subjects were then given two injection days of morphine sulphate during which they were also given an injection of naloxone hydrochloride (1 MPK). All animals received the first injection series of 1.25 MPK morphine, while nine subjects who completed all phases of the experiment were then selected to enter the 2.5 MPK injection series of morphine. In addition to these animals, nine other subjects were chosen to be tested solely on the 2.5 MPK series and also entered in Experiment VI (Clonidine and ICSS) of this thesis.

All injections were administered subcutaneously at the nape of the rat's neck. The injections were give 20 minutes prior to the beginning of an experimental session. This was done to insure that each agent had sufficient time to be absorbed into the CNS. A twenty minute period was selected as the post-injection interval on the basis of the opiate and ICSS literature in which a time frame of between 20-45 minutes was employed by researchers interested in maximizing morphine's excitatory effects (Adams & Lorens, 1973; Lorens & Mitchell, 1974; Marcus & Kornetsky, 1974; Jackler et al., 1979; Nelson et al., 1980). Morphine sulphate injections given subcutaneously will reach peak concentrations between 20 to 30 minutes post-injection and will be maximally effective about 50-70 minutes post-injection (Goodman & Gillman, 1979). Since the experimental sessions in this experiment were approximately 98 minutes long (one hour and 38 minutes), a twenty minute injection interval would place the maximal morphine effect within the first third of the experimental period.

All injection dosages were calculated to produce a constant concentration which was equivalent with the dose requirement. Each animal's injection varied on the basis of adjusting the volume according to their body weight. Each animal was tested on each drug day at each site. The order of which site presented first was determined by a counterbalancing procedure.

After completing the 1.25 mpk morphine paradigm, animals were again given three more days of saline followed by a fourth injection day of either the d- or l-isomer of amphetamine at 1.0 mpk. This sequence was repeated until each animal obtained two injection days at this dose for each isomer. After completing this series of morphine and amphetamine injections, all animals were then entered into the 1.25 mpk morphine and 1.0 mpk d- and l-amphetamine paradigm for a second set of administrations.

Animals who finished the first morphine and amphetamine dosage series were then entered into sequence of 2.5 mpk morphine and 2.0 mpk d- and l-amphetamine injections which followed the identical format of that described for the first dosage administrations.

If at any time during the experiment an animal failed to emit control rated during their saline control days then the following procedure was followed:

1) If an animal failed to "sample" from the lever during an experimental period, then after one minute had elapsed and in the absence of at least two responses, the lever was retracted from the chamber. After 30 seconds, the lever was reintroduced and the session commenced again. If the S again failed to sample, then the lever was retracted a second time and the 30 sec. procedure was repeated again. The lever entering the chamber

and the stimulus light onset should serve as a conditioned stimulus to elicit a response from the subject towards the lever.

2) If an animal continued to be a non-responder subsequent to the lever retraction procedure, then a priming stimulus was administered to the subject only when the lever was being extended on the third time, and only when the subject was approaching the lever area. There was never any attempt to deliver completely non-contingent "priming" stimuli to the organism.

3) If an animal still failed to respond, then it was removed from the test apparatus. The test apparatus and electrode cables were then examined, and the electrical stimulation parameters and equipment were investigated for possible malfunctions. Animals were then tested in another chamber as a last recourse.

4) Animals who continued to fail to emit control rates during saline injection days were removed from the experiment and again tested for their thresholds by a modified method of limits. If the threshold had shifted, or if the electrode tip under tip was mechanically impaired, then the S was tested on their second tip. If an animal then returned to the paradigm, it was retested on all phases of the

experiment, e.g., stabilization, baseline, etc., prior to reentry into the drug paradigm.

5) If an animal only displayed one day of atypical control rates, then that day was recorded, but the test day condition was repeated until control rates were recovered for that subject.

The data to be analyzed, consisted of the following data points:

baseline stabilization	(8 days)
pre-drug saline	(6 days)
morphine	(6 days)
morphine naloxone	(2 days)
post-drug saline	(3 days)

In general, the data was analyzed by using a series of two-way analyses of variance with repeated measures on all factors. The design chosen was one in which the variable of day was tested within the drug condition by C-T interval by site. The post-hoc analyses were either Duncan a posteriori tests or by a multiple comparisons procedure for orthogonal polynomials. These analyses were first performed on the morphine and saline raw scores to determine if there was an overall effect of drug. This was done by combining the ICSS rates obtained across all seven C-T intervals. The next analysis examined this data for the existence of an interaction of drug condition by group (self-stimulation site). A multivariate procedure (MANOVA) was also employed

to determine the influence of drug condition and site on each of the C-T intervals independently. In this analysis, the C-T intervals served as multiple dependent variables factored by the condition of drug and site tested in days.

The morphine within-site and between-site C-T data was analyzed by use of this MANOVA procedure in which each of the seven C-T intervals (0.0 - 5.0 msec.) served as a dependent measure. This MANOVA contained four main factors: 3 between and 1 within factor. This four factorial design employed the between factors of Group (ICSS sites), Condition (locus of the C and T pulses, e.g., between versus within-site, and the order of pulse presentation), and Drug (saline vs morphine). The only within factor was subject.

Between-Site C-T Function

Procedure

All subjects first completed the within site C-T procedure which was described before entering the between-site condition.

The C-T "split pulse" technique hopefully proved to be a useful tool in measuring interactions. In one condition the C pulse was delivered to the LC at its within site intensity, and the T pulse delivered to either MFB, or FF, CC electrode. In the reverse condition the C pulse was delivered to the MFB, CC, etc., at its within site intensity and the T pulse delivered to the LC at its within site

intensity. For each pair, nine days of C-LC, T-MFB and nine days of C-MFB, and T-LC alternating in an a-b-b-a manner was tested over the seven C-T intervals each day. Which C-T interval were presented on a given day was determined by a latin square design.

The order of stimulation for each of the ICSS sites was determined by a counterbalanced, over the two six day series, of morphine and naloxone in combination. Thus, this abba procedure was repeated until each animal had received three injection days for each of the two C-pulse delivery conditions, e.g., C-pulse delivery to the LC first or the second (HYP) site first.

The current intensity chosen for each site was that same current employed by the within-site C-T condition for that specific site. The C-T intervals were again the values of 0.5, 0.8, 1.2, 1.5, 2.5, 5.0 msec., and a condition in which the T-pulse was omitted. The C-C intervals ranged from a minimum of 15 msec. to 60 msec. during the pre-baseline period. As in the within-site condition, the CC value which was most effective in producing a C-T function in which the ICSS response rates were below operant levels at 0.0 msec. and maximal at 5.0 msec. was chosen as the value for this interval. As in the within-site condition, all animals, with the exception of two, used the same C-C interval of 30 msec. These values were the same C-C values employed in Experiment I for each animal. The two animals

with variants in their C-C interval also had concomitant current threshold changes during their within-site drug testing phase. This S C-C interval was chosen to produce equal rates with those obtained during baseline control days in the within-site C-T condition.

The importance of the C-C interval for the interpretation of C-T interval response rate data has been emphasized by the work of Yeomans (1979) and others (Yeomans, Bielajew and Shizgal, 1979).

An interaction between ICSS sites was defined as the occurrence of response rates in the "split" pulse condition which were greater than the combined response rates obtained at the corresponding C-T interval in the within-site conditions for these same sites. The condition in which the C-pulse was omitted was chosen as the reference point for the definition of this neurophysiological interaction since this was the interval in which simultaneous stimulation of both sites occurred at their within-site intensities. This would then be the point of maximal current spread according to a current "spread" hypothesis, and summation therefore maximal and additive. To compare differences between the two C-pulse conditions over different C-T intervals, a two-way analysis of variance was performed.

Drug Administrations

After completion of this procedure, animals were administered sub-cutaneous injections of .9% saline for three days followed by six injection days of either 1.25 or 2.5 mg/kg of morphine sulphate. The effects of these injections were observed on the response rates of animals on each of their between site conditions, e.g., C = LC, C = 2nd site (HYP). All subjects then received on injection day naloxone HCl at 1.0 mg/kg and morphine sulphate at either 1.25 or 2.5 mg/kg. Which dose of morphine was administered, was determined according to a counterbalanced ABBA design. The reversibility of a morphine effect on ICSS and of naloxone indicated whether or not the observed effect is opiate specific. Animals then received three more injection days of saline followed by a four day sequence of D + L amphetamine.

Subjects were then returned to baseline control rates of responding during a six day injection series of .9% saline during which pulses were again "split" between electrode loci. The effects of 1.25 and 2.5 mg/kg of morphine sulphate was observed in this "split" pulse condition. This morphine series was again followed by two injection days of morphine plus naloxone, and a second four day d- and l- amphetamine isomer screen.

After completion of the first amphetamine injection sequence at 1.0 mpk, all animals then entered a second

morphine injection paradigm at 1.25 mpk. This morphine paradigm was described earlier. Following the completion of this second morphine 1.25 injection series, animals were then entered into a second series of the d- and l-amphetamine isomer screen at 1.0 mpk.

Nine subjects who finished the entire within-site and between-site C-T experiments were also entered into a second dosage condition in which they received an additional series of morphine (2.5 mpk in a 2 mg/ml solution) followed by a series of d- and l-amphetamine isomer injections at dosage of 2.0 mpk (2 mg/ml). These drugs were administered in the same manner as described earlier for the 1.0 mpk series of amphetamine in the present experiment. The order of C- and T-pulse delivery as well as the order of drug administration were again determined by a counterbalanced design.

Experiment II

Subjects

Twenty-four Sprague-Dawley rats were maintained as described in Experiment I. The apparatus, surgery, and histology were also as described in Experiment I.

Procedure

Animals were sterotaxically implanted with bi-polar electrodes aired at various diencephalic self-stimulation sites as described in Experiment I. All subjects were

tested after surgery using the monophasic pulse-pair technique described in Experiment I.

Drug Administrations

All subjects tested through ICSS rate stabilization, then entered the drug administration paradigm. This paradigm consisted of three days of .9% saline injections followed by a fourth injection day of naloxone hydrochloride (Endo Labs). The dosage of naloxone to be administered (1.0, 10.0, or 40.0 mg/kg) was determined by a counterbalanced design. This sequence of naloxone and saline was repeated until all animals completed three tests at each dose level. All subjects were then given three more days of saline followed by a fourth injection day of morphine sulphate at 1.25 mg/kg.

In a second phase of this experiment, to examine for a reverse tolerance to naloxone, all animals were then given a two week recovery period after which they were restabilized to baseline control rates of responding. Nine of these subjects then received three days of saline followed by a fourth injection day 1.0 mg/kg of naloxone. Subsequently, animals received three more days of saline followed by a three day series of 80 mg/kg of naloxone each day. On the final day of the experiment Ss were then again administered 1.0 mg/kg of nalone.

Experiment III

Subjects

Six subjects which had completed the within-site and the between-site ICSS studies in the present thesis (Experiment I) entered the clonidine paradigm after a three week hiatus. This inter-test interval was given in order to allow these animals to return to their pre-drug saline control rates of responding and to minimize any post-drug changes in receptor biochemistry. An additional 13 animals which had been implanted earlier in this thesis, but which had not been used in the earlier experiment were also employed in this study. The care and maintenance of all animals was as described in Experiment I of this thesis. The surgery, apparatus, and histology were also as described in Experiment I.

Procedure

After completing the shaping and baseline procedures outlined in Experiment I, all animals were entered into the drug administration schedule described below.

Drug Administrations

All animals received ten days of saline baseline testing days prior to the first drug administration. The drug administration schedule was that of three days of .9 percent saline administered subcutaneously followed by a

drug day injection sequence of .05, .1 or .5 mpk of clonidine (Rhone Co.) hydrochloride. All injections were equated in volume by using a concentration which reflected the dosage being administered to an animal. Animals repeated this sequence until they received three injections at each dosage level. The dosage to be administered on a given day was determined by a latin square design. Following this injection series, animals were then given three more days of saline followed by an injection day of morphine sulphate at 1.25 mpk.

Animals were then given three more injection days of saline followed by a fourth day in which they were injected with morphine sulphate (1.25 mpk) in combination with clonidine. This series of saline and clonidine plus morphine was repeated until each animal received clonidine at each of the three dosage levels. The dose of clonidine to be administered on a given day was determined by a counterbalanced design.

Animals completing this sequence of drug administrations then received three more injection days followed by a fourth injection day of morphine sulphate (1.25 mpk) plus naloxone (1.0 mpk). This series of injections was repeated until animals received two injections days of morphine in combination with naloxone.

Any animal completing the within-site C-T clonidine and morphine paradigm was entered into a between-site "split"

pulse procedure as described in Experiment I. These animals were then administered nine days of baseline in the "split" pulse condition (see Experiment I), and then entered a drug administration regimen which consisted of: 1) three days of saline; 2) nine days of the "split" pulse condition under morphine; 3) three days of saline; 4) nine days of the between-site condition under clonidine at the three dose levels; 5) three days of saline; 6) six days of clonidine (.1 mpk) administered in combination with morphine (1.25 mpk). The order of dosage administration and C-pulse delivery was determined by a counter-balance design. The order of C-T intervals was determined by a latin square design.

RESULTS

Histological

Twenty-four Ss completed the within-site C-T experiment for morphine at the 1.25 mpk dosage while nine subjects finished the 2.5 mpk morphine condition. There were thus 33 pairs of electrode tips to be localized at the end of the experiment. Twenty-three subjects had their stimulating electrode tips located in the nucleus locus coeruleus region while 10 subjects had electrodes localized in the area of the dorsal noradrenergic bundle around the periventricular gray. The exact location of each electrode tip for hypothalamic and brain stem placements appears for each animal in Table I. Hypothalamic placements included the medial forebrain bundle (n=8), the fields of Forel (n=12), the Cru Cerebri and internal capsule (n = 6), and the Zona Incerta (n = 7). For the purpose of future analysis, the ZI and FF placements were grouped together into the LC - FF group (Bodnar et al., 1980) and formed what Bodnar called the dorsal far-lateral hypothalamic group. A diagrammatic section from Konig and Klippel (1963) and one from Palkovitz and Jacobwitz (1974) for each level of the pons and hypothalamus appears in Figures 1 and 2. These sections contain the placements for each animal outlined in a circle. Figures 3 and 4 are representative sections of actual histologically prepared sections for both the pontine and hypothalamic placements. Figure 5 is a plate derived

from the work of Simantov et al. (1977) which depicts the variations in opiate receptor and enkephalinergic fiber density in four comparable plates from Konig and Klippel (1963) and the fluorescent histochemical maps of Palkovitz and Jacobwitz (1974).

Behavioral

The current intensities for all groups of Ss combined irrespective of site ranged between 100-650 microamps. The group of LC placements contained ICSS current intensities which ranged from 100 to 300 microamps, the fields of Forel animals displayed current levels of between 400 to 550 microamps, while the CC group placements ranged from 150 to 500 microamperes.

The effect of the C-T interval on response rates was first examined by formulating a composite index of response strength over all C-T intervals. First, the combined rates of responding over all C-T intervals were summed together for all subjects irrespective of site into a CSUM variable. This CSUM variable was subjected to a one-way analysis of variance in which CSUM was tested against the seven C-T intervals. This 1 x 7 ANOVA revealed a significant effect of C-T interval on response rate ($F=7.859$, $df = 1, 222$, $p < .0001$) as measured by the CSUM variable. Furthermore, the response rate C-T function was found to contain a significant linear component ($F = 14.20$, $df= 1$, $p < .0000$)

df= 1, $p < .0000$) and significant non-linear component ($F=1.792$, df = 121, $p < .0029$) ($r^2 = .71$). The data points employed in this analysis were obtained from the last 4 pre-saline ICSS within site baseline days for each of the animals who completed the paradigm. A Duncan Multiple Range analysis further revealed that the lower C-T interval values of 0.0, .5, and .8 msec. significantly differed with a value of 1.2 msec., and all four of these collectively (0.0 to 1.2 msec.) significantly differed with the larger C-T interval values of 1.5, 2.5 and 5.0 msec. (Duncan Multiple Range Test, $p < .05$).

Thus, in summary, the overall C-T response rate function exhibited the properties of differential responding at low versus high C-T intervals. This function displayed a non-linearity at the extreme values of 0.0 to 0.5 msec. and 2.5 to 5.0 msec., while maintaining linearity in the midrange values of 0.8 to 1.5 msec. An inspection of Figures 7 through 12 reveal this relationship between response rates and C-T interval during the saline (open circles) C-T response rate functions for each of the four ICSS of LC, MFB, CC and FF.

The differential effect of ICSS site of stimulation (LC, MFB, CC, FF) on the C-T response rate function was investigated by employing the CSUM variable. This CSUM variable was compared between each of the four ICSS sites in the present study by employing a 1 x 4 factorial analysis of

variance. This analysis revealed a significant difference between ICSS sites with respect to their overall C-T responding ($F = 9.8$, $df = 3, 198$, $p < .00001$). A Duncan Multiple Range analysis further indicated that the overall LC response rates were significantly different than both FF and CC response rates, that both CC and MFB response rates were significantly different than LC, and that the MFB significantly differed with LC and FF rates, while CC rates were significantly different than FF and LC response rates (Duncan Multiple Range Test, $p < .05$).

A further analysis of each individual C-T interval with respect to site was performed using a repeated measures MANOVA in which each of the 7 C-T intervals served as a dependent variable. This analysis was followed by a multiple comparison procedure of orthogonal polynomials to determine the relative difference between each mean response rate by site by C-T interval. This MANOVA analysis demonstrated a significant difference between groups at the C-T intervals of 1.2, 1.5, 2.5, and 5.0 msec. The response rates of subjects at 0.0 and 0.5 msec. displayed no significant differences between groups during their pre-saline baseline conditions (MANOVA, $p < .05$).

Morphine Within Site Effects

The effects of morphine at 1.25 mpk and 2.5 mpk for both representative individual Ss and the three treatment

groups are displayed graphically in Figures 6 to 12. In these Figures, the mean response rate for each group of subjects at each site is plotted as a function of the seven C-T intervals in msec. The open circles represent the pre-drug saline control days while the closed circles represent either 1.25 or 2.5 mpk of morphine sulphate depending on the drug condition illustrated in the legend. The closed triangles represent the injection days on which both morphine and naloxone (1.0 mpk) were given in combination. The vertical crossed lines illustrate the standard error of the mean at each C-T interval.

The effects of morphine on the C-T response rate function were statistically analyzed by the implementation of a repeated measures multivariate analysis of variance. In this procedure, each of the seven C-T intervals were tested as dependent variables by the independent measures of site stimulation, drug condition (morphine vs. saline) and dose (1.25 and 2.5 mpk). Naloxone reversibility of morphine's effects was defined as having occurred when the C-T response rate function under naloxone in combination with morphine did not differ by greater than one standard error of the mean with respect to the pre-morphine saline control day rates of responding. In all the following analysis, the first two days of post-drug saline baseline days were eliminated from the analysis in order to control

for any "carry over" effects between post-drug saline days and their previous drug condition treatment days.

The multivariate analysis of morphine's effect on each of the C-T intervals revealed a significant difference between morphine elicited response rates across C-T intervals as compared to their pre-drug saline control rates of responding (MANOVA, $F = 7.4$, $df = 8,408$, $p < .0001$). This overall main effect on the C-T interval was present at both the 1.25 and 2.5 mpk drug dosage administrations. Since this analysis produced seven source summary tables (one for each C-T interval) with corresponding orthogonal polynomial comparison tables, the author chose to enhance the clarity of the analysis by reducing the number of dependent measures.

In order to control for the variability between treatment conditions, which was produced by different control rates of responding, the 1.25 mpk data was converted to a percentage change value according to the formula:

$$\text{Percentage Change} = \frac{(\text{saline rates} - \text{drug rates})}{\text{saline rates}} \times 100$$

A one-way analysis of variance for repeated measures revealed that the four sites of LC, FF, CC and MFB significantly differed with each other with respect to their percentage change from baseline values (ANOVA, $F = 7.3$, $df = 3, 408$) at the 1.25 mpk. This difference was also found at

the 2.5 mpk dosage using the same procedure (ANOVA, $F = 5.71$, $df = 3, 112$, $p < .01$). A Duncan Post-Hoc analysis revealed that at the 1.25 mpk dose level the LC was significantly different than the MFB in percent change scores, while both of these were significantly greater than the FF and CC. (Duncan Multiple Range, $p < .05$).

Individual ANOVAS were performed for each subject. All ICSS supporting sites had a significant main effect of C-T interval. Morphine effects were also found to be significant on C-T interval and the drug by group interaction effect. There was also a significant overall effect of the drug condition. The observed morphine effects on ICSS rates were classified into three patterns according to Nelson (1977): facilitations, depressions, and no effect.

The individual data for each single S was also analyzed using a MANOVA procedure for repeated measures. This analysis yielded a series of summary source tables for each animal. The effects of morphine at 1.25 and 2.5 mpk are summarized below. Some representative data for the LC-MFB, LC-FF, and LC-CC animals appears in Figures 6, 8 and 10. These representative subjects (110 and 46N) data are plotted as mean responses per minute as a function of the C-T interval in msecs. The closed circles represent morphine injection doses, while the open circles represent saline control days.

In the LC group of placements at 1.25 mpk, 19 of 23 subjects demonstrated a significant increase at 3 or more C-T intervals, while four subjects demonstrated increments of at least two intervals. All 10 of the dorsal noradrenergic placements displayed significant response rates increases at 4 or less C-T intervals. Five of the LC subjects demonstrated a significant decrease in four or fewer intervals. These S had electrode placements that were ventral-lateral or ventral-medial to the LC.

Subjects were sub-divided on the basis of their LC electrode placements in LC-FF group of subjects. This was done in order to attempt to parcel out the variance in this group's LC morphine scores at 1.25 mpk. These LC group of placements displayed smaller and fewer significant response rate facilitations as a group when they were compared to the other two groups of LC placements (MANOVA, p .05). The basis for this differentiation was formulated on the findings of Nelson (1977) who demonstrated that morphine induced rate facilitations could be obtained from animals with electrodes implanted in or lateral to the nucleus coeruleus or dorsal noradrenergic bundle. Conversely, animals with placements which were located both ventral-medially and ventral=laterally to this region demonstrated rate depressions.

Five subjects were separated from the LC group of placements who demonstrated response suppressions in four or

more intervals (46M, 57N, 390, 71P, and 790) at both doses of morphine. These subjects also had their electrodes localized in either the pariaqueductal gray (PAG), just dorsal to the PAG or in the region ventral and medial to the nucleus locus coeruleus. Three other subjects who demonstrated response suppressions at at least two intervals but were not included in this LC-PAG group had electrodes in the brachium conjunctivum, the dorsal tegmental nucleus, and just ventral to the nucleus of the mesencephalic V. The data for the five LC-PAG subjects as a group under 1.25 mpk of morphine is depicted in figure 12. The remaining 15 subjects in the LC-FF groups are plotted in the lower portion of figure 12.

In the MFB group, six subjects displayed response rate increases while two demonstrated decrements at five or fewer C-T values. In the CC group, three subjects showed rate increments at two intervals, while two subjects demonstrated no effect at any interval. One CC subject demonstrated a suppressive effect at two intervals.

The FF group of placements had eight subjects who showed response rate increments at five or three intervals, two subjects demonstrated ICS rate decrements at two C-T intervals, while two Ss showed no effect.

All subjects tested with the 1.0 mpk dose of naloxone in combination with morphine sulphate demonstrated response

rates which approximated saline control rates by plus or minus one standard error of the mean.

In five of 23 subjects, naloxone not only reversed the morphine effect, but further lowered rates significantly below 50% of their control rates (ANOVA, $F = 6.0$, $df = 2, 23$, $p < .001$). This effect was significantly greater in the MFB Ss as compared to either FF or CC placements (ANOVA, $F = 3.16$, $df = 2, 23$, $p < .0001$).

2.5 mpk Administration of Morphine Sulphate Results

Of the nine subjects who finished the 2.5 mpk paradigm, five had placements in the dorsal noradrenergic bundle while the remaining four subjects had placements in the LC. Of these four LC placements, three of these were more medial and impinged on the aqueduct, while the last was localized more ventrally.

Three subjects had medial forebrain bundle placements, two Ss had H2 fields of Forel placements, and the remaining two placements were in the Zona Incerta. Only one cru-cerebri placement was used at the 2.5 mpk dosage level. Of the nine animals who completed the 2.5 mpk dose, six of these entered the paradigm for the first time while the remaining three subjects had previously undergone the 1.25 mpk dosage administration, and the remaining within site and between site paradigm.

In summary, eight of the nine LC placements in the locus coeruleus produced response rate facilitations in the morphine condition of 2.5 mpk. These facilitations were found to be significant using the aforementioned MANOVA procedure. These facilitations were found to occur at three or more intervals across animals.

Six of nine hypothalamic and far-lateral hypothalamic placements demonstrated response rate facilitations. At a 2.5 mg/kg dose of morphine, all MFB subjects (n = 8) showed rate facilitations which were significant at three or more intervals. One ZI and two FF Ss demonstrated a significant response rate increment at two intervals while the remaining ZI animal facilitate only at the 0.5 msec. C-T interval. The CC subject demonstrated a significant response rate suppression at two intervals.

The injection of naloxone plus morphine also eliminated the effects of morphine sulphate, whether depression or facilitations in all cases. The criteria of plus or minus one standard error return to baseline rates were again employed as the criteria for determining the efficacy of naloxone reversal.

Within-Site D- and L-Amphetamine

Figures 13, 15 and 17 illustrate the mean response rate for each group of ICSS subjects (e.g., LC, FF, etc.) plotted

as a function of the C-T interval in msec. for each of the drug conditions of d-amphetamine, l-amphetamine and the saline control condition. Also included in this series of figures are representative data for the individual subjects in the LC, MFB and LC-FF conditions. The LC-CC subject data were not clearly represented by any particular pattern of responding, so therefore these figures for representative subjects are omitted from these figures. The data for each of these drug conditions was analyzed by employing a multivariate analysis of variance in which the drug condition at each dosage level was tested for each of the seven C-T intervals. In this MANOVA for the 1 mpk data, the overall Hotelling F-test was found to be significant ($F = 5.08$, $df = 49$, $p < .0001$). Furthermore, this overall ANOVA revealed a significant effect of the drug condition d-amphetamine as compared to control saline rates of responding ($F = 5.8$, $df = 49$, $p < .0001$), as well as between the d-amphetamine condition versus the l-amphetamine condition ($F = 2.91$, $df = 165$, $p < .05$). These effects were significant for each of the multivariate dependent variables of C-T interval.

A comparison of the individual treatment groups was performed on the MANOVA data for the amphetamine conditions by implementing an orthogonal polynomial multiple post-hoc comparison. This analysis illustrated that the LC animals' saline rates were significantly different than those of the

CC animals; the saline control rates of the FF group were also significantly different than the CC group (Orthogonal Polynomials Contrast, $p < .01$). The LC animals control saline rates were also significantly different than the FF group, while the MFB animals did not significantly differ with the LC saline rates, but did differ from the FF and CC placements (Orthogonal Polynomials Contrasts, $p < .01$). The LC saline control rates did not significantly differ between the three treatment groups of LC-MFB, LC-CC and LC-FF.

This three factorial MANOVA for repeated measures was also examined for the specific effects of drug and group membership on the individual C-T interval dependent measures. This analysis can be summarized as follows: 1) there was a significant effect in the group by drug interaction condition between l-amphetamine and group membership ($F = 2.05$, $p < .0001$). This effect was significant at the C-T intervals of 0.8 to 5.0 msec. ($F = 13.76$, $df = 7, 4, 8$, $p < .0001$), but not for the 0.0 or 0.5 msec. values; 2) in addition, the main effect of the drug between the eignevalue roots of 5, 6 and 7 were found to significantly differ from each other ($F = 6.9$, $df = 49$, $p < .001$). These eignevalue roots represented differences between the LC saline ICSS rates, the MFB saline control rates, and those induced by l-amphetamine in the locus coeruleus ($F = 6.3$, $F = 3.9$, $df = 49$, $p < .001$); 3) the group parameter was also found to be significantly different

between the LC-MFB animals and both the LC-FF and LC-CC groups at all C-T values; 4) all treatment groups, e.g., LC-CC, LC-MFB, LC-FF, significantly differed from one another at all conditions at a C-T value of 1.2 msec. and at all subsequent higher C-T values (Orthogonal Polynomial Contrasts $p < .0001$).

A repeated measures MANOVA was also performed on the individual subject data under the d- and l-amphetamine drug conditions. This analysis indicated a significant d- or l-amphetamine effect in all subjects tested over days. This analysis by subject is further examined in a subsequent section.

An overall analysis of variance was also performed on the group data using the CSUM variable. This test revealed a significant d-isomer effect in both the LC and MFB of the LC-MFB group, and in the LC sites of the LC-CC and LC-FF groups. The LC-FF animals similarly demonstrated a significant d-isomer, but not an l-isomer, facilitation effect with respect to their saline control rates of responding in the FF site. All LC placements in all groups demonstrated a significant L-isomer rate facilitation effect.

A percentage change score (PCS) was again employed in a series of repeated measures ANOVAS in order to equate the three treatment groups on the basis of baseline control rates of responding, and thus reduce the intersubject

variance. The percentage change scores for each group were based on the CSUM variable data point, and this value was entered into the PCS formulas. The PCS for each treatment group of subjects at each site under the conditions of the d- and l-isomer of amphetamine at 1.0 mpk and of morphine at 1.25 mpk are depicted in Table 4. The 2.0 mpk data was excluded from this analysis, as was the 2.5 mpk morphine data, since not all Ss received all of these doses in repeated measures design fashion. The 2.0 mpk and 2.5 mpk doses of amphetamine and morphine will be discussed in a separate group design paradigm.

Table 4 clearly illustrates that the LC-MFB animals demonstrated the greatest percentage change scores across all drug conditions. Furthermore, the d-isomer showed a greater potentiation in rates in the d-isomer condition than in the l-isomer condition for the LC groups in the LC-MFB and LC-CC groups, but not for the LC-FF subjects. An analysis of variance performed on this data revealed that the LC change score under morphine was significantly greater in the LC placements in the LC-MFB group than in the other two groups of LC placement groups, e.g., LC-FF, etc. However, the LC morphine PCS as a group were significantly greater than the morphine PCS in all other groups (ANOVA, $p < .01$; Duncan Post Hoc, $p < .05$). The d-isomer PCS was significantly greater than l-isomer PCS in the LC groups of the LC-MFB and LC-CC animals, but not in the LC-FF subjects.

The d-isomer and l-isomer PCS scores were also equipotent and not significantly different in the FF and MFB groups (Duncan Post Hoc, $p < .05$). The LC-CC placements demonstrated significant rate facilitations which were greater in their PCS values in both the LC and CC as compared to the PCS values obtained under the l-isomer condition.

In the nine subjects which received both the 1.25 mpk and 2.5 mpk morphine dosage levels, the differential effects in PCS was maintained between the d- and l-isomer in the LC groupings. The PCS under morphine was also significantly greater in the LC group as compared to the other treatment conditions (Duncan Post Hoc, $p < .05$). However, at the 2.0 mpk dose, the MFB percentage change score under d-amphetamine was significantly greater than l-isomer, while the CC l-isomer percentage change score increased significantly over the l-isomer response at 1.0 mpk, but this l-isomer effect was still significantly different than the d-isomer at 2.0 mpk. When the 1.0 and 2.0 mpk PCS values were compared in an ANOVA design for unequal sample sizes, using the PCS scores as the dependent measure, this analysis showed that the LC and MFB PCS did not significantly differ between their 1.0 mpk and the 2.0 mpk doses, under either the d- or l-isomer. The PCS for the FF and CC groups were significantly greater for both the d- and l-isomer, as compared to the MPK PCS values, but the PCS

under morphine at 2.5 mpk did not significantly differ with the PCS obtained at 1.25 mpk.

The present study employed the electrode tip classification scheme discussed by Nelson (1977). It became apparent in that study that a simple dichotomy between d- and l-sites was not possible. This was particularly true in the l-isomer data. The present study and other authors (Nelson et al., 1977; Tempel, 1982) had difficulty in distinguishing the d-sensitive and l-sensitive sites with respect to the efficacy of the l-isomer. Thus, l-amphetamine failed to significantly increase ICSS rates in all tips as compared to their saline control rates of responding. Therefore, a three category classification was employed to delineate the effects of these isomers. These categories included those 1) tips which demonstrated a significant d-isomer effect which was not significantly different than the l-isomer (L+), but in which the l-effect was significantly different than saline control days 2) those tips in which there was significant d-effect as compared to the l-effect, but in which the l-effect (L-) was not significantly different than saline 3) those tips in which the d-isomer was significantly stronger than the l-isomer, and both isomers showed significant differences from saline control rates (D+).

This classification resulted in 14 of the LC placements being labelled (D+). 14 being called (L-) and 5 tips were differentiated as (L+).

The MFB group contained five rats who were classified as (D+), 2 which were (L+) and 1 which was (L-). In the FF-ZI placement group 1 was (D+), 14 were deemed (L-), and 4 labelled (L-). The CC placements contained no (D+), 4 (L-) sites, 1 (L+) area, and 1 subject in which L was greater than the d-effect. The results of arranging these tips by classification scheme showed that norphine facilitations occurred more frequently in the D+ tip population and in the absence of a morphine-induced rate depression. This effect was independent of which dose of morphine was examined. The L placements were ones which produced significant depressions under morphine in at least 1 C-T interval.

These neuroanatomical sites were further examined statistically on the basis of their responsivity to the d- and l-isomer of amphetamine according to Nelson's (1977) criterion discussed earlier. The results of this division revealed that 31 out of 33 animals displayed a significant d-response which was greater than the l-isomer response. The total number of D placements was significantly greater in the MFB and LC than they were in the ZI, FF and CC. Of the LC subjects 28 out of 33 demonstrated a significant d-response which was greater than the l-isomer response and in which the l-effect was significantly different than saline

control rates (D+). In the MFB, 7 out of 8 animals displayed a significant D+ relationship, while 5 of 19 FF-ZI Ss, and 1 of 7 CC subjects was classified as D+. (MANOVA for repeated measure, $p < .05$).

Furthermore, the LC placements were examined with respect to neuroanatomical locus to determine the nature of the differential responsivity of the LC grouping in the LC-FF animals and those of LC-CC and LC-MFB subjects. This analysis revealed a greater number of ventral medial LC placements and DNB placements in the LC-MFB and LC-CC subjects than in LC-FF LC placements.

To further delineate this PCS phenomena, a multiple linear regression analysis was performed on the PCS values to determine which predictors were the best indicators of the PCS morphine facilitations. In the MLR, the value of each PCS for each site was included as predictors, as were the baseline and saline control rates of responding. Several sequential MLR analyses were performed in order to select out any covariant factors. The final regression equation revealed that for the LC-MFB subjects, the d-amphetamine at each site effect was the best predictor of morphine rate facilitations at each respective site; for the LC-FF subjects, the LC and FF l-isomer PCS was the best predictor of each of these respective sites; for the LC-CC group, the LC was best predicted by the LC d-isomer response; and the CC morphine facilitation was best

predicted by the LC d-isomer PCS and then the CC d-isomer PCS (see Table 5).

C-T Between-Site Morphine Interaction Analysis

The C-T data for the between-site "split" pulse condition was examined in order to determine if there was an overall effect of the locus of the C-pulse presentation and the order of site stimulation. An interaction between sites was defined as occurring when the response rates obtained when the C and T pulses were divided between two electrode sites was greater than the sum of responding obtained at each ICSS locus individually during the C-T interval of 0.0 msec. (T-pulse is omitted). The dependent measure in this analysis was the sum of responding obtained over the seven C-T intervals (CSUM variable). Each of the three treatment groups was examined in a series of repeated measures ANOVAS using the CSUM variable. These ANOVAS investigated the relationship between the following conditions: 1) the condition in which the CSUM variable represented the combination of response rates obtained at each site at a C-T value of 0.0 msec. in the within-site single ICSS function condition; 2) the condition in which the CSUM variable represented the combination of response rates obtained when the C-pulse was delivered to the LC site first and the T-pulse to the second site summed across the seven C-T intervals; 3) the condition in which the CSUM variable

represented the combination of response rates obtained when the C-pulse was delivered to the second site in a treatment group first (e.g., FF) and the T-pulse to the LC site second summed across the seven C-T intervals; 4) the condition in which the CSUM variable represented the individual within-site response rates obtained at a C-T value of 5.0 msec. for the LC and each of the designated treatment group's second site.

The control rates employed in this analysis were those obtained during the 18 day saline interaction baseline period, and those rates obtained under comparable control saline days during the within-site morphine paradigm. The results of these analyses revealed that for each treatment group of LC-MFB, LC-FF and LC-CC, there was a significant C-T between-site interaction effect. Thus, all animals in each group displayed a significantly greater rate of responding across C-T intervals when the C and T-pulses were "split" between sites, than when each ICSS site was stimulated independently (C-T = 0.0 msec.)

For the LC-MFB animals, the overall interaction effect was significantly different than the C-T control rates obtained by combining the C-T interval response rates obtained at 0.0 msec. in the LC with 0.0 msec. rates obtained from the MFB ($F = 179.34$, $df = 2, 123$, $p < .000$). Furthermore, the rates differed with control rates irrespective if the LC or the MFB was stimulated first. The

rates of responding when the C-pulse was delivered to the LC first, also did not significantly differ from the interactions produced when the MFB was stimulated first. However, all rates obtained across C-T intervals were significantly different than those obtained at a C-T interval of 5.0 msec. for both the LC and the MFB within-site condition.

The LC-FF and LC-CC animals also demonstrated significant ICSS interactions between sites using the CSUM variable as a dependent measure ($F = 75.59$, $df = 2, 270$. $p < .000$; $F = 158.3$, $df = 2, 95$, $p < .000$). However, this effect in these groups depended upon which site was stimulated first. Thus, this effect was significantly greater for the condition in which the C-pulse was delivered to the LC first, than when the second site (FF or CC) was stimulated first.

A series of MANOVAS for repeated measures and a trend analysis was also performed on this interaction data in which the seven C-T intervals each functioned as a dependent measure. The results of these procedures were: first, the C-T response rate function in the interaction between-site condition demonstrated an overall significant linear trend at 0.0 and 0.5 mse. C-T intervals; secondly, a cubic component which entered into the function at between 1.2 msec. to 1.5 msec.; and finally, a linear component at the

C-T values of 2.5 to 5.0 msec. (Orthogonal Polynomials, $p < .000$).

To more specifically delineate the locus of the interaction effect between sites, the author analyzed the data with a series of MANOVAS for repeated measures in which the seven C-T intervals were tested by group and by the locus of the C-pulse (e.g., C-pulse LC versus C-pulse MFB) as well as by the within-site C-T condition at each site. This three-factorial design revealed that the LC-MFB group did not demonstrate significantly different interactions between the condition in which the C-pulse was delivered to the MFB first at only the C-T values of 1.2, 1.5, 2.5 and 5.0 msec. (Orthogonal Polynomials, $p < .000$). However, this group's interactions did significantly differ with the within-site rates obtained at each site over all seven C-T intervals.

The LC-FF subjects demonstrated significantly greater rates when the LC was stimulated first than in the reverse condition when the FF site was stimulated first at C-T interval values of 0.0 to 2.5 msec. The between-site interaction condition rates in which the LC first received a C-pulse also significantly differed with both the within-site C-T functions at these same values. The between-site condition in which the C-pulse was delivered to the FF first significantly differed with the within-site conditions in

the LC and the FF only at values of 2.5 and 5.0 msec. (Orthogonal Polynomials, $p < .000$).

The LC-CC animals demonstrated significantly greater rates when the LC and not the CC was stimulated first at the C-T interval values of 0.8 to 2.5 msec. These animals also demonstrated significantly different rates between the within-site functions at the LC and CC and those obtained in which the LC was stimulated first and those in which the CC was stimulated first at C-T values of 0.0 to 5.0 msec.

In general, the LC-MFB interactions were greater than those obtained in the LC-FF condition and those obtained in the LC-CC condition (Orthogonal Polynomials, $p < .000$). while LC-FF interactions were not significantly different than LC-CC interactions. However, in all cases the C-pulse in the LC condition was greater than the C-pulse in the FF or CC condition. Furthermore, when the C-pulse was delivered to MFB first, the response rate functions obtained were always significantly greater than those obtained when the C-pulse was delivered to either the FF or the CC first.

Morphine Overall Effect

Each of the treatment groups was also examined individually with a series of 2 x 4 MANOVAS for repeated measures in which group membership was held constant in each analysis. The within-variable in this analysis was nested in days and the subject variable was excluded from the

procedure. This analysis provided an equal N in all cells for all Ss within a group, and a series of orthogonal polynomials was performed after each analysis for both the Conditions and Drug Factors. This MANOVA provided for a specific analysis of each group's specific LC and second site placements.

In this analysis, each of the seven C-T intervals again functioned as dependent variables which were tested by Condition and Drug. The Condition Factor represented the locus of the C and T-pulses and their order of presentation, while the Drug Factor delineated the saline and morphine conditions. The Condition (COND) Factor contained 4 levels: 1) C and T-pulses both within the LC 2) the C and T-pulses both within the second site 3) the C and T-pulses "split" between sites with the C-pulse being delivered to the LC first and 4) the C and T-pulses "split" between sites with C-pulses being delivered to the second site first.

The procedures revealed that the LC-MFB group demonstrated an individual group MANOVA significant overall F-ratio ($F = 1.8$, $df = 2, 806$, $p = .015$) for the COND by Drug interaction effect. This effect was significant only at the C-T intervals of 1.2 and .5 msec. ($F = 4.106$, $df = 1, 826$, $p = .00071$; $F = 2.7$, $df = 1, 826$, $p = .046$). The overall effect of Drug was also found to be significant ($F = 13.633$, $df = 7, 820$, $p < .000$) and this effect was specific at all seven C-T interval values ($F = 44$ to 68, $df = 6, 808$,

p = .000). The condition effect was also found to be significant (F = 2.5, df = 21, 806, p = .000). This effect was significant at all seven C-T intervals value (F = 10.7 to 60.34, df = 7, 811, p = .00001).

The within-site and between-site condition were significantly different between 0.0 to 1.2 msec. (Orthogonal Polynomial, p = .001). The within-site conditions and the between-site condition in which the C-pulse was delivered to the LC first at the C-T values of 1.5 to 5.0 msec. (Orthognonal Polynomials, p. < .000). The effect of Drug was significant over all C-T intervals while the Condition by Drug interaction was found to be significantly different at 1.5 to 5.0 msec. (Orthognonal Polynomials).

Morphine Between-Site Effects by Group and by C-T Interval

Figures 18 to 23 depict the mean response rates obtained under saline (open circle) and those obtained at either 1.25 or 2.5 mpk of morphine sulphate (closed circles) plotted as a function of the C-T intervals in msecs. Figures 18, 20 and 22 are representative data for single subject data for each of the three treatment conditions of LC-MFB, LC-FF and LC-CC. Each of these figures illustrates the condition in which the C-pulse is delivered to one of the three second sites (MFB, FF, CC) first. The group data for each of the three treatment conditions also appears in

the remaining figures in this series (Figures 19, 21, and 23). The closed triangles in this group data represents the effects of naloxone hydrochloride at the dosage of 1.0 mg/kg.

The effects of morphine on C-T responding were first examined by collapsing these scores across groups C-pulse condition in order to address the question of whether there was an overall drug effect of morphine versus the saline control rates on the C-T interaction conditions. Thus, a three factorial MANOVA of group by drug revealed that there was an overall significant effect of the drug condition (morphine) on the C-T response rates interactions produced between sites (Hoetelling, $F = 2656$, $df = 14, 563$, $p < .000$). There was a significant drug effect at each C-T value. In addition, there was also a significant drug by group interaction at the C-T values of 1.2 to 5.0 msecs. The effect of group was also significant ($F = 5.43$, $df = 14, 563$, $p < .000$). The LC-MFB group significantly differed from the LC-FF and the LC-CC groups, while the LC-FF differed from the LC-CC and LC-MFB groups ($F = 5.37$, $df = 14, 563$, $p < .000$; $F = 2.2$, $df = 6, 178$, $p = .049$) (Dimension reduction analysis). This group effect was significant for all seven C-T values ($F = 19.37$ to 29.85, $df = 2, 569$, $p = .0001$). An orthogonal polynomial analysis revealed that for all groups there was a significant effect at all C-T intervals for the drug and group effect. There

was a significant drug by group interaction effect for the LC-MFB and LC-FF at the C-T values of 0.8 to 5.0 msec. (Orthogonal Polynomials, p = .000, .048, .021, .019, .036).

The LC-FF group demonstrated a significant overall effect of Drug (F = 3.0, df = 7, p = .006), Condition (F = 14.6, p < .000) and the Drug by COND interaction (F = 1.5, p = .06). For the Drug condition there was a significant difference at all seven C-T interval values (F = 9.1 to 17.67, p < .001). The condition effect was significant at the C-T values of 0.0 to 5.0 msec. (p = .0007, .0010, .0005). COND was significantly different for all within-site and between-site levels at C-T values of 0.0 to 1.2 msec., while the drug effect was significant at all seven C-T intervals (Orthognonal Polynomials). There was no significant COND by Drug interaction effect.

For the LC-CC group there was a significant drug effect overall (F = 2.284, df = 7, 143, p = .031), Condition Factor (F = 6.973, df = 21, 425, p < .000), and COND by Drug interaction (F = 3.341, df = 21, 425, p < .000). This COND by drug effect was significant at all seven C-T intervals (F = 2.67, 5.7, 7.98, 8.88, 6.72, 4.182, 4.69, df = 3, 149, p = .049, .001, .000, .000, .007, .004). The drug effect was only significant at the C-T interval of 0.8 msec. (F = 10.19, df = 1, 149, p = .002), while the COND factor was significant at all seven C-T intervals (F = 4.74, 6.85,

4.02, 21, 85, 18.61, 20.36, 25.63, df = 3, 149, p = .003, .000).

Orthogonal contrasts revealed significant differences between all within and between-site levels of COND at the C-T values of 1.2 msec. or greater (p = .000). Within-site of LC and CC C-T functions were significantly different with the between-site condition in which the C-pulse was delivered to the LC first at C-T values of 0.5, and 0.8 msec. At the values of 0.0 only the LC and the condition in which the C-pulse was delivered to the LC first differed from each other. The drug effect was significant only at the interval of 1.2 msec. The COND by drug interaction was significant at the C-T values of 0.8 msec. and greater.

The LC-FF group analysis revealed a significant effect of Drug (F = 1.57, df = 21, 251, p = .05) at C-T values of between 0.0 and 1.2 msec. (F = 2.83, 3.6, 3.05, 2.88, df = 3, 269, p = .031, .031, .014, .029, .036). Orthogonal contrasts revealed that the drug was significantly different between the Condition Factor when the C-pulse was delivered to the LC and the within-site LC C-T function at 0.0 to 1.2 msecs. The within-site LC function was

The between-site C-T data under morphine and saline were converted to percentage change scores as detailed earlier. The three treatment groups of LC-MFB, LC-FF and LC-CC were then analyzed by employing these PCS values in the analysis of variance in which the PCS values were tested

against the two between-site conditions of C = LC and C = 2nd site. This analysis revealed that the morphine PCS scores for the LC-MFB group did not significantly differ when the C-pulse was delivered to either the LC or MFB first (Reliability ANOVA, $p < .05$). Both the LC-FF and the LC-CC group demonstrated a significant differential effect depending on whether or not the C-pulse was delivered to the LC first. For the LC-FF group, the morphine effect was greater when the C-pulse was delivered to the LC first (Reliability $p < .05$) while for the LC-CC group the effect was greater when the C-pulse was delivered to the CC first.

A multiple linear regression analysis was performed on the percentage change scores for the between-site C-T data. In this analysis, the between-site condition PCS values were used as an outcome or criterion variable while the within-site C-T morphine, d- and l- amphetamine isomer and saline control days data were used as the predictors. The results of this analysis appear in Table 13. The PCS values for the within-site morphine test days were the best morphine predictor of the between-site condition in which the C-pulse was delivered to the MFB first was the morphine within site condition for the MFB. This was followed by the within morphine LC condition and then the within site d- and l-isomer effects on the MFB. The same distribution of predictors was seen in the between condition in which the C-pulse was delivered to the LC first.

In the LC-FF group, the morphine within-site values for the LC were found to be the significant best predictors of both between-site conditions, e.g., C = LC versus C = FF (MLR, $F = 7.5$, $df = 3,268$, $p < .01$). The condition in which the C-pulse was delivered to the FF first was also sensitive to the LC d- and l-isomer data and then the d- and l-data for the FF followed as predictors. However, the order of predictors for the condition in which the LC was stimulated first revealed that the LC and FF d-isomer data preceded the l-isomer effects in these sites.

For the LC-CC group, the within-site LC morphine was the strongest predictor of the between-site morphine interaction effects when the C-pulse was delivered to LC first. However, this effect was not significant. The order of predictors was then the d-isomer effects in the LC and CC followed by the l-isomer effects. In the reverse condition, when the C-pulse was delivered to the CC first, the CC within-site morphine data was followed by the morphine within-site data. These predictors were then followed by the LC d-isomer and CC l-isomer data. The beta weight relations in all cases for all treatments suggested that the relationship between the morphine interaction effects and the within site morphine effects were inversely related to each other. This inverse relation was small (-.1) and suggested that slightly smaller morphine facilitations in the within-site condition were associated with a somewhat

larger between-site C-T effect. However, the small r2 values suggest a predictable derivation from linearity as suggested by the trend analysis.

Between-Site D- and L-Amphetamine

An analysis of variance performed on the C-T interval data points revealed that both the main effects and the interaction effect of Drug and Group (site of stimulation) were significant at each of the three parameters (ANOVA, $p < .00001$, $p < .001$). This analysis indicated that the effect of amphetamine on ICSS depended on both the isomer which was administered and the ICSS site being stimulated during an experimental session.

A post-mortem analysis (Duncan Test) also revealed the following relationships:

1)that the overall effect of the d-isomer was always significantly greater than the l-isomer effect on ICSS in each of the different C-pulse conditions e.g., C-pulse delivered to the LC versus C-pulse delivered to HYP site.

2)that there was a significant d-isomer rate facilitating when responding under amphetamine was compared to that obtained during saline control days.

3)that the effects of the l-isomer of amphetamine were significantly different than saline control

rates only when this comparison was performed at the 1.2 msec. data point.

An analysis of the effect of group membership (ICSS site grouping) on ICSS rates revealed that the LC-MFB group significantly differed with both the LC-FF and LC-CC groups. The LC-FF group also significantly differed with the LC-CC group, but only at the long C-T intervals (1.5 to 5.0 msec). The overall effect of the group by drug interaction indicated that the LC-MFB group demonstrated a significantly greater d-isomer effect than their l-isomer effects across C-T interval values (Duncan Test $p < .05$). The LC-FF and LC-CC groups demonstrated a significant difference between the d- and l-isomers of amphetamines only at the short duration C-T intervals and at the longer C-T intervals when the C-pulse was delivered to the LC first. The order of C-pulse did not serve to differentiate the LC-MFB group in that the d-isomer effect was always greater in magnitude than the l-isomer effect across C-T intervals. The LC-FF group could also be differentiated on the basis of the d-isomer, but again only when the C-pulse was delivered to the LC first at a CT of 1.5 msec.

Multivariate Analysis

A repeated measures multivariate analysis of variance was also performed on this d- and l-isomer amphetamine data in order to determine the specificity of this drug on

individual C-T intervals. Responses per minute is plotted as a function of each of the seven C-T intervals for each of the two C-pulse conditions, e.g., C = LC, pulse delivered to the LC etc.. The values obtained from the overall Hoetelling F-test performed on each of the C-T intervals by subject ranged from F = 5.6 to F = 6.7 (MANOVA, $p < .000001$). The effect of condition (C-pulse location) was significantly different between groups at the value of 0.8 msec. ($F = 4.2$, df = 77,569, $p = .049$) msec. and at 1.2 msec. ($F = 5.1$, df = 77,569, $p = .05$). The drug by subject interaction was also found to be significant in the univariate ANOVA of the MANOVA procedure ($F = 4.5$, $p = .032$). This effect was significant for the C-T values between 0.5 to 1.5 msecs. ($F = 3.3, 4.66, 4.77$, $p < .043, .05, .047$).

The 2 mpk dosage of d- and l- amphetamine in the between-site condition was difficult to analyze due to small number of animals who had completed the entire paradigm from each of the treatment groups. Thus, the unequal and insufficient number of cases allowed for only a within subject individual analysis of the three treatment groups: LC-MFB (N = 4), LC - FF (N = 2), and the LC-CC (N = 3). A reliability repeated measures analysis of variance using the CSUM variable revealed that the d-isomer and the l-isomer rates under the two doses of this drug were not significantly different from each other. However, the d-

isomer and the l-isomer at the 2 mpk dosage level was effective in producing a rate facilitation in two of the LC-MFB animals and in one LC-CC subject (10 P,11P, and 1 Q) when such facilitations were not obtained at the 1.0 mpk dosage level in these same animals.

In this analysis the main effects of group membership, condition (locus of the C-pulse), and the drug effect (saline versus the d- or l-isomer of amphetamine) were tested using each of the seven C-T intervals as dependent measures. This procedure revealed that the overall effect of group by condition by drug was significantly different at the shorter duration C-T intervals of 0. 0 thru .08 msec. ($F = 2.62$, $df = 14, 164$, $p = .002$). The principal component which best discriminated this effect that of C-pulse location (Condition). Thus, the best delineation of the d- and l-isomer effects could be accomplished by the examination of the order in which the C-pulse was delivered to either the LC or HYP site ($F = 2.5$, $df = 14, 166$, $p = .003$). The effect of group membership alone was not significant. However, when the variable of subject was entered into a nested within-subjects repeated MANOVA in addition to those mentioned earlier, this variable was found to be significantly different at each of the 7 C-T interval values ($F = 2.21$, $df = 77,569$, $p < .00001$).

The data for both representative single subjects and that of the three treatment groups under the d- and l-isomer

of amphetamine at each dose level appears in figures 24 thru 27.

A multiple linear regression analysis was also performed on the d- and l-isomer interaction data in the between-site "split" pulse condition. The percentage change scores (PCS) obtained during the administration of the two isomers and saline control days (see the within-site CT condition for a description of this PCS score) functioned as the criterion variables in this analysis. The predictor variables in this regression included: 1) the within-site morphine PCS values: 2) the within-site d- and l-isomer data; 3) the locus of the electrode placement; 4) the within-site saline CSUM values; 5) the between-site C-T "split" pulse PCS values obtained in each C-pulse condition; as well as 6) the drug dosage. The criterion variables were also separated on the basis of C-pulse locus such that for each of the two C-pulse conditions under each of the two isomers of amphetamine there existed a separate regression analysis.

In all cases, the best predictor of the d-amphetamine interaction response rates in the LC-MFB and LC-FF groups was the between-site percentage change scores obtained under morphine when the C-pulse was delivered to the LC first ($F = 11.64$, $p < .01$). However, the LC-CC group failed to demonstrate a significant predictor for either isomer in the between-site condition. This failure to obtain a

significant result may be in part due to the small number of subjects in this regression procedure.

An analysis of variance performed on the LC-MFB PCS values revealed that the d-isomer was significantly more effective than l-isomer in its rate faciliatory effect on ICSS behavior regardless of which site was stimulated with the C-pulse first (Reliability ANOVA, $p < .05$). This differentiation between isomers was also true for the LC-FF animals, but was only found to be significant when the LC was stimulated first. The LC-CC group of animals exhibited a d-isomer effect which was greater than the l-isomer only when the C-pulse was delivered to the CC first (ANOVA, $p < .05$).

The LC-MFB group demonstrated a significant increase in their ICSS rates under the d-isomer of amphetamine in both of the C-pulse conditions. However, the PCS scores in each of these C-pulse conditions did not significantly differ from each other in magnitude (Reliability ANOVA, $p < .05$). In contrast, the LC-FF group demonstrated a significantly greater rate facilitation under d-amphetamine when the LC was stimulated with the C-Pulse first. This differentiation was also true for the l-isomer of amphetamine. The LC-MFB group demonstrated little or no effect to the l-isomer in either C-pulse condition. The LC-FF group demonstrated a significant increase in their PCS values under the l-isomer of amphetamine only when the C-pulse was delivered to the FF

first. Thus, the percentage change in ICSS rates relative saline control rates under the l-isomer was always greater when the FF, but not the LC received the C-pulse first. The LC-CC group of placements also demonstrated a significantly greater d-isomer effect on ICSS than an l-isomer effect. However, the d-isomer had no effect when it was administered in during the condition in which the C-pulse was delivered to the LC first. In contrast, the d-isomer significantly increased ICS response rates when the C-pulse was delivered to the CC first. The l-isomer of amphetamine had no significant effects in either C-pulse conditions.

Histological

Eleven of the 24 subjects had electrodes localized in or impinging directly on the MFB areas. Four of these Ss had placements in which the electrode tips were impinging on the medial aspect of the MFB, while four were located inside the medial forebrain bundle itself. These animals were called the MFB group. Of the remaining animals, eight subjects had placements just dorsal to the dorsal perifornical area just ventral to the fasiculus mammillothalamicus (VMT). The last seven subjects had more lateral placements in which the stimulating electrode was impinging either on the pars reticulate of the internal capsule, the fields of Forel or the zona incerta.

Behavioral

An examination of the MFB animals revealed that five of the eight as demonstrated a significant response suppression at 1.0, 10.0, 40.0 and 80.0 mpk (Wilcoxon Test, $p < .05$) while three Ss evidenced this suppression at 10.0, 40.0 and 80.0 mg/kg. Figure 29 illustrates mean responses per minute plotted as function of the C-T interval in msec. for a representative MFB subject (32 L) at 1.0, 10.0 and 40.0 mpk doses of naloxone. Figure 32 depicts the 80 mpk dose for this representative subject. The entire MFB group of placements is illustrated in figure 28. The suppressive effects of naloxone were significantly greater at the 80 mpk dose for the MFB group.

The VMT-IC animals showed no significant suppressive effects (Fig. 30) on ICSS rates except for two subjects who evidenced a significant suppression at 40 mpk. Some representative data for the VMT-IC subjects appears in figure 31 (35 L). This subject (35L) demonstrated very little responsivity to naloxone even at the 80 mpk dosage level (Figure 32). The suppressive effects of naloxone on response rates were significantly greater for the MFB animals than for the VMT-IC animals at all doses (ANOVA, $p < .05$).

This opiate reactivity was characterized in the present experiment by an animals response to morphine. Animals

which showed a response to morphine which was in the form of ICS rate facilitations, also demonstrated a response suppression to naloxone.

In ten out of 11 MFB Ss, morphine sulphate injections (1.25 mpk) injections significantly increased ICSS response rates while no such response facilitations were evidenced in the VMT-IC animals. These faciliatory effects occurred in the same C-T intervals as those in which naloxone had a suppressive effect on ICSS rates (ANOVA, $p < .05$).

There was also a tendency for some Ss, to who an increased sensitivity to naloxone at 1.0 mg/kg after receiving the injection series of three days of naloxone at 80 mpk. However, this reverse tolerance was only found to be significant in one MFB S, rat 32 L. This pattern was not characteristic in general of either MFB or VMT-IC animals.

Experiment III

Results

Figures 32 and 33 represent mean response per minute plotted as a function of the C-T interval for each of the separate electrode placement groups. In these figures, saline control rates are represented by the open circles while the closed circles represent the different doses of clonidine and those of naloxore. Each of the animals in each of the groups demonstrated a significant response rate

facilitation at the 1.25 mpk dose of morphine (Anova, $p < .05$) with the exception of the animals with placements localized in the cru cerebri. This rate facilitation was reversible by the 1.0 mpk injection of naloxone.

A group multivariate analysis which employed the seven C-T intervals as the dependent measures demonstrated a suppressive effect of clonidine on ICSS rates at each of the three dosage levels (.05, .1, and .5 mpk). The main factor of drug and the overall F statistic for Group (site of stimulation) were found to be significant in this analysis (MANOVA, $p < .05$). This effect was present across the 7 C-T intervals in the within-site condition.

An analysis of variance employing the CSUM variable further supported the existence of a significant response suppressive effect (ANOVA, $p < .05$). This response suppressive effect was found to be significantly different between the LC and MFB group of placements when they were compared to the FF and ZI group of animals who completed the paradigm (Duncan Multiple Comparison, $p < .05$). No CC placement animal demonstrated a significant decrease to clonidine at any dose.

Those animals entering the between-site C-T interaction condition under morphine and clonidine administrations were analyzed in a similar fashion to the with-site condition. The "split" pulse condition was available for only the LC-MFB group of placements since they were the only animals to

survive the entire paradigm. Mean responses per minute is plotted as a function of the C-T interval for the LC-MFB in the between-site "split" pulse drug conditions in Figure 33. The data from these animals was analyzed with the MANOVA repeated measures procedure using each of the seven C-T intervals as dependent variables.

Morphine administrations of 1.25 mpk significantly increased the ICSS rates obtained from the LC-MFB group of placements in the "split" pulse condition. This morphine induced rate enhancement was significant in both C-pulse conditions (MANOVA, $p < .01$). The magnitude of the ICSS rates facilitations under either saline or morphine did not significantly differ between C-pulse conditions.

Clonidine (.1 mpk) administered in combination with morphine (1.25 mpk) abolished the morphine induced ICSS rate facilitations at all C-T intervals but the 0.0 msec. interval when the C-pulse was delivered to the LC first (MANOVA, p .05). Furthermore, clonidine significantly depressed ICSS rates below saline control levels at 1.2 and 1.5 msec. These rate depressions were also not significantly different than the saline control rates emitted from both the LC and the MFB at 1.5 msec. in the within-site C-T condition. Morphine in combination with clonidine did not significantly affect ICSS rates when the C-pulse was delivered to the MFB first.

Clonidine (.1 mpk) when administered alone produced a significant ICS rate decrement across all C-T intervals as compared to saline control rates (MANOVA, p .001). This decrease in ICSS was observed only in the condition in which the C-pulse was delivered to the LC first. There was no significant difference between saline and clonidine effects on ICSS rates when the C-pulse was delivered to the MFB first.

GENERAL DISCUSSION

The present study demonstrated that the within-site C-T response rate changed following the administration of morphine sulphate. These effects of morphine were found to be site-specific on ICSS responding. Morphine sulphate at 1.25 mpk and 2.5 mpk primarily produced a significant increase in C-T response rates from animals with placements in the dorsal noradrenergic bundle, the dorsal-medial aspect of the locus coeruleus, the medial forebrain bundle/lateral hypothalamic area and the fields of Forel/Zona Incerta. Animals with electrodes located in the cru cerebri/internal capsule showed little alteration in their response rates under this drug at the same doses. All morphine induced effects on ICSS were reversible by naloxone at 1.0 mpk. The pattern of morphine responsivity was greater in magnitude for the LC and MFB group of placements. These subjects displayed the greatest percentage change in response rate than did the other group of placements. Subjects with placements localized in the fields of Forel displayed a response to morphine which was greater than those exhibited by the cru cerebri/internal capsule group of placements. These morphine induced facilitations were more highly correlated with areas which were sensitive to the d-isomer of amphetamine and which also had been demonstrated to contain rich concentrations of catecholamines and multiple catecholaminergic projections (particularly

noradrenergic) (Ungerstedt, 1977; Lindval and Bjorklund, 1974; Palkovitz and Jacobwitz, 1975). The density of enkephalins or opiate receptors did not appear to be influential in determining the morphine response in these subjects. In fact, self-stimulation sites which were high in their endogenous opiod density (CC) demonstrated virtually no response to morphine administration, while conversely those areas which were low in endogenous opiod levels (LH/MFB) demonstrated a large response to morphine administrations (Simantov et al., 1977; Pert el al., 1977; Pert and Snyder, 1974).

C-T Trend Analysis

The results of the orthogonal polynomial analysis and a trend analysis obtained from the procedures for repeated measures at each of the seven C-T intervals revealed three prominent trends across both the baseline and saline-drug condition data. Firstly, there was predominantly a quadratic or cubic component in the C-T values which was greater than 1.5 msec. The C-T values of 0.0 msec. to 0.8 msec. were best represented by a linear or quadratic function, while those between 0.8 and 1.5 msec. were primarily quadratic. Thus, the data for the saline control days C-T function could be best represented by third order polynomial:

$$Y=ax+by^2+cz^3$$

Secondly, the delineation of C-T trends under morphine revealed that morphine drug effects were significantly greater in the range of 0.8 msec. to 1.5 msec. The drug effects at 0.0 to 0.5 msec. were less frequent and lower in magnitude, while those between 2.5 msec. and 5.0 msec. fell midway between the two other C-T classes. This functional triad might represent the combination of interaction of three components in the C-T function intimated by Coons et al. (1977). Thus, the first factor might represent summation (linear component), the second (quadratic trend) might indicate refractoriness, and the third might reflect neurochemical kinetics such as K_d or Beta Max (cubic component).

Naloxone hydrochloride, when administered by itself, produced a significant site-specific effect on ICSS rates of responding. This agent suppressed rates of responding across all doses in the medial forebrain bundle group of placements while reducing these rates only at the higher (40-80 mpk) doses in animals with placements in the ventral mammillothalamic area, the internal capsule, the zone incerta, and the fields of forel. This naloxone effect on ICSS rates decreased in a dose responsive fashion for the MFB animals. The effect of naloxone in decreasing ICSS rates was correlated with the occurrence of a morphine response rate facilitation in that area. These morphine induced facilitations occurred in the same C-T intervals as

naloxone suppression of ICS rates. There was only one observed case of a naloxone sensitization produced by multiple sequential administrations of this drug.

In agreement with work of Bodnar (1979), the between-site C-T data obtained under both saline and morphine administrations in the present thesis also support the existence of two discrete ICSS systems. The present study's data also suggests that these systems are differentially controlled by the nucleus locus coeruleus. Thus, the nature of the observed ICSS interaction was determined by the order in which the LC was stimulated by the C-pulse. Animals with electrode tips localized in the LC and the medial forebrain bundle demonstrated a non-directional or symmetrical interaction in which the order of C-pulse delivery did not affect the magnitude of the neurophysiological interaction. Conversely, animals with placements determined to be in the LC and the dorsal far lateral hypothalamic area (FF,CC) displayed an asymmetrical or directional neurophysiological interaction. In these animals, the magnitude of the ICSS interaction was always greater when the C-pulse was delivered to the LC first.

The d- and l-amphetamine isomer within site C-T data also suggested several relationships between the different electrode placements: First, in general, the d-isomer of amphetamine produced a greater facilitation in ICSS response rates than did the l-isomer irrespective of where the

electrode placement was histologically localized in a subject. However, when the percentage change scores between drug induced response rates and saline control rates were examined for the d- and l-isomer data, a differential responsivity was demonstrated between animals who had placements in the LC and MFB. These animals demonstrated a greater percentage change score than the other group of placements. This effect was particularly strong in animals who had demonstrated a response facilitation to morphine sulphate at either dose. Second, the LC and MFB who both demonstrated response rate facilitations to morphine were also found to display a significant d- and l-isomer effect which also significantly differed in magnitude from each other. Third, response to the d-isomer was a significant predictor of morphine ICSS rate facilitations and vice-versa. Finally, the l-isomer was relatively ineffective in producing a significant response rate facilitation at the 1.0 mpk dose level. If a response facilitation was exhibited by an animal under the l-isomer, it rarely approximated the magnitude of the rate facilitations produced by the d-isomer.

The present study demonstrated that a greater percentage of animals with placements in the LC and the MFB exhibited significant facilitations in response rate under both the d-isomer and l-isomer of amphetamine that did ZI and CC placements. Furthermore, morphine-induced rate

facilitations were also greater in the LC and MFB groups than they were in the animals with CC and ZI-FF placements. Similarly, the percentage change scores also demonstrated a significant differential in isomer responsivity between treatment conditions. Thus, the LC and MFB placements exhibited significantly greater responsivity as measured by the PCS scores at the 2.0 mpk dosage, LC-CC subjects demonstrated significant isomer differentiation in which the d-isomer response was greater than the l-isomer response at both sites, while LC-FF and LC-MFB animals showed this differentiation only at the 2.0 mpk dosage level. The LC-FF group additionally demonstrated an equipotent facilitation rate under the d- and l-isomer at the 1.0 mpk dosage level. This differentiation was further supported by the MLR analysis which revealed that in the LC-placements, the response to morphine was best predicted by the PCS obtained under d-amphetamine in the locus coeruleus; the morphine facilitations in the MFB were best predicted by the d-isomer PCS obtained in the MFB, while the FF morphine PCS was best predicted by the l-isomer PCS from the FF. In the CC placements and in the LC placements from the LC-CC group, the LC PCS was the best predictor for the morphine facilitations from both sites. The l-isomer PCS was also the best predictor for morphine facilitations in the LC placements in the LC-FF group. This differential may be partly explained by the differential number of PAG and more

ventral-medial placements around the LC in this group, and the larger number of placements in or dorsal-lateral to the LC and DNB placements in the LC grouping of the other ICSS groupings. Nelson (1977) has, in fact, reported this dorsal-lateral versus ventral-medial differentiation in the LC with respect to morphine responsivity. The LC is a heterogenous grouping of cells which include noradrenergic, Gabba, substance P, and high density opiate receptor areas (Simantov et al., 1977; Pert, Kuhar and Snyder, 198_; Palkovitz and Jacobawitz, 1974; Lindval and Bjorkland; Cooper, Bloom and Roth, 1983). It is not surprising then that a differential electrode placement in this nucleus might lead to differential opiate and/or opiate responsivity. The importance of such a medial-lateral versus dorsal-ventral distinction has been discussed by Ellman et al., (1976) and others (Ellman et al., 1979; Ackerman et al., 1976).

In general, LC-MFB animals demonstrated significant elevations in their rates of responding under d-amphetamine and under l-amphetamine when these drugs' rates were compared to their respective saline control day rates. However, in the LC-MFB, there was also a significantly greater number of animals who demonstrated a greater response to the d-isomer than to the l-isomer of amphetamine than there were in any of the other groups. Animals with placements in the FF, ZI and CC tended to demonstrate a

significant d-isomer effect which was greater than the l-isomer, but in which the l-isomer was not significantly different than baseline saline control rates (L-). The CC placements showed the least responsivity to the d-isomer in terms of total absolute rates of responding, and in the number of subjects displaying a significant rate of facilitation. This group contained only one subject who demonstrated both a significant d- and l-isomer effect in which both isomers were able to produce a significant response rate facilitation over saline control rates of responding. The differential PCS scores for the LC-CC group in which the CC PCS was greater under d-amphetamine than in any other group, and for any other isomer, may be an artefact of the low number of CC and IC subjects in the study. Furthermore, CC subject response rates were low and more variable than in any other of the treament conditions. This "floor" effect coupled with the large intra- and inter-subject variability may account for the discordant d-isomer effect and PCS scores. Thus, although subjects demonstrated large facilitations from baseline, the intra-day variability may have led to a failure to obtain significance in all but one subject (1 Q).

One possible mechanism for the current findings may be due to their differential NE and DA concentrations. Thus, both the d- and the l-isomer had significant rate facilitating effects in high NE sites of the LC and MFB,

while DA sites such as the CC demonstrated virtually few significant d- and l-isomer facilitations which were different from saline control rates or different between isomers. Tempel (1982) also reported a differentiation between MFB-PF (perifornical) animals and those of the ZI, CC and FF. Both Tempel (1982) and Nelson (1977) reported difficulty in demonstrating an l-isomer effect at the 1.0 mpk dose. The present study also reports a greater number of L-subjects in the FF, CC and ZI at 1.0 mpk dose, and similarly had problems with the l-isomer at the 1 mpk dosage level.

The present study's findings are also in agreement with those of Liebman and Segal (1977) and those of Nelson (1977). These authors also demonstrated that sites which were facilitated by morphine were also facilitated by d-amphetamine. The data of Tempel (1982) also demonstrated an effect of the d- and l-amphetamine isomers, and further illustrated that the d-isomer was more effective in elevating response rates than was the l-isomer in MFB-PF animals. An LC lesion also failed to influence the responsivity of MFB-PF but not FF, ZI or CC animals to morphine. The present study also supports the work of Nelson (1977) in that differential pharmacological responses measured by ICSS can sometimes serve to be a better predictor of the morphine faciliatory effect, than more traditional histological neuroanatomical verification

techniques. Thus, the d- and l-amphetamine isomer screen, when employed with a 1 mpk or 2 mpk dose, is a reliable method for differentiating electrode sites and their underlying neurochemical substrate.

These two ICSS systems could also be differentiated on the basis of their response to the drugs of abuse. The type of morphine effect observed on these interactions was dependent upon the nature of the electrode placements. Thus, the non-directional interactions produced in the LC-MFB animals were equally potentiated by morphine sulphate administrations in the two C-pulse conditions. The directional or asymmetrical interactions produced in the LC-FF and LC-CC groups of electrode placements were differentially affected by morphine sulphate administrations penchant on the order of C-pulse presentation to the locus coeruleus. The magnitude of the morphine induced facilitations on ICSS rates were always greater in these animals when the C-pulse was delivered to the LC first. This data clearly implicates the primacy of the LC and possibly noradrenersic mediation of ICSS in this network projecting from the nucleus locus coeruleus to the dorsal far lateral hypothalamic area. Thus, the electrical stimulation of the LC and possibly its noradrenergic projections are important variables in the mediation of morphine's faciliatory effects on ICSS interaction rates obtained in the LC-DFLH, but not the LC-MFB ICSS network.

These two ICSS networks could also be differentiated on the basis of their response to the d- and l-isomer of amphetamine. Again, in this set of studies, the nature of the isomeric effect on ICSS rates was determined by the nature of the ICSS interaction. In general, the d-isomer produced equal ICS rate facilitations in the two C-pulse conditions for the LC-MFB group of placements. However, the LC-DFLH groups demonstrated a response which was dependent on the order of C-pulse presentation to the nucleus locus coeruleus. Furthermore, the d-isomer effect on ICSS rates in the within-site condition served to be a good predictor of the morphine rate facilitating effect in both the within and between-site conditions. These effect sizes were strongest when the sites involved in the prediction were noradrenergic in content. This was also in agreement with the finding that the LC-MFB (two primarily noradrenergic sites) demonstrated the greatest percentage change scores in the between site condition under the d- and l-isomer amphetamine administrations. The influence of both the noradrenergic system and the LC were then both supported by the amphetamine isomer data.

The locus coeruleus and the medial forebrain bundle are both CNS areas which are richly innervated by norepinephrine as well as by other neurochemicals (Ungerstedt, 1977; Lindval and Bjorklund, 1974; Palkovitz and Jacobwitz, 1974; Simantov et al., 1977). The d-isomer of amphetamine

produced rate facilitations from these sites which were equipotent and symmetrical in the C-T "split" pulse condition of the present study. The l-isomer had little or not effect on these LC-MFB interactions regardless of the C-pulse condition. This d- and l-isomer differentiation is similar to that reported for the electrical stimulation of a single site in isolation (Philips and Fibiger, 1973; Liebman and Butcher, 1974; Ellman et al., 1977).

The fields of Forel and the Cru Cerebri both contain high densities of catecholamines (Ungerstedt, 1977; Lindval and Bjorklund, 1974; Palkovitz and Jacobwitz, 1974). The Cru Cerebri is particularly rich in dopamine receiving projections from nigro-striatal system emanating from the A-9 nucleus, the substantia nigra (Ungerstedt, 1977). Both of these areas displayed interactions with the locus coeruleus which were asymmetrical under both isomeric conditions. Thus, the order of C-pulse delivery to the locus coeruleus determined the effect of the d- and l-isomer of amphetamine on the between-site interactions. The effects of the d- and l-isomer of amphetamine on the between-site interactions was similar to those produced by morphine sulphate at 1.25 and 2.5 mpk in the first experiment of the present thesis. Both the amphetamines and morphine selectively affected the between-site ICSS rates. The effect of these drugs on ICSS rates was determined by the type of ICSS interaction observed at a given pair of self-stimulation loci.

If a pair of sites had been categorized as being symmetrical then the effect of both morphine and the isomer of amphetamine was to produce an equipotent rate facilitation in both C-pulse conditions. Conversely, if a pair of ICS sites had been categorized as being asymmetrical then morphine and the isomers of amphetamine produced a rate faciliatory effect in only one of the C-pulse conditions.

The d-isomer potentiation of the LC-MFB between-site C-T interactions and the failure of the 1-isomer to effect ICSS rates are findings which suggest a strong noradrenergic influence in this network (Philips and Fibiger, 1973). The d-isomer effect on C-T response rates was greater in magnitude than those of the 1-isomer for both the LC-FF and LC-CC group of placements. However, these effects depended upon the locus of the C-pulse. The influence of the dopaminergic system in these areas may be serving a smaller modulatory effect as evidenced by the partical effects by the 1-isomer on ICSS rates. These effects of the 1-isomer may be dependent on the densities of endogenous dopamine in these areas (Philips and Fibiger, 1977; Coyle and Snyder, 1973).

One possible interpretation of these differential site-specific effects on the ICSS interactions might be that the type of effect observed was determined by the relative state of transynaptic influences at the time of stimulation. Thus, the more selective the influence of the noradrenergic

system at each site in an interaction at the time of electrical stimulation than the more likely that dopamine neurons more distal to the site of stimulation will affect ICSS rates under the d-isomer. For example, the DA releasing or reuptake inhibiting effects of the l-isomer would have to be sufficient to "push" a system further than could be expected by the use of electrical stimulation alone.

The LC-MFB interactions have been explained as resulting from the activation of two separate ICSS systems (Bodnar, 1977). The medial forebrain bundle when stimulated could affect both dopamine and noradrenergic mechanisms. This fiber path influences could extend to the DFLH, the substantia nigra, or the dorsal aspect of the DA A-10 nucleus via path neuron or fiber of passage connectivity (Millhouse, 1969; Ungerstedt, 1977; Lindval and Bjorklund, 1974; Palkovitz and Jacobwitz, 1974; Liebman and Butcher, 1977). Thus, the effects of the l-isomer on ICSS rates would be minimal if it occurred at all since the output of this system was already maximally high. The LC-FF and LC-CC groups of placements since they shared a common termination point (Bodnar, 1977) would be expected to be more differentially sensitive to the isomers of amphetamine depending on the order of C-pulse presentation. The isomeric effect should then be contingent on whether or not

a dopaminergic or noradrenergic site was stimulated with the C-pulse first.

The effect of the d-isomer according to a transynaptic hypothesis (Berberg, Stevens, and Franklin, 1976) should be greater when a dopaminergic area is being activated by ICS, and conversely the l-isomer should have a greater effect when a noradrenergic system is being sted by ICS. Therefore, according to this model, electrical stimulation of two sites which were either both d- or l-isomer sensitive should produce a greater effect than stimulating a combination of sites which were d- and l- sensitive respectively. This prediction was made by Steiner's laboratory which suggested that if one site was rich in DA and the other was rich in NE than the resultant interaction would be greater than one produced by stimulating two areas which were alike in their catecholamine content (Steiner and Ellman, privileged communication; Ackerman et al., 1976; Ellman et al., 1975).

The data from the present study did not bear the predictions from the transynaptice hypothesis. The magnitude of the interactions between the d- sensitive LC and the d- sensitive MFB was always greater than observed between the LC and FF sites (l- sensitive). Similarly, the LC-FF interactions were always greater in magnitude than those obtained between the LC and the l-sensitive CC. Thus, electrical stimulation of a d-sensitive and a l-sensitive

site produced a smaller interaction than one obtained between two d-isomer sensitive sites.

Both the LC-CC and the LC-FF group of placements demonstrated a d-isomer effect when the DFLH site was stimulated first (CC or FF). However, this effect was found to be significantly different between C-pulse conditions (LC receives the C-pulse) in only the LC-CC group of animals. This l-isomer had virtually no effect, with the exception of the LC-FF group, in affecting ICSS rates. Furthermore, in all three groups the d-isomer effect was always greater than the l-isomer's effect on ICSS rates.

In contrast to the transynaptice predictions, the d-isomer seemed to be more effective in facilitating ICSS rates in primarily noradrenergic areas while the l-isomer was only effective in the condition in which the C-pulse was delivered to a DA containing site first. This data suggests a greater noradrenergic sensitivity and supports the notion of a dopaminergic modulatory role in ICSS behavior.

In summary, the d- and l-isomer of amphetamine produced effects in the C-T between-site condition which were similar to those seen under the administration of morphine sulphate. This similarity in activity between the two classes of drugs of abuse is further supported by the multiple regression data in the present study. This regression analysis indicated that the d-isomer was the best predictor of the

between-site morphine interactions in NE sites, while the l-isomer was more often the best predictor in DA sites.

The administration of clonidine also served to differentiate the influence of the noradrenergic system. The effect of this agent on the within-site C-T function was found to be site-specific for each of the ICSS placement groups. Further, these influences were both dose responsive and dependent on the noradrenergic content of the ICSS areas. This influence of the noradrenergic system, as well as the putative primacy of the LC in the interaction condition, were also reflected in the data obtained from the LC-MFB group of placements in the C-T "split" pulse condition while being administered varying doses of clonidine.

Clonidine's effects were also site-specific in this LC-MFB between site condition. These animals which under control saline conditions displayed symmetrical and non-directional interactions demonstrated an asymmetry in their response to both clonidine administered alone, and to morphine administered in combination with clonidine. Clonidine's effect was specific to the C-pulse condition. This alpha-2 adrenergic agonist decreased ICSS rates when the C-pulse was delivered to the LC first (highly NE and opiate dense), but not in the condition in which the C-pulse was delivered to the MFB first.

The noradrenergic mediation of both morphine faciliations and ICSS interactions were both illustrated by the administration of clonidine in combination with morphine. This agent eliminated or reduced morphine's faciliatory effects when the C-pulse was delivered to the LC, but not the MFB first.

Morphine rate enhancements of ICSS behavior were abolished in the between-site condition when the C-pulse was delivered to the LC first, but not in the reverse C-pulse condition in which the medial forebrain bundle was electrically stimulated first. Thus, the LC-MFB interactions which had been demonstrated to have primarily symmetrical and non-directional ICSS rate facilitations under saline and morphine demonstrated an asymmetry in their response to clonidine plus morphine. These results suggest that both the morphine induced and "split" pulse produced ICSS rate facilitations were dependent on the noradrenergic locus coeruleus in the LC-MFB group of placements. This effect of clonidine on both the C-T interactions and morphine rate induced facilitations are most likely mediated by the presynaptic alpha-adrenergic autoreceptors in the locus coeruleus. The LC's mediation of these effects is further supported by the fact that this area is also dense in both endogenous opiate and norepinephrine concentrations (Simantov et al., 1977; Lindval and Bjorklun, 1974; Sammatini and Grattiani, 1976).

The modulation of morphine's excitatory effect on the LC-MFB interactions could also be explained by the influence clonidine on some other alpha-2 adrenergic projections extending from more posterior sites situated in the brainstem. For example, Katz et al. (1975) has suggested that the adrenergic projections from the C-1 through C-10 adrenergic nuclei might be the mediators of ICSS control. These neuroanatomical pathways have been clearly delineated by the histochemical work of Hokfelt and others (Hokfelt et al., 1976).

This clearly suggests the existence of some presynaptic influence on the noradrenergic system which are essential for the maintenance of morphine's rate enhancement effect on ICSS. However, since clonidine's effect, either alone or in combination with morphine, were dependent on the prior stimulation of the locus coeruleus, then the possibility of a non-adrenergic and non-catecholaminergic system in the LC-MFB interaction condition. Thus, the electrical stimulation of the locus coeruleus was not both necessary and sufficient for the maintenance of either an ICSS interaction or a morphine induced rate facilitation when the C-pulse was delivered to medial forebrain bundle first.

One possible explanation for the effects of clonidine on the LC-MFB interactions might be related to this agent's ability to interfere with the "temporal" parameters governing the LC's lead time to the MFB. This could be

achieved by alterations in the presynaptic membrane's permeability produced by some form of autoreceptor feedback at the level of the LC. For example, the activation of the autoreceptor population most probably changes this nucleus's stimulation threshold to an incoming stimulus.

The results of C-pulse condition in which the MFB was stimulated first also may depend on that of a descending poly-synaptic pathway from the MFB to the LC (Bodnar, 1977). The administration of Clonidine's effects on membrane permeability might be delayed activation of the LC which would allow the MFB descending pulse enough lead time to overcome the clonidine inhibition. This effect would result from the synchronizing of the delayed LC pulse with that of first descending pulse from the MFB and allow for temporal and spatial summative effects to occur at the postsynaptic membrane.

The present study's data also support the results of other laboratories which have reported an interaction between the catecholamines and endogenous opiates. For example, the amphetamines can have their effects attenuated by the administration of an opiate antagonist such as naloxone (Kornetsky et al., 1977). These findings and those of the present study suggest that the noradrenergic and enkephalinergic or endorphine endogenous opiod systems are neurochemically, neurophysiologically and behaviorally interactive. These ideas are borne out by the present

experiment since morphine's rate facilitating effects were at least partially dependent on some noradrenergic mediation from the locus coeruleus. It is well established that nucleus is richly innervated by norepinephrine, opiod receptors and enkepahlinergic projection (Simantov et al., 1977; Pert et al., 1979). The medial forebrain bundle which was little affected by clonidine during the "split" pulse interaction condition under morphine administrations, is also sparsely supplied by opiate receptors.

This interaction between catecholamines and the endogenous opiods in ICSS behavior has been documented by other studies in the literature. Glick et al. (1975) and Olds (1979) produced results which argued that both the lateral hypothalamus and the medial forebrain bundle were important in the mediation of morphine's reinforcing effects. Cerebral injections of morphine into lateral hypothalamic ICSS sites increased the cellular firing rates in this area (Olds and Niehus, 1979). The iontophoretic application of morphine to the nucleus locus coeruleus directly reduces the firing rates in this area (Berd and Kuhar, 1977). Thus, both the LC and the MFB are differentially affected by the direct administration of morphine at the cellular level. This dissociation of morphine effects on the primarily noradrenergic loci of the LC and the MFB are further supported by the work of Tempel (1982). This author demonstrated that LC lesions did not

affect MFB ICSS or ICSS rate facilitations produced under morphine.

Similarly, clonidine not only abolished or reduced the LC-MFB interactions to control levels, but the administration of this agent alone sometimes reduced the between-site condition rates to below those observed under saline control rates observed in the within-site LC and MFB ICSS conditions. Again, these effects were only observed in the condition in which the C-pulse was delivered to the LC first.

In conclusion, the present studies data and those observed under morphine and the amphetamine isomers support the notion that the noradrenergic system is selectively involved in the maintenance of the ICSS interactions and also in the production of morphine response rate facilitations. This selectivity was clearly determined by the nature of the ICSS interaction and the order and locus of C-pulse presentation.

The suggested existence of two separate ICSS systems such as the LC-DFLH in the present study raises the issue of whether or not all these loci subserve identical mechanisms of reward (Grauer and Thomas, 1982). Grauer and Thomas (1982) suggest at least two classes of self-stimulation sites: 1) MFB ICSS related areas which are characterized by rapid rates of responding, hyperactivity and general arousal; 2) limbic system related ICSS structures which

yield lower rates, and are accompanied by hyperactivity. The MFB related sites such as the nucleus accumbens septi are not affected by deprivation conditions (Rolls, 1974; Olds and Olds, 1965). Steiner and Ellman also proposed the existence of two reward systems; one which was specific and the other non-specific (Ellman and Steiner, 1971) in the modulation of reward threshold and goal directed behavior. The non-specific system was postulated as being embodied in the LC and SN, while the specific system was believed to emanate from the ventral tegmental area and follow more classic limbic pathways.

The present study also suggests the existence of two discrete ICSS systems which emanate from the locus coeruleus. The differential relationship between this nucleus with the septum and the medial forebrain bundle have been delineated behaviorally, neuroanatomically, and neurochemically. The work of Farber et al. (1974), Mattiace et al. (1977), and Tempel et al. (1982) have also clearly demonstrated the differential effects of the LC on ICSS behavior maintained from the septum and the medial forebrain bundle by electrolytic and radio-frequency lesioning of this nucleus.

One possible conjecture from the two network ICSS literature might be that of the existence of a neural intergration center for ICSS reward. This area might receive both noradrenergic and dopaminergic (and or

cholinergic and enkephalinergic etc.) inputs signal the properties of the ICSS to higher levels of CNS organization. For example, Keene (1977) and Keene and Casey (1975) demonstrated the existence of at least two "affect" coding ICSS receptive fields in the intralaminar nucleus of the thalamus. These nuclei were believed to receive inputs from the nucleus gigantocellularis in the medulla via the MFB (Keene and Casey, 1974). These thalamic cells were also believed to signal the hedonic and fear reducing properties of a stimulus. Thomas et al. (1981) have a similarly suggested that the lateral septum and its projections were associated with fear reduction while those from the MFB with their hedonic properties.

The pulse-pair technique can provide the future researcher with an excellent method for further differentiating the neural connectivity between ICSS and thalamic units by the implementation of the "split" pulse technique. Further research could employ this technique and its delineation of discreate neuro-behavioral pathways in conjunction with pharmacological manipulations to expand the present investigators work. In the final analysis, the pulse-pair technique could serve to identify the relationship between incoming stimuli, their stimulus properties and their functional relationship to higher order "affective" receptive fields. However, more importantly, the present study provides the behavioral pharmacologist

with a sensitive methodology for predicting and measuring the effects of psychoactive agents on the CNS.

The results of the clonidine and morphine studies proved to be exciting and unexpected findings. These "split" pulse studies point out a clear differentiation of ICSS behavioral networks and their underlying neurochemistry which demonstrate a resolution only possible with C-T technique. For example, although morphine administrations produced an equipotent rate facilitation in both C-pulse conditions, the neurochemical substrate governing these facilitations were differentially affected following clonidine administration in combination with morphine. The prior activation of the LC under clonidine and morphine was ineffective in maintaining the morphine induced rate faciliatory effects. This inhibition of clonidine suggests that CA systems, particularly NE, were an important component in maintaining morphine's rate excitatory effects when the LC but not the MFB was stimulated first. Thus, the neurochemical mechanisms underlying morphine facilitations from these two sites were mediated by at least in part different neurotransmitter systems. The MFB is a heterogenous nuclear area, and it is not surprising that morphine's neurochemical effects might be less dependent on noradrenergic mediation.

This type of neurochemical differentiation on the basis of a behavioral "marker" system provides a baseline from

which other classes of pharmacological agents could be employed to delineate role of other receptor populations in the LC and MFB. Furthermore, the logic of this analysis using the "split" pulse technique could similarly extend to the asymmetrical interactions, as well as interactions between other pairs of neural loci.

Table 1

Anatomical Site, Monaminergic Nuclei or Fibers, and Representative Konig and Klippel Stereotaxic Atlas Section for Each Hypothalamic ICSS Site

SUBJECT	ANATOMICAL SITE	MONAMINERGIC NUCLEI OR FIBERS	KONIG&KLIPPEL SECTION
78 N	Medial Forebrain Bundle	Mixed	35 B
	Internal Capsule	Nigro-Striatal DA System	
79 O	Medial Forebrain Bundle	Mixed	35 B
	Perifornical	Dorsal NE Bundle	35 B
11 O	Medial Forebrain Bundle	Mixed	35 B
	Perifornical	Dorsal NE Bundle	35 B
15 O	Medial Forebrain Bundle	Mixed	35 B
	Internal Capsule	Nigro-Striatal DA System	
57 N	H2 Fields of Forel	Dorsal NE Bundle Nigro-Striatal	36 B
	Ventral Mammothalamic	Dorsal NE Bundle	
46 N	Zona Incerta	Dorsal NE Bundle Nigro-Striatal System	36 B
	H2 Fields of Forel	Dorsal NE Bundle Nigro-Striatal System	
39 O	Zona Incerta	Dorsal NE Bundle Nigro-Striatal DA System	36 B
	Ventral Mammothalamic	Dorsal NE Bundle	

SUBJECT	ANATOMICAL SITE	MONAMINERGIC NUCLEI OR FIBERS	KONIG&KLIPPEL SECTION
72 N	H2 Fields of Forel	Dorsal NE Bundle Nigro-Striatal DA System	35 B
75 P	Zona Incerta	Dorsal NE Bundle Nigro-Striatal DA System	35 B
7 Q	Cru Cerebri	Nigro-Striatal DA System	35 B
71 P	Internal Capsule	Nigro-Striatal DA System	35 B
	Dorsal Medial Forebrain Bundle	Mixed	
83 P	Zona Incerta	Dorsal NE Bundle Nigro-Striatal DA	37 B
	H2 Fields of Forel	Dorsal NE Bundle	
58 P	H2 Fields of Forel	Dorsal NE Bundle Nigro-Striatal DA	37 B
95 O	H1 Fields of Forel	Dorsal NE Bundle	40 B
11 O	Medial Forebrain Bundle Dorsal Medial Forebrain Bundle	Mixed	35 B
79 N	Zona Incerta Internal Capsule	Dorsal NE Bundle Nigro-Striatal DA System	34 B
92 O	Perifornical Medial Forebrain Bundle	Dorsal NE Bundle Mixed	32 B
97 O	H2 Fields of Forel	Nigro-Striatal DA System Dorsal NE Bundle	32 B
10 P	Internal Capsule	Nigro-Striatal DA System	32 B

SUBJECT	ANATOMICAL SITE	MONAMINERGIC NUCLEI OR FIBERS	KONIG&KLIPPEL SECTION
11 P	Zona Incerta	Dorsal NE Bundle Nigro-Striatal DA System	36 B
	H1 Fields of Forel	Dorsal NE Bundle	
16 P	Cru Cerebri	Nigro-Striatal DA System	36 B
	H1 Fields of Forel	Dorsal NE Bundle	
31 P	Zona Incerta	Dorsal NE Bundle Nigro-Striatal DA System	34 B
61 P	Dorsal Medial Forebrain Bundle	Mixed	39B
	H2 Fields of Forel	Dorsal NE Bundle Nigro-Striatal DA	
1 Q	Internal Capsule	Nigro-Striatal DA System	35 B
73 P	H2 Fields of Forel	Nigro-Striatal DA System Dorsal NE Bundle	35 B

Table 2

Differential Densities of Opiate Receptors, Norepinephrine and Dopamine for Each of the Groups of ICSS Loci

SITE	OPIATE REACTIVITY	DOPAMINE DENSITY	NOREPINEPHRINE
Locus Coeruleus	HIGH	LOW	HIGH
Medial Forebrain Bundle	LOW	HIGH	HIGH
Cru Cerebri	HIGH	HIGH	LOW
Fields of Forel	LOW	LOW	HIGH

Table 3

Breakdown of Individual Subjects Who Entered the C-T "Split" Pulse Interaction Condition for Each of the Three Treatment Conditions

GROUP	SUBJECT NUMBER
LC-MFB	(Includes both the LC and MFB Placements)
	78O, 11O, 15O, 31P, 92O, 71P
LC-FF	(Includes ZI,FF and DFLH Placements)
	46N, 57N, 39O, 79N, 21P, 61P, 73P, 83P, 79O
LC-CC	(Includes IC and CC Placements)
	1Q, 1OP, 16P

Table 4

Percentage Change Scores for Each of the Amphetamine Isomers and Morphine (1.25 mpk) for Each of the Three Groups of ICSS Sites

GROUP	SITE	PERCENTAGE CHANGE SCORES		
		AMPHETAMINE		MORPHINE (1.25 MPK)
		D	L	
LC-MFB	LC	51	41	91
	MFB	46	43	61
LC-FF	LC	38.9	40	67
	FF	39	40	57
LC-CC	LC	69	20	68
	CC	68	27	48

Table 5

Multiple Regressions Summary for Each of the Three Treatment Groups and Their Associated Best Predictor Variables, Beta Weights, and F-Values, Rho-Square Values, and Associated Probability Levels

GROUP	SITE	PREDICTOR (PCS)	BETA	F	DF	PROBABILITY
LC-MFB	LC	LC D-AMPHET.	-.18	2.119	8,679	.05
	MFB	MFB D-AMPHET.	-.04	2.93	8,679	.01
KC-FF	LC	LC L-AMPHET.	-.06	3.42	8,865	.01
	FF	FF L-AMPHET.	-.06	3.38	8,865	.01
LC-CC	LC	LC D-AMPHET.	-.07	2.100	8,280	.05
	CC	CC D-AMPHET.	-.03	3.65	8,280	.01

Table 6

Summary of Multiple Linear Regression Predictor Variables, F-Values, Beta Weights, and Associated Probabilities for Each of the Three Treatment Groups Using the Percentage Morphine Scores as a Criterion in the Between-Site C-T Interaction Condition under Morphine 1.25 mpk

GROUP	CONDITION	PREDICTOR (WITHIN SITE)	BETA	F	DF	PROB. MORPHINE % CHANGE
LC-MFB	C=MFB	Within MFB (M)	-0.133	11.06	*12,614	11.68
		Within LC (M)	-0.130	11.124		
		Within MFB (D)	-0.7520	3.75		
r^2 = .084		Within MFB (L)	-0.7516	3.746		
Mult = .294		Within LC (L)	-0.7254	3.490		
		Within LC (D)	-0.7124	3.369		
	C=LC	Within MFB (M)	-0.229	10.88	*12,614	11.2
r^2 = .08		Within LC (M)	-0.127	10.52		
		Within MFB (D)	-0.0733	3.54		
Mult = .28		Within MFB (L)	-0.0732	3.53		
		Within LC (L)	-0.0706	.301		
		Within LC (D)	-0.0694	3.186		
LC-FF	C=FF	Within LC (M)	-0.11076	10.972	12,873	9.85
		Within FF (M)	-0.11075	10.969		
r^2 = .06		Within LC (L)	-0.07601	5.227		
		Within LC (D)	-0.06382	3.699		
Mult = .245		Within FF (L)	-0.05644	2.896		
		Within FF (D)	-0.05543	2.794		
	C=LC	Within LC (M)	-0.216	10.6	12,6873	13.8
r^2 = .101		Within FF (M)	-0.126	10.42		
		Within LC (D)	-0.065	3.75		
Mult = .36		Within FF (D)	-0.064	3.54		
		Within FF (L)	-0.061	.01		
		Within LC (D)	-0.060	3.75		
LC-CC	C=CC	Within LC (M)	-0.125	4.649	12,275	13.9
r^2 = .092		Within CC (M)	-0.119	4.245		
		Within LC (D)	-0.084	2.155		
Mult = .304		Within CC (L)	-0.0775	1.808		
		Within CC (D)	-0.074	1.657		
		Within LC (D)	-0.060	1.144		
	C=LC	Within LC (M)	-0.09	2.822	11,276	9.02

2	Within CC	(M)	-0.09	2.577
r = .081	Within LC	(D)	-0.0671	1.308
Mult = .294	Within CC	(D)	-0.061	1.097
	Within CC	(L)	-0.058	1.005
	Within LC	(L)	-0.057	0.0911

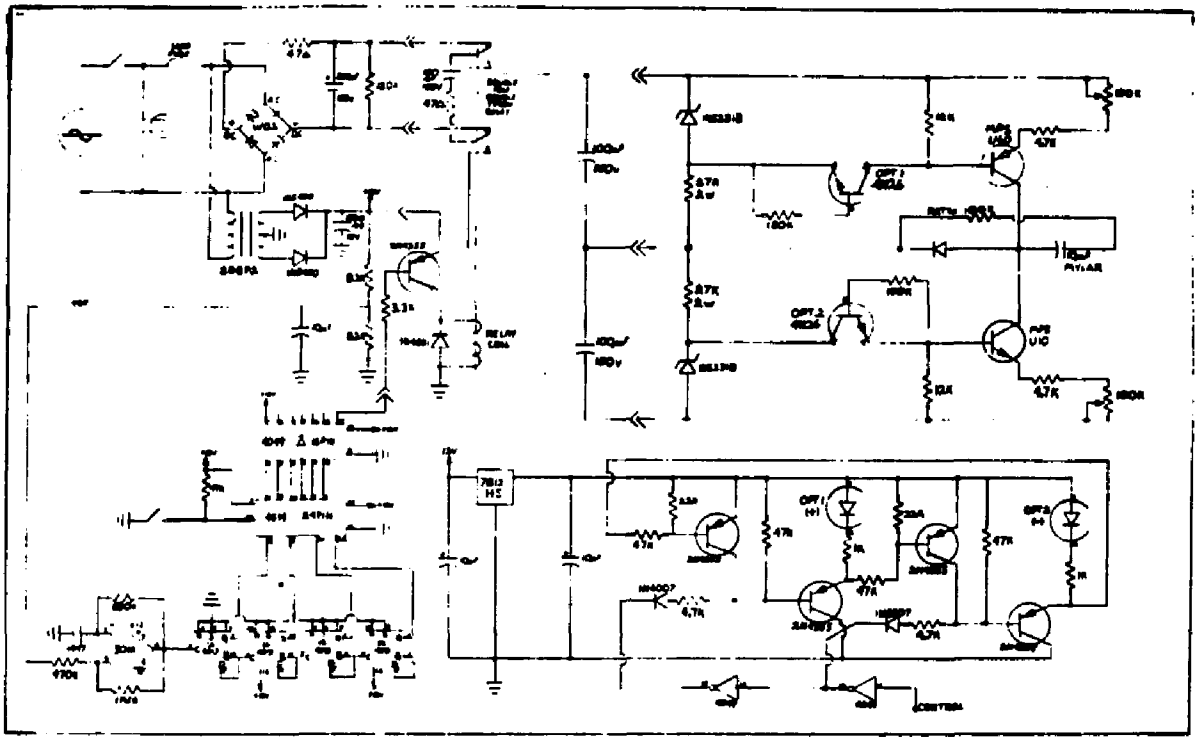


Diagram 2. Schematic diagram for multi-channel optically isolated constant current stimulator.

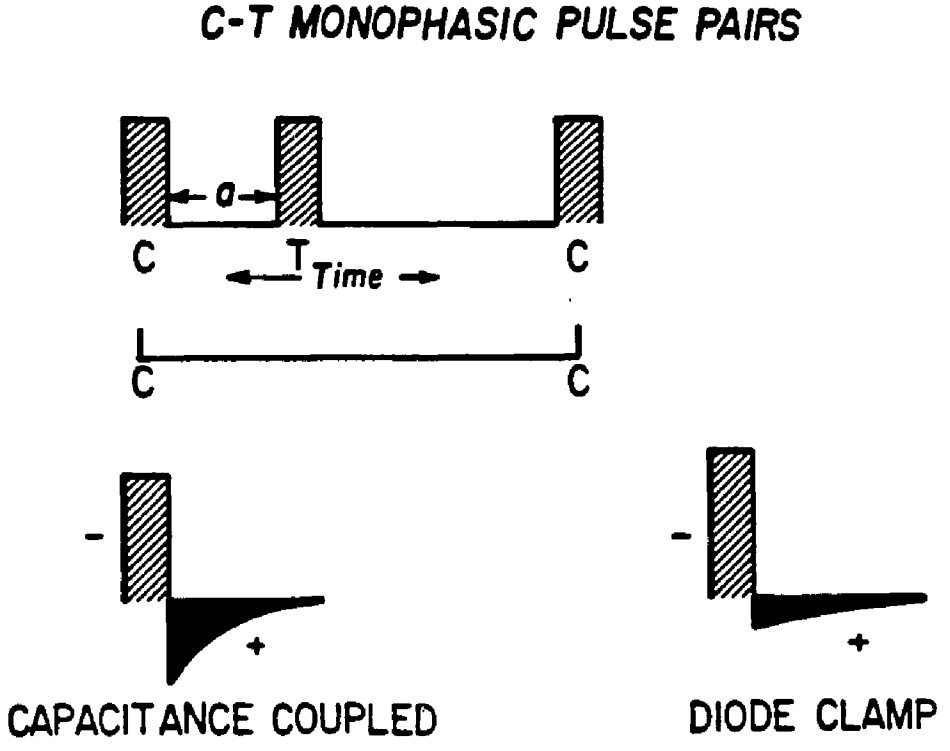


Diagram 1. C-T monophasic pulse pairs. Comparison of capacitance coupled and diode rectified current limitation.

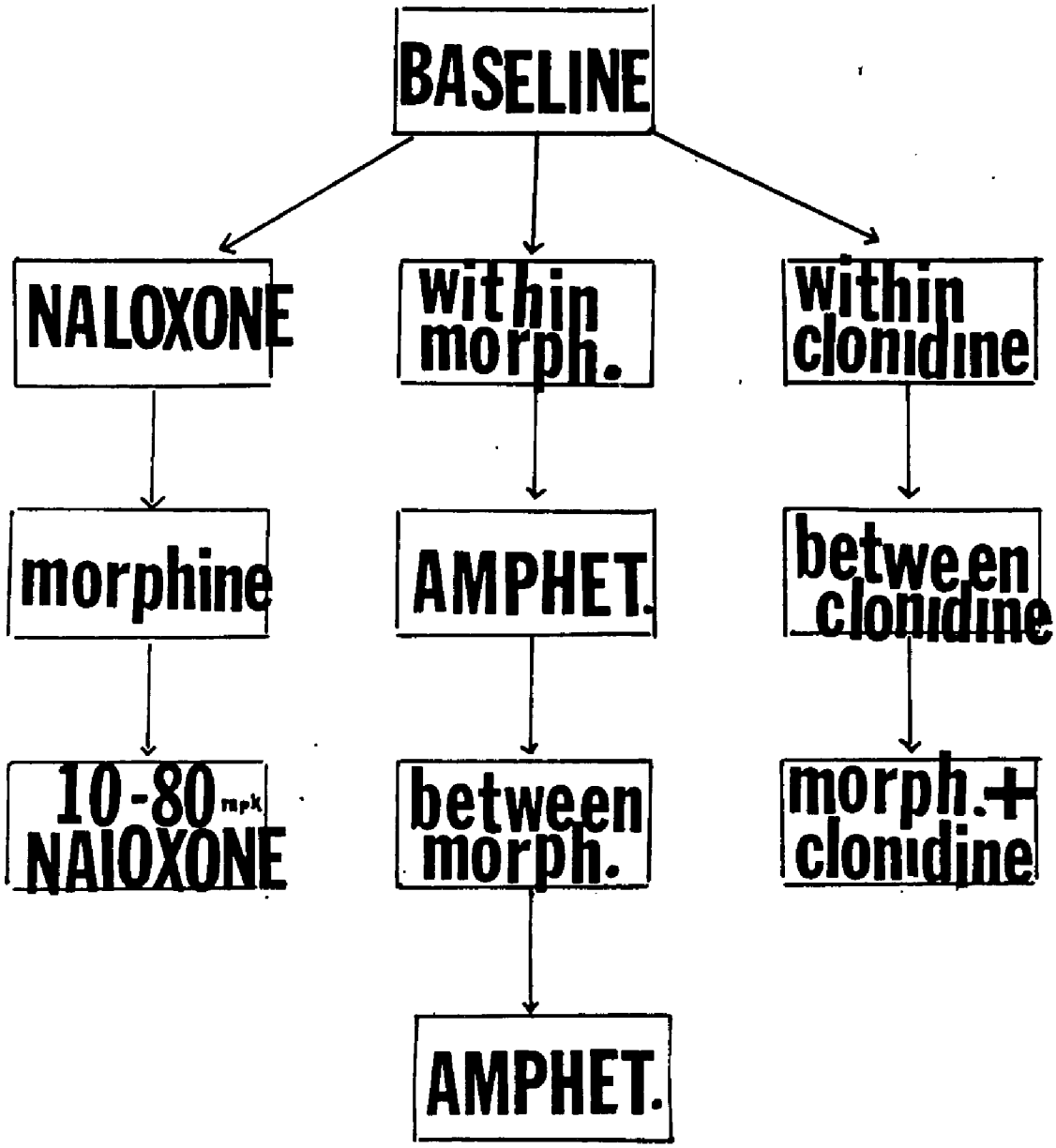


Diagram 3. Flow chart of experiments.

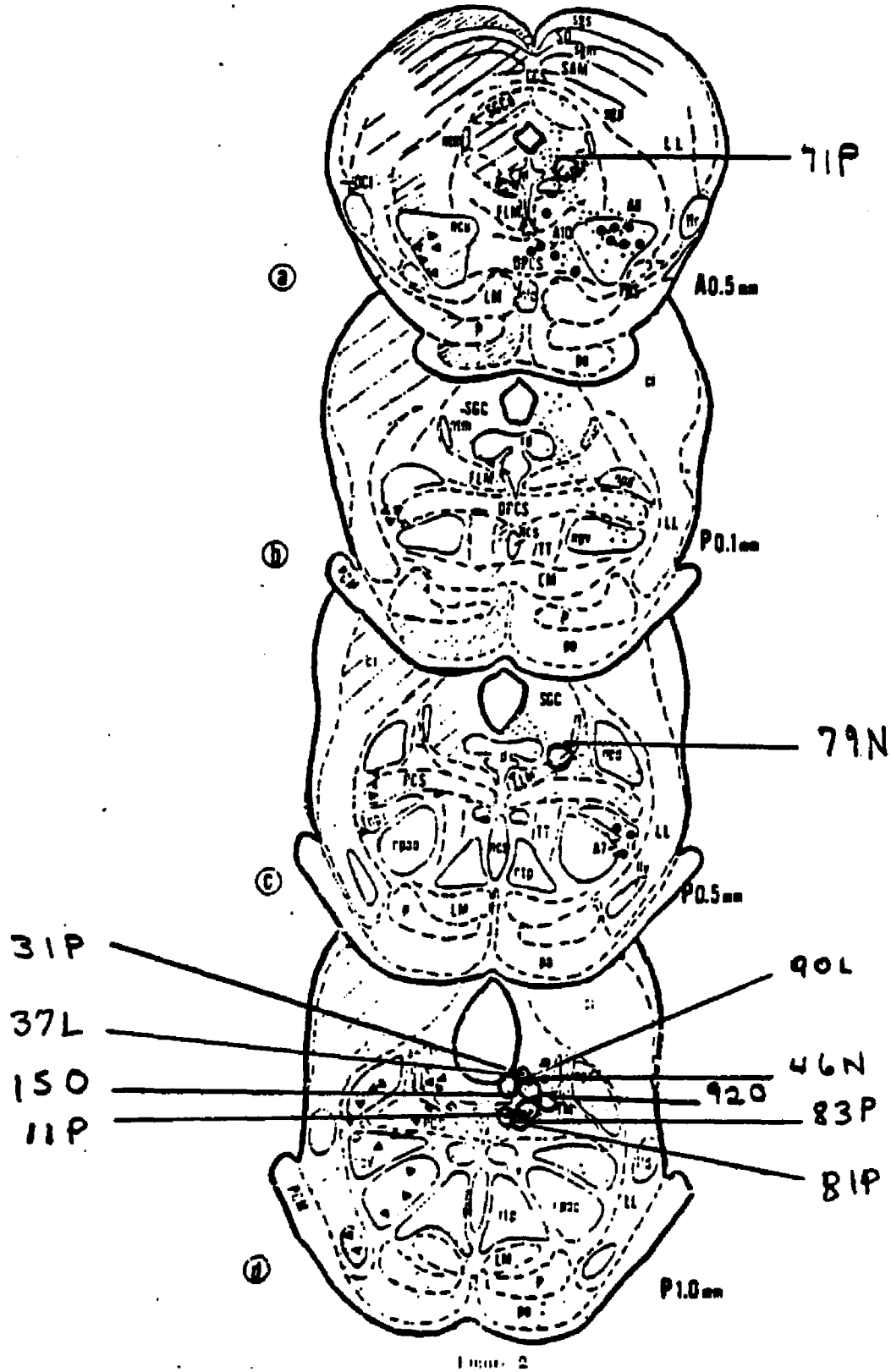
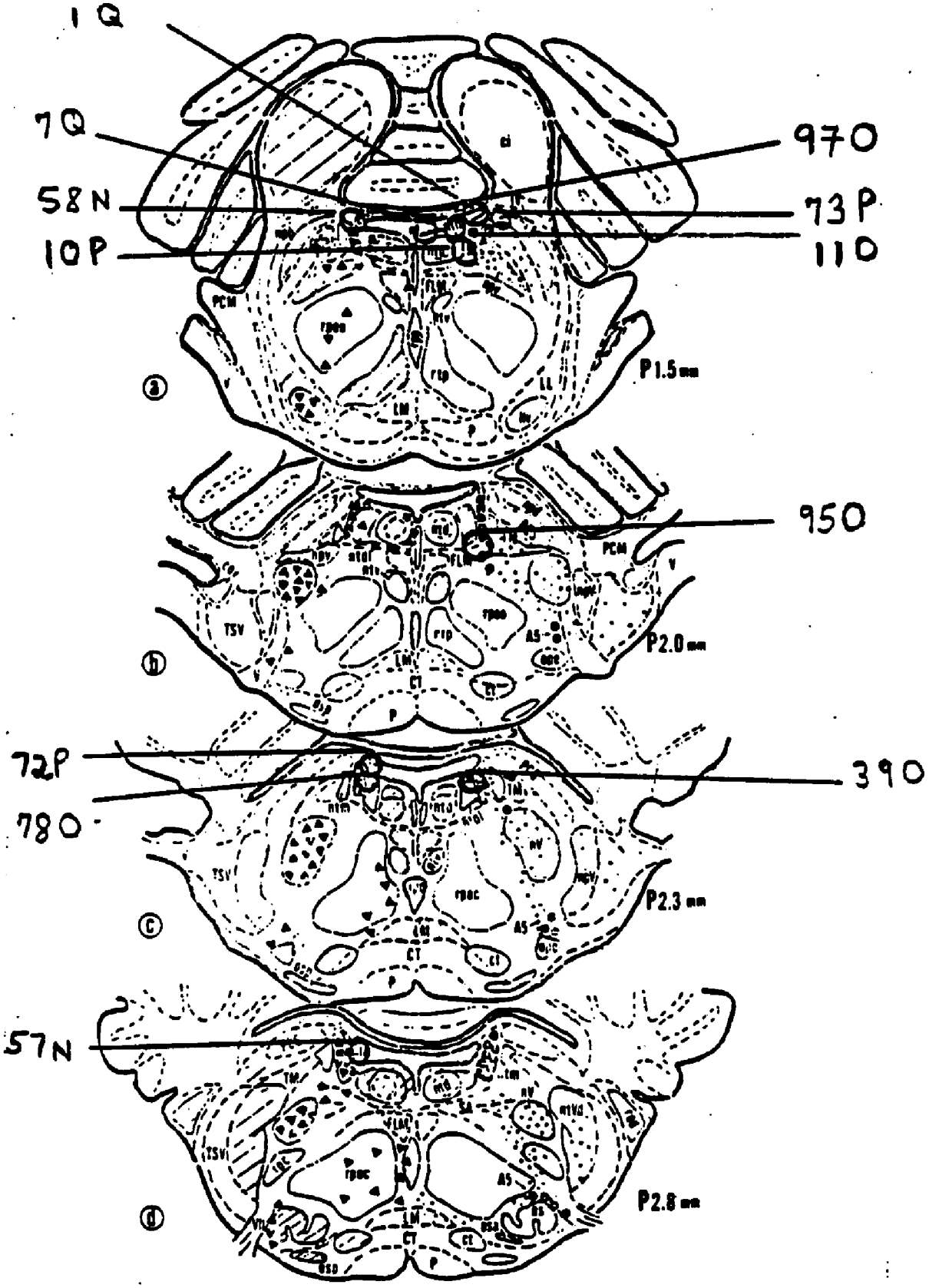
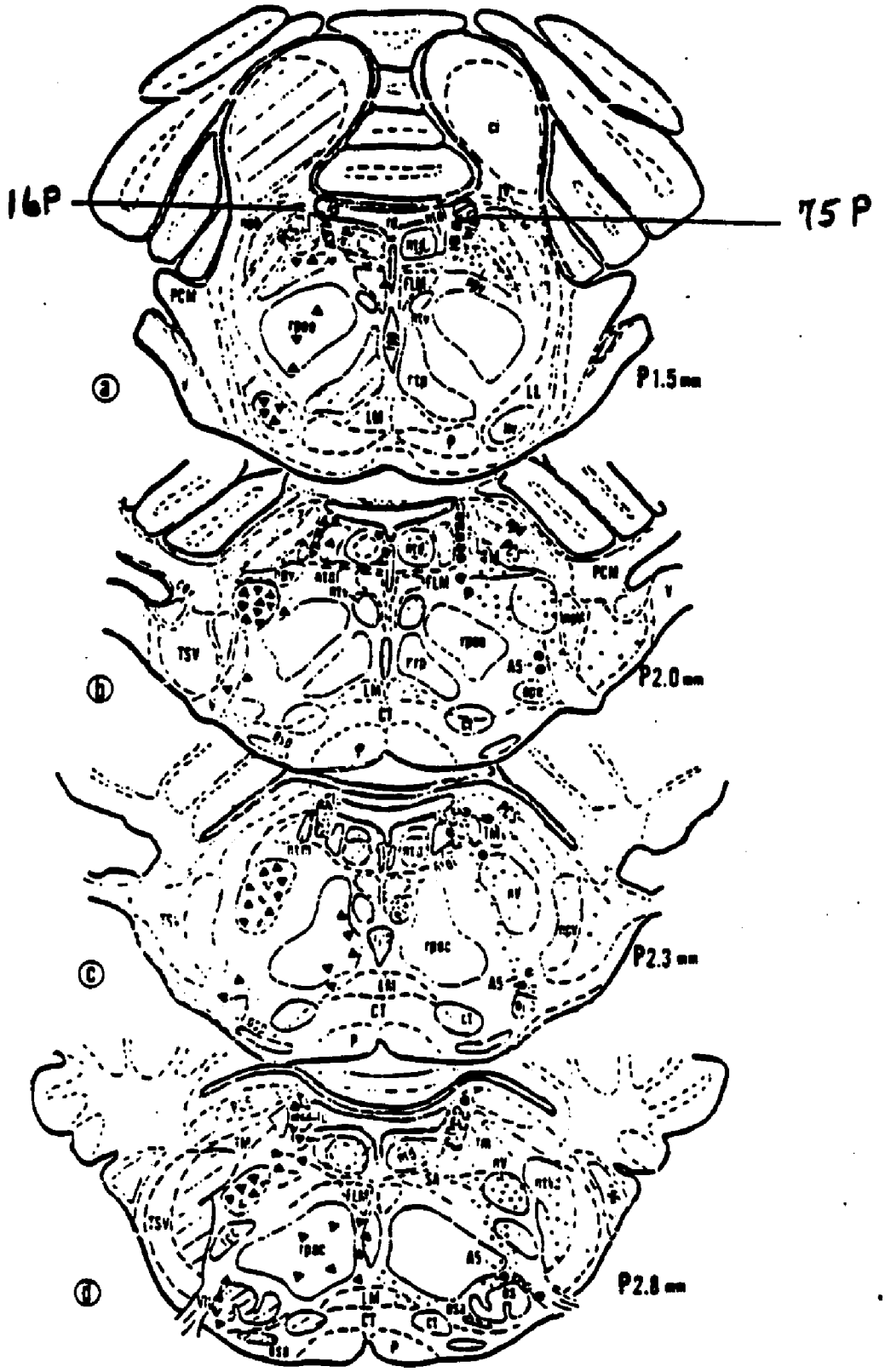


Figure 1. Representative stereotaxic sections from the atlas of Palkovitz and Jacobwitz (1974) illustrating the location of electrodes in the area of the nucleus locus coeruleus and the dorsal noradrenergic bundle.


1Q
7Q
58N
10P
97O
73P
11O
95O
72P
78O
39O
57N
P1.5 mm
P2.0 mm
P2.3 mm
P2.8 mm


16P
75 P
P1.5 mm
P2.0 mm
P2.3 mm
P2.8 mm

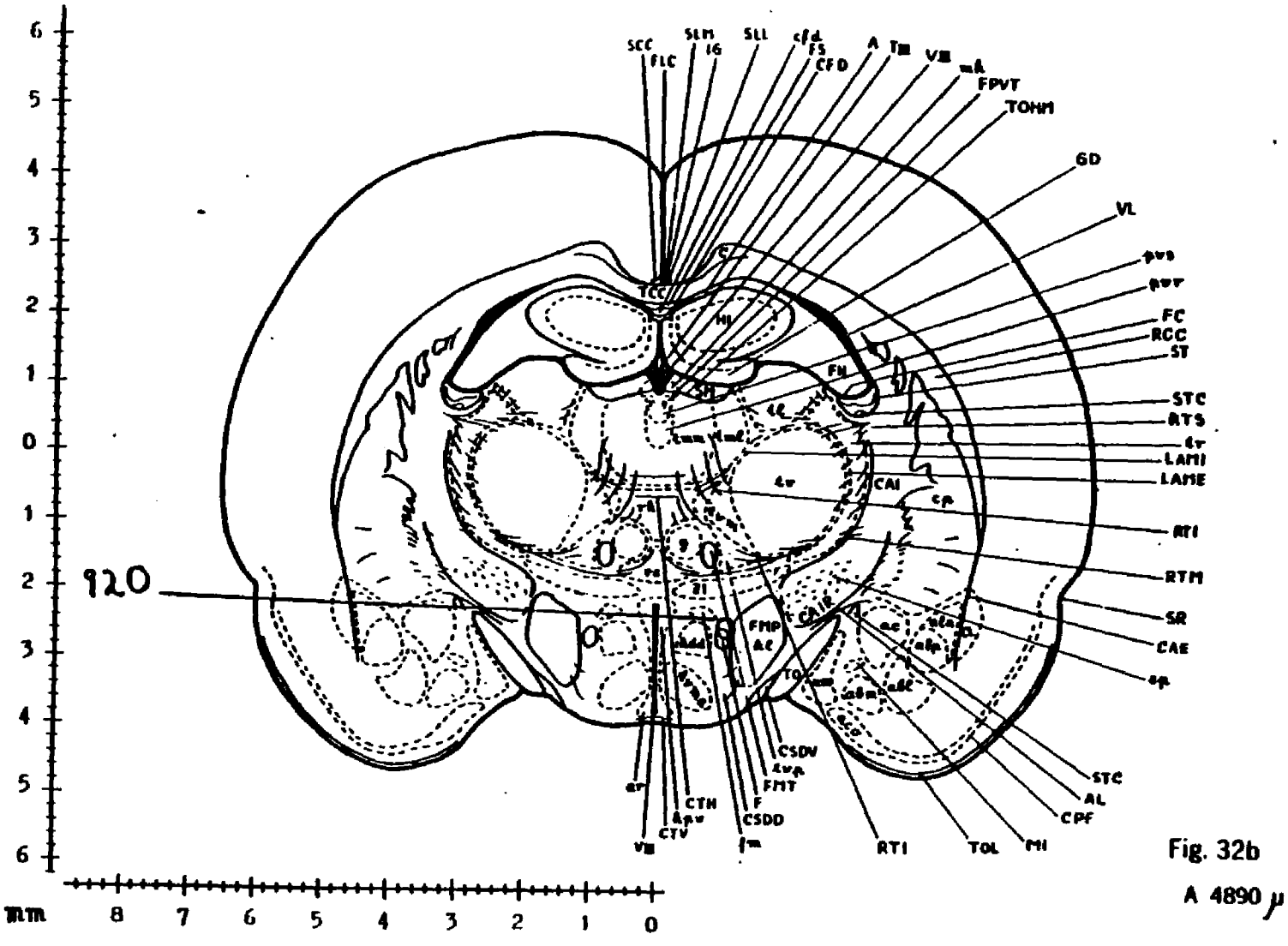


Fig. 32b

A 4890 μ

Figure 2. Representative stereotaxic sections from the atlas of Konig and Klippel (1963) illustrating the location of electrodes in the areas of the medial forebrain bundle and the dorsal far lateral hypothalamus.

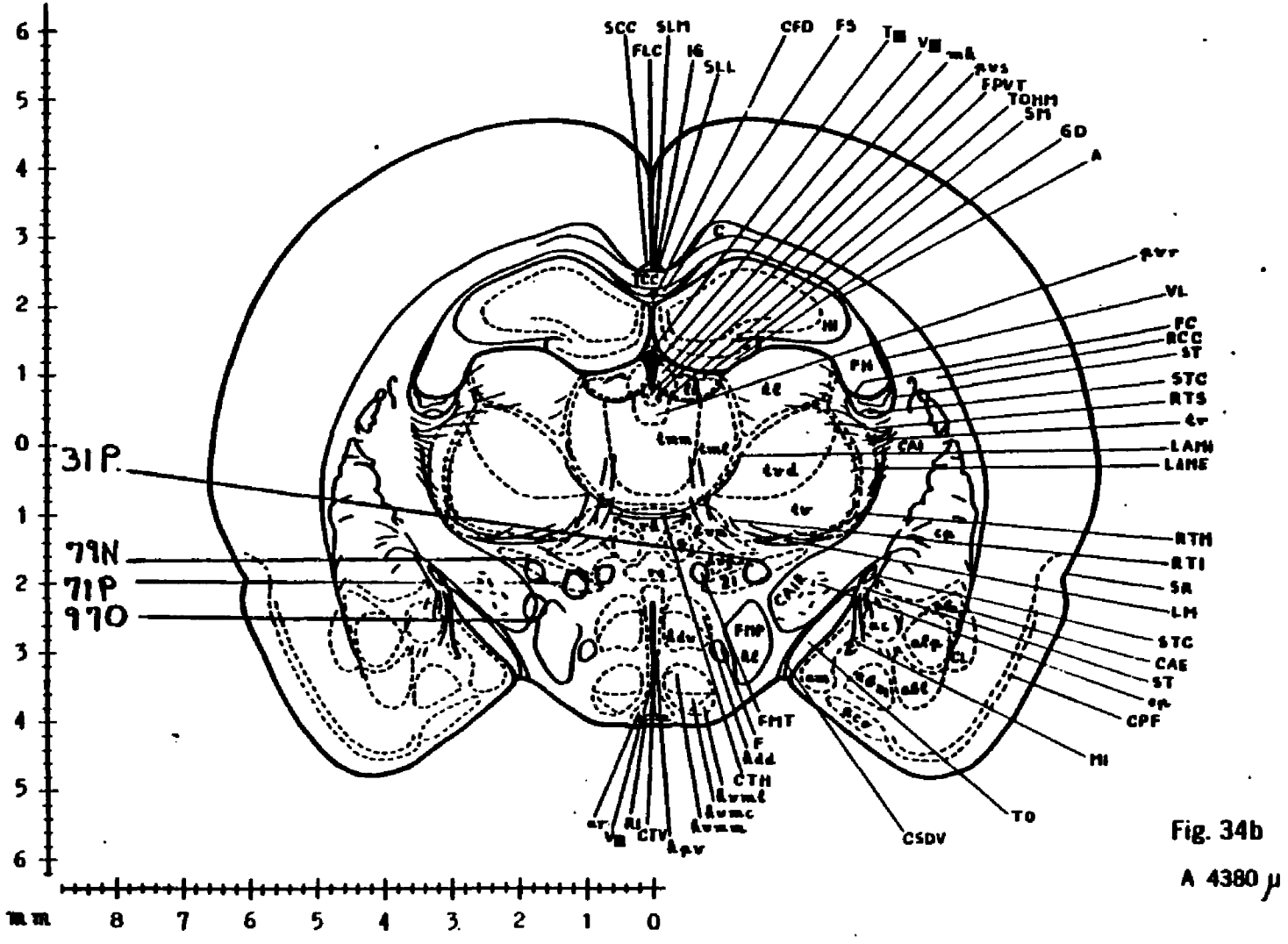


Fig. 34b

A 4380 μ

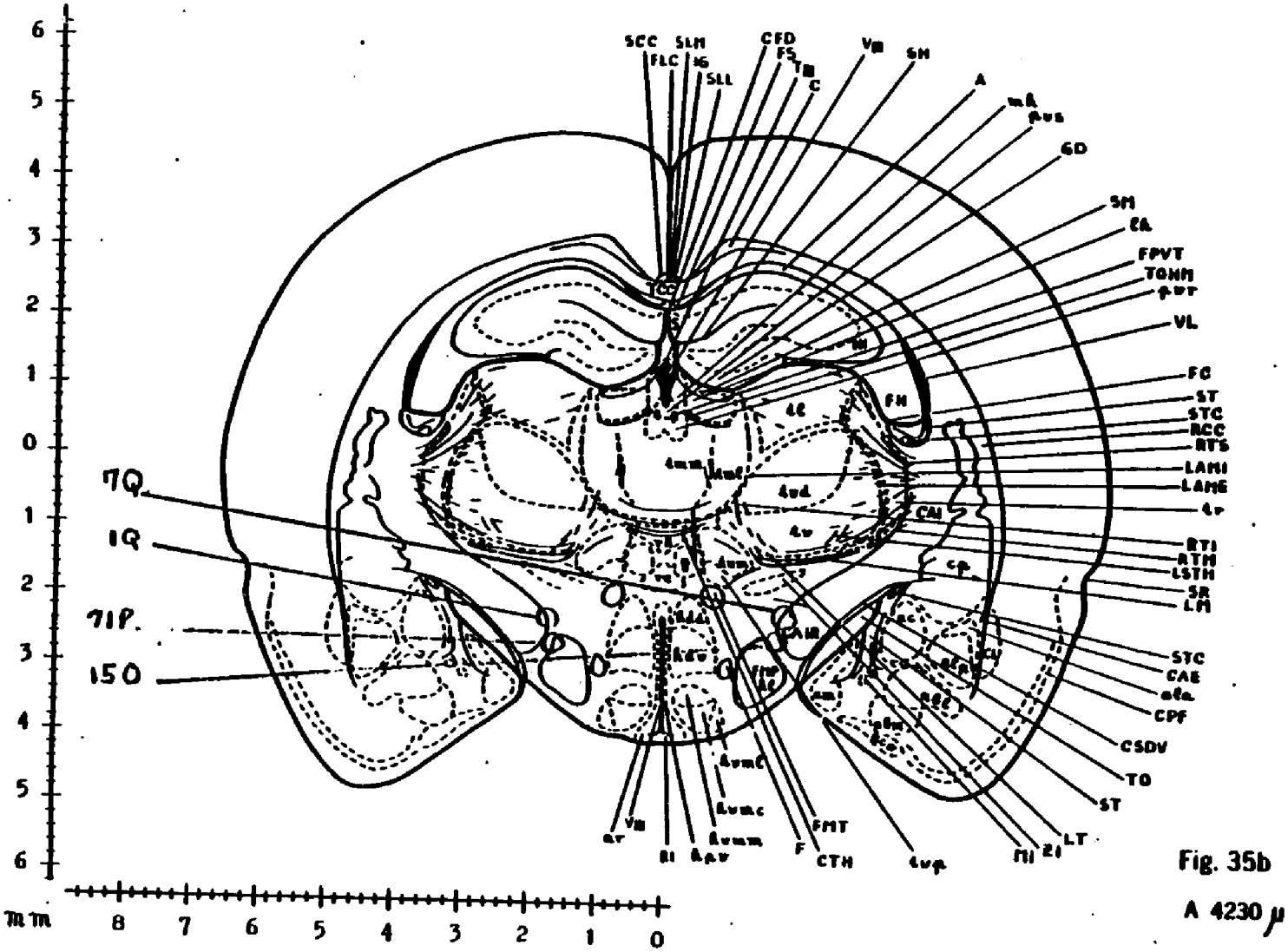


Fig. 35b

A 4230 μ

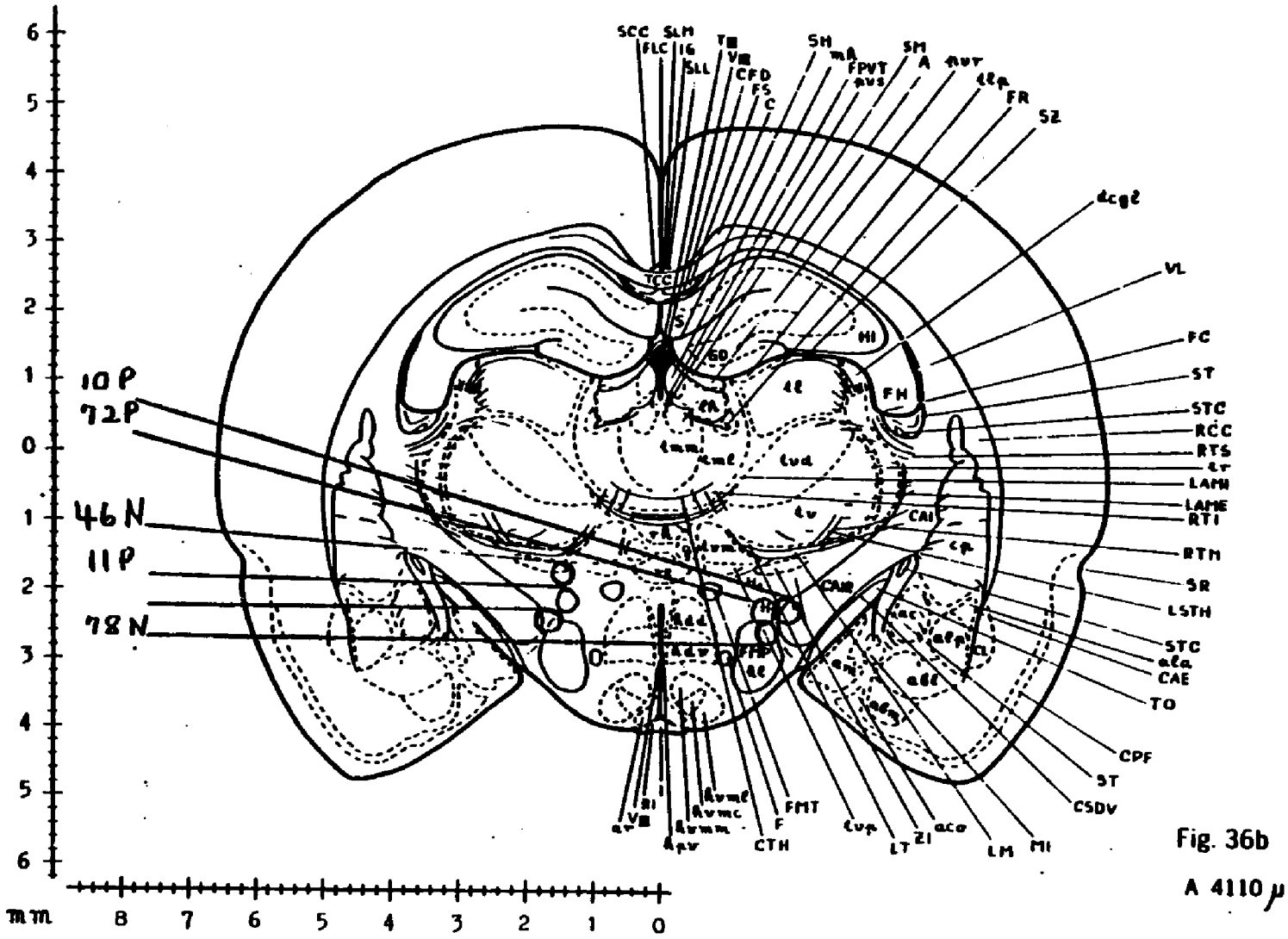


Fig. 36b

A 4110 μ

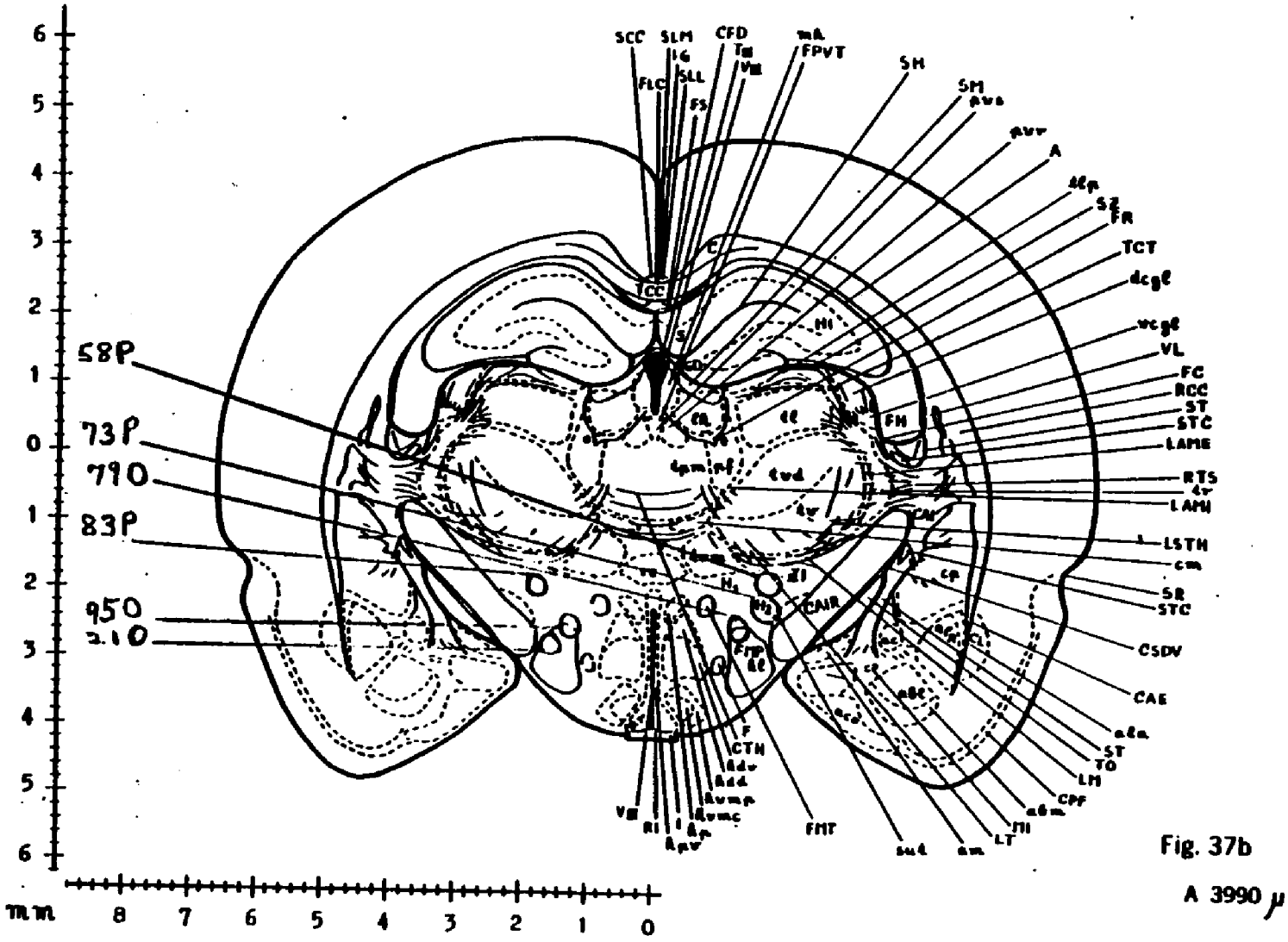


Fig. 37b

A 3990 μ

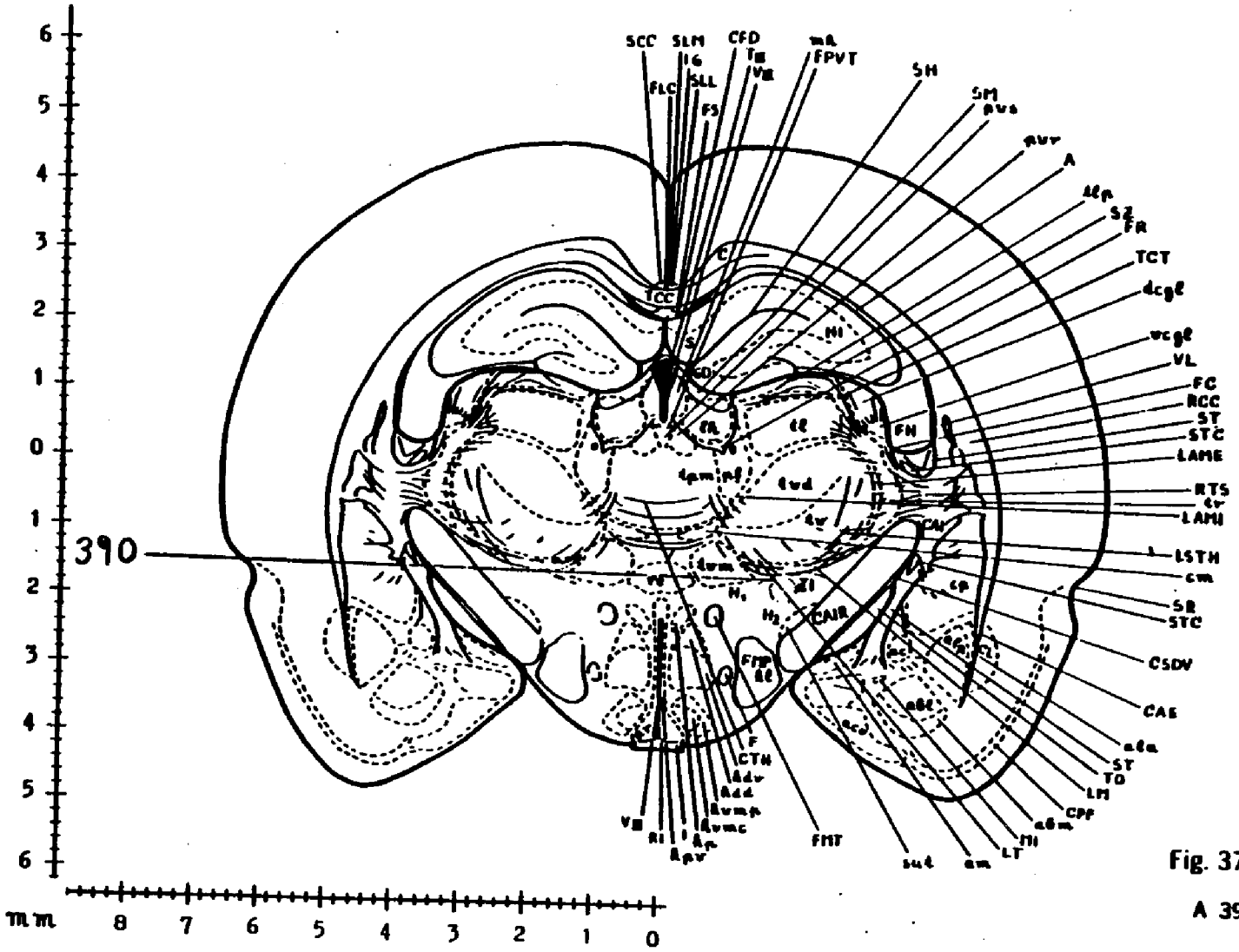


Fig. 37b

A 3990 μ

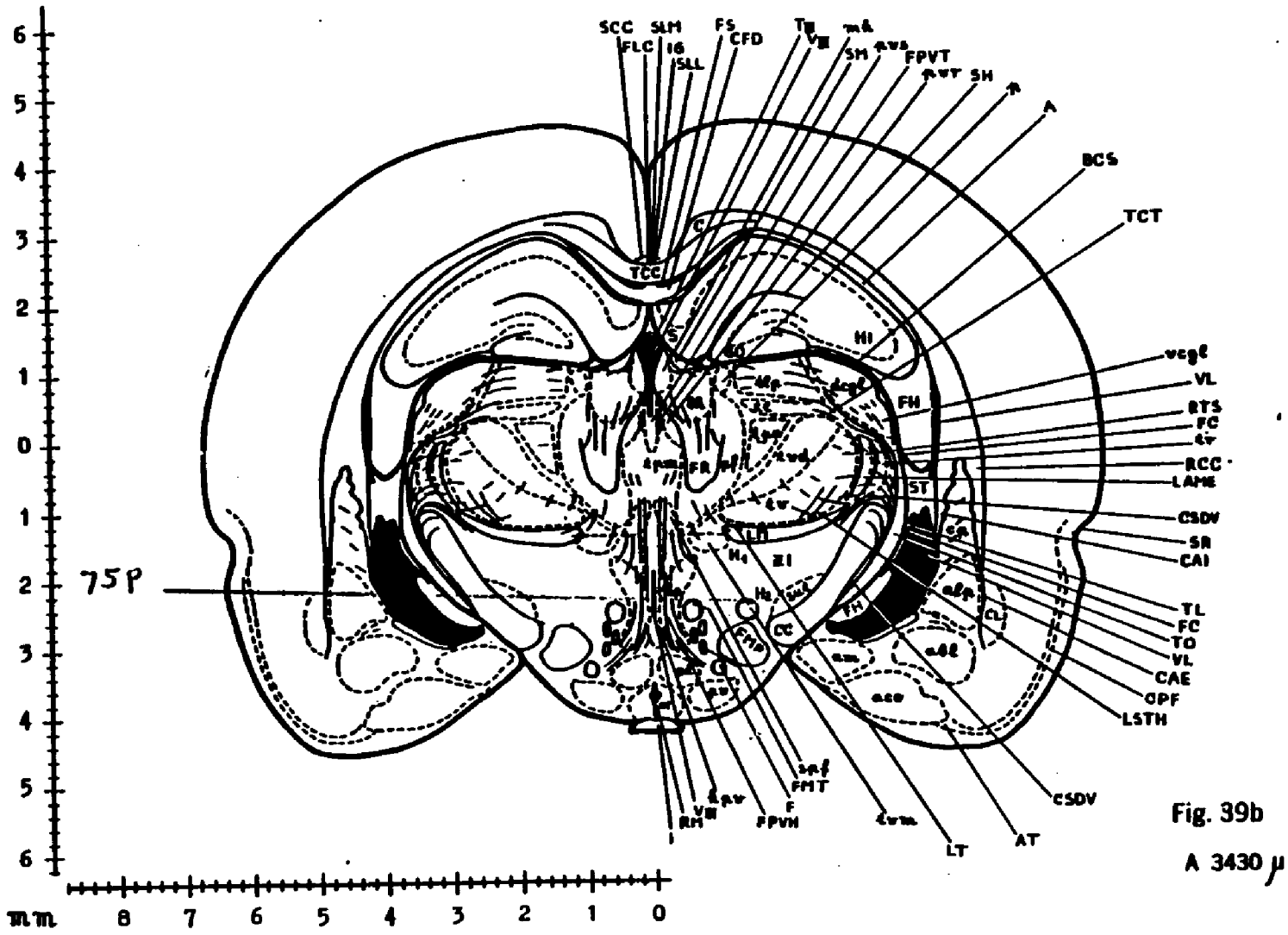
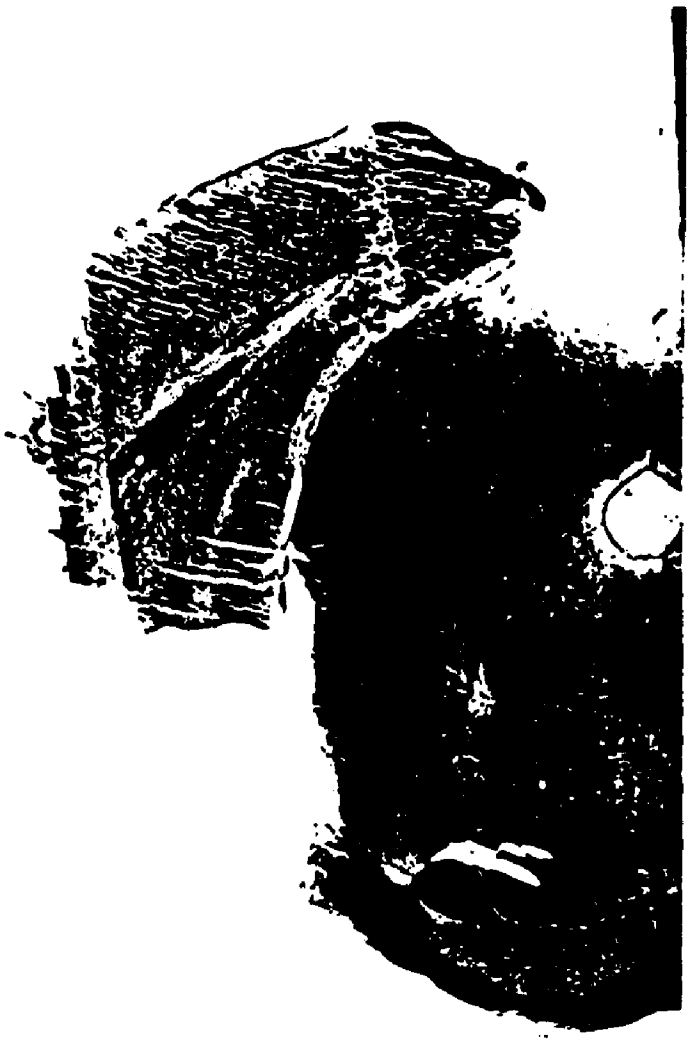
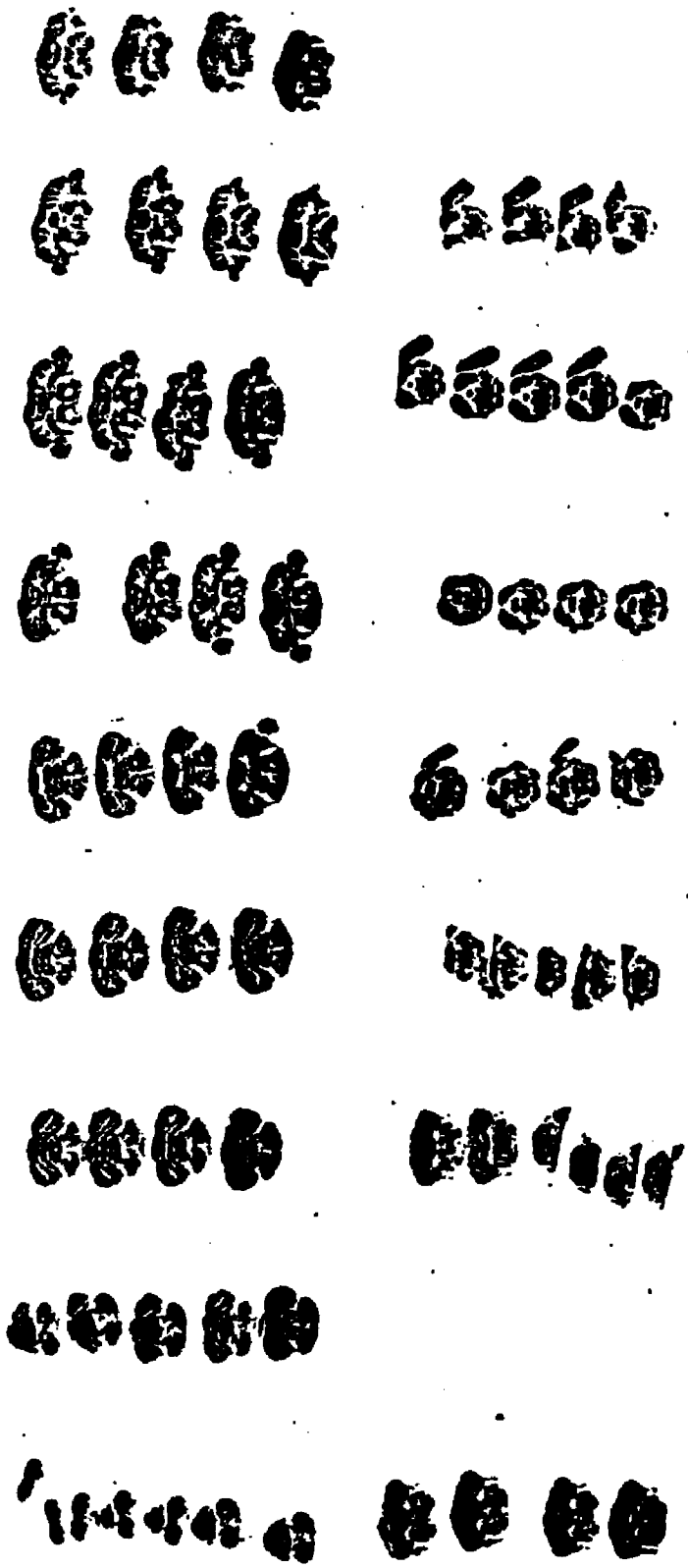


Fig. 39b

A 3430 μ

Figure 3. Representative histological photomicrographs for the animals with electrodes localized in the area of the nucleus locus coeruleus and the dorsal noradrenergic bundle.





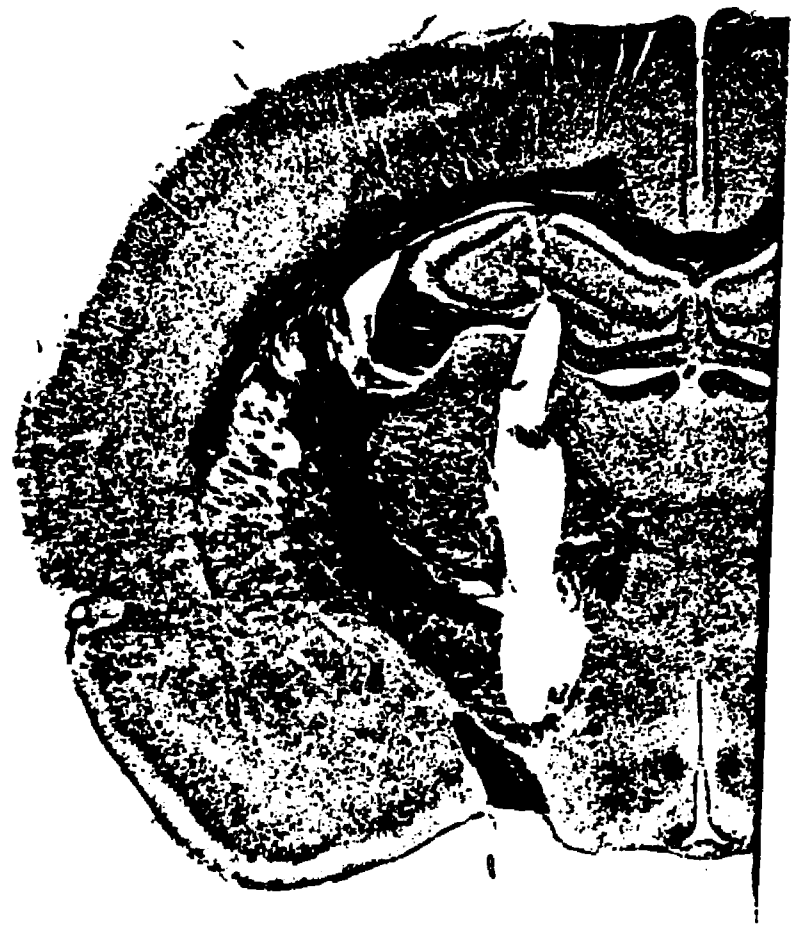
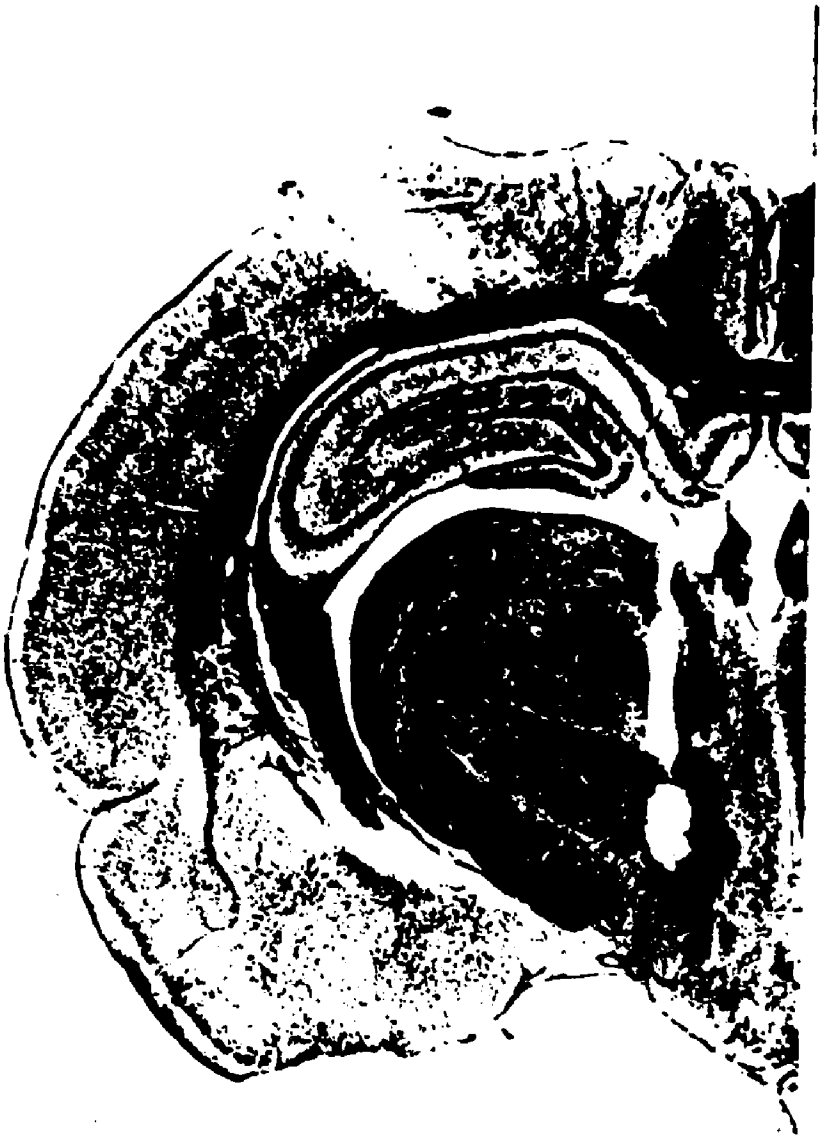
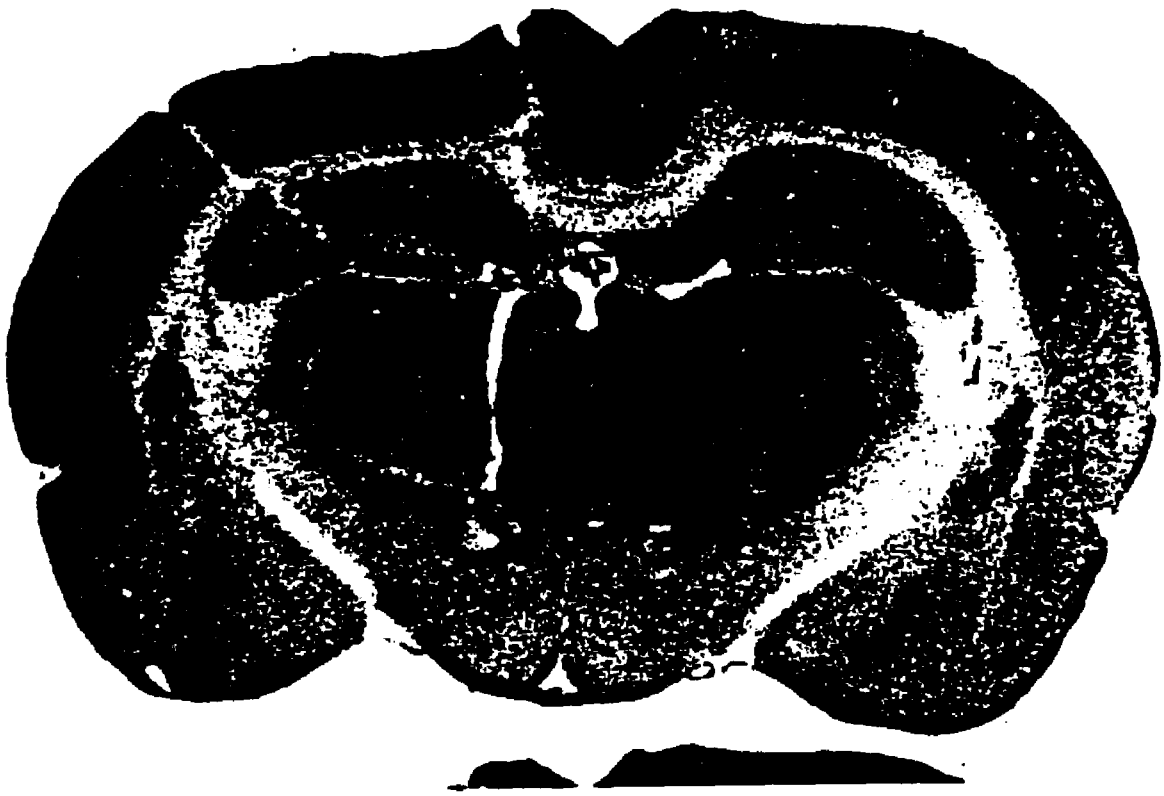
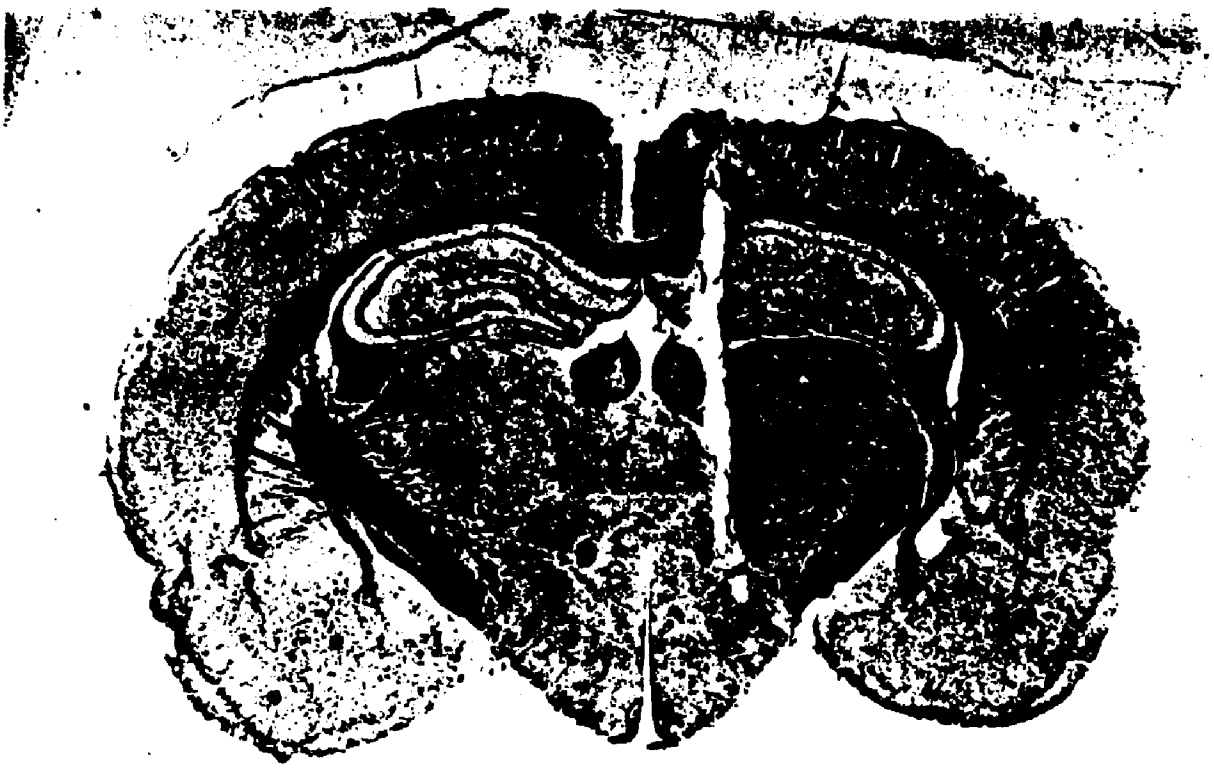
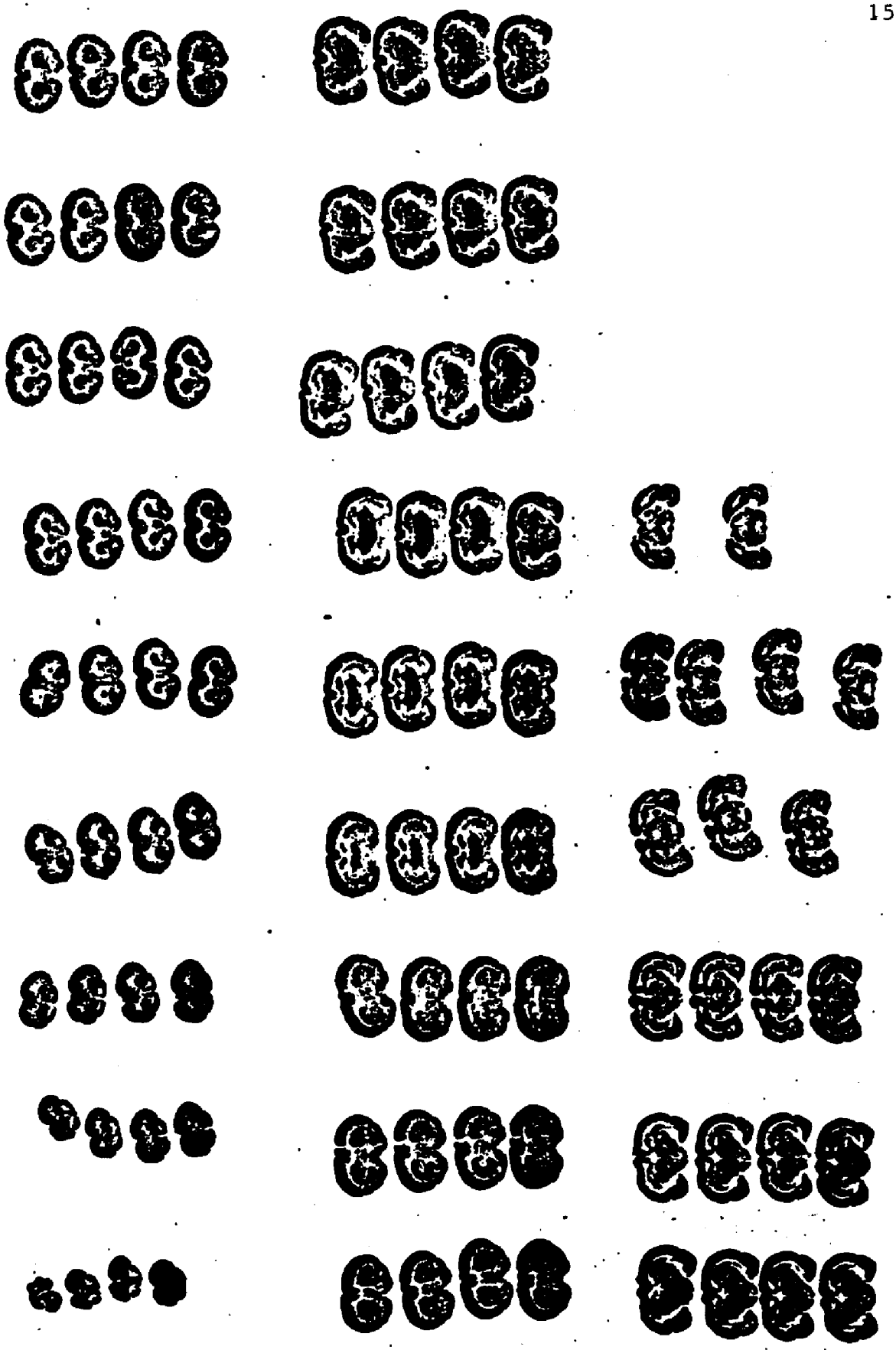


Figure 4. Representative histological photomicrographs for the animals with electrodes localized in the area of the medial forebrain bundle and the dorsal far lateral hypothalamus (Fields of Forel and Cru-Cerebri).









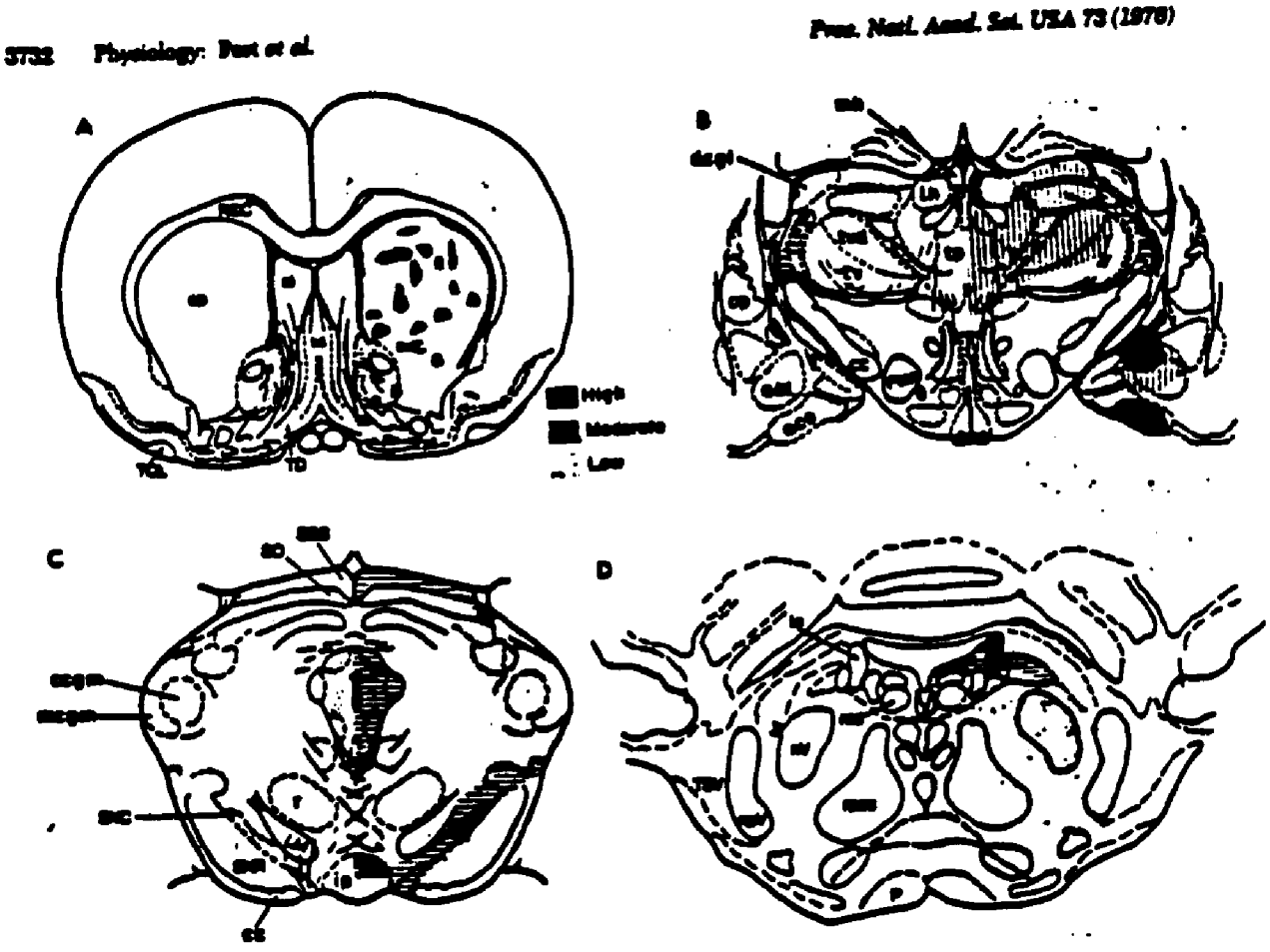


Figure 5. Representative sections from immuncytohistochemical, receptor binding and stereotaxic flourscent atlases illustrative of the ICSS sites in the present study.

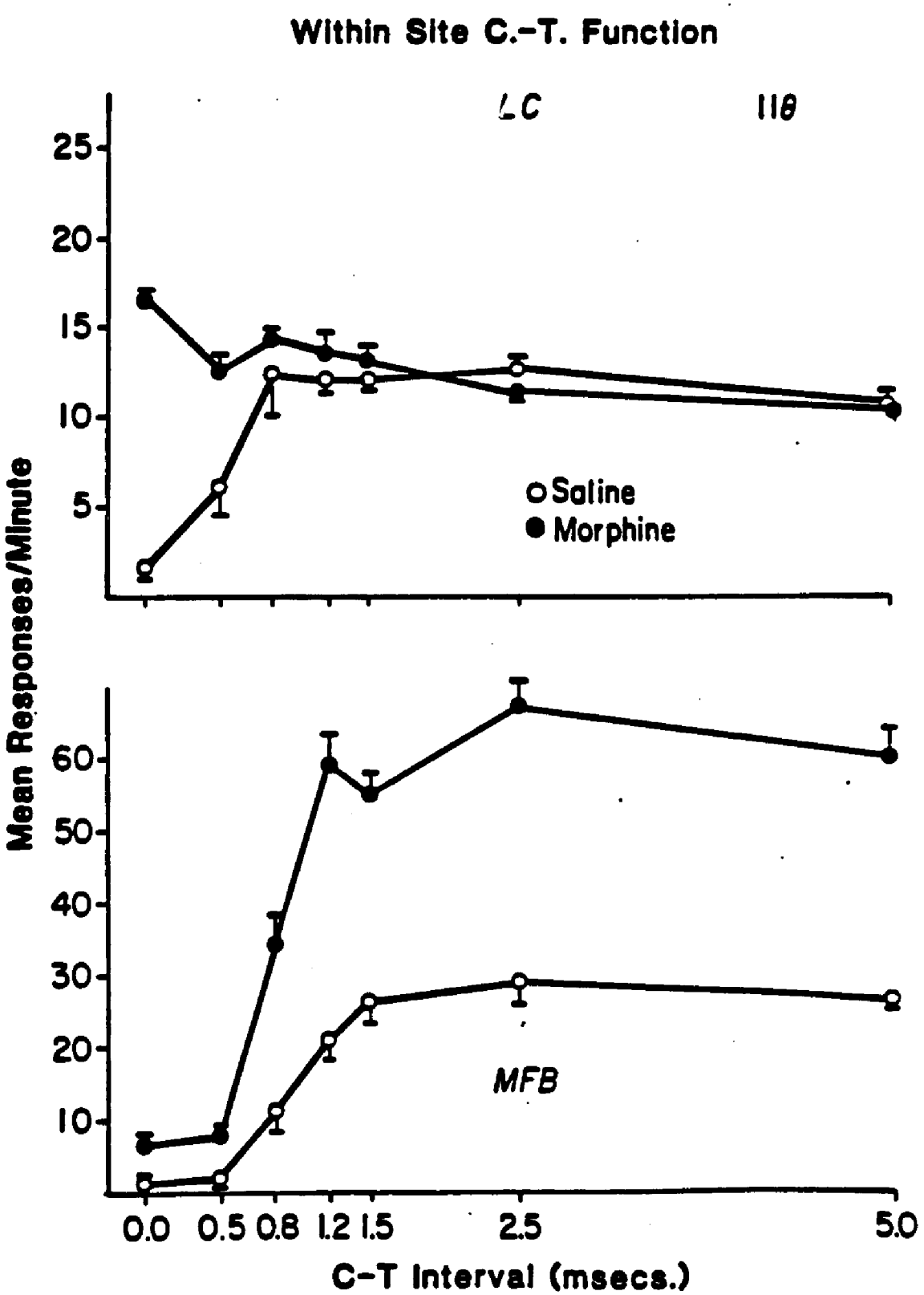


Figure 6. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 110 from the LC-MFB group of placements in the morphine and naloxone Within-Site C-T condition.

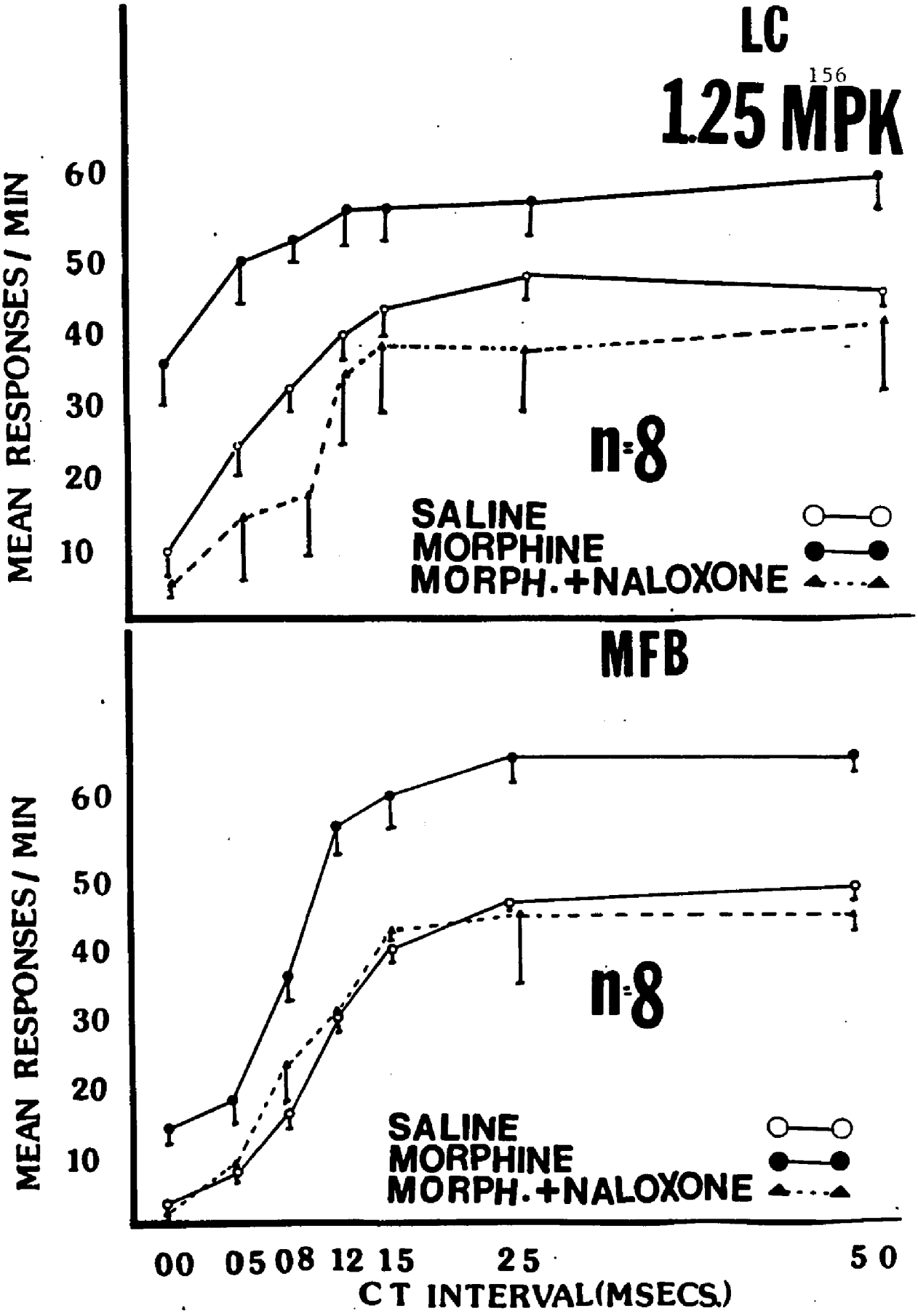
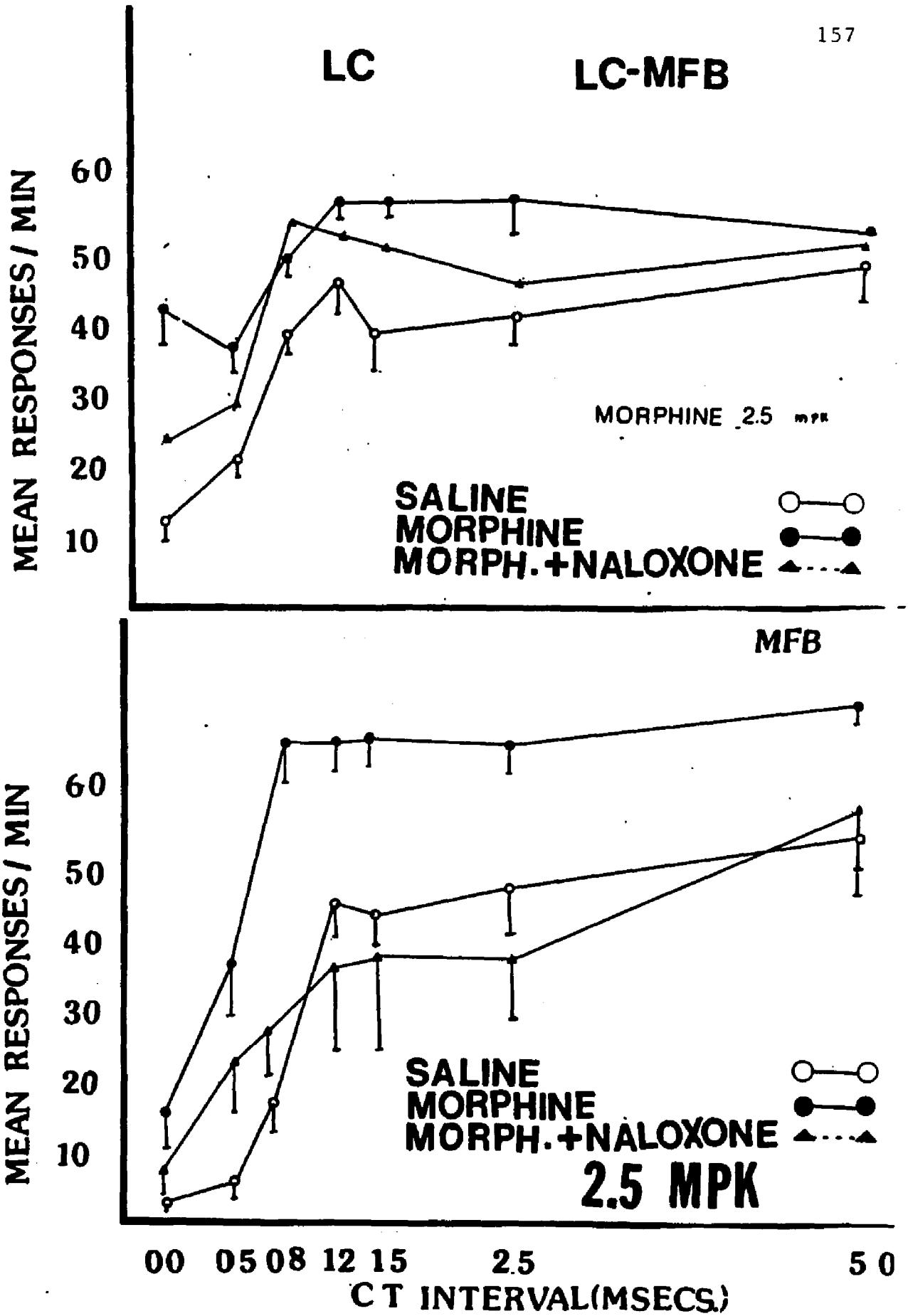


Figure 7. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-MFB placements in the morphine and naloxone Within-Site C-T condition.


LC
LC-MFB
MEAN RESPONSES / MIN
60
50
40
30
20
10
MORPHINE 2.5 mpk
SALINE
MORPHINE
MORPH.+NALOXONE
MFB
MEAN RESPONSES / MIN
60
50
40
30
20
10
SALINE
MORPHINE
MORPH.+NALOXONE
2.5 MPK
00 05 08 12 15 2.5 5 0
CT INTERVAL(MSECS.)

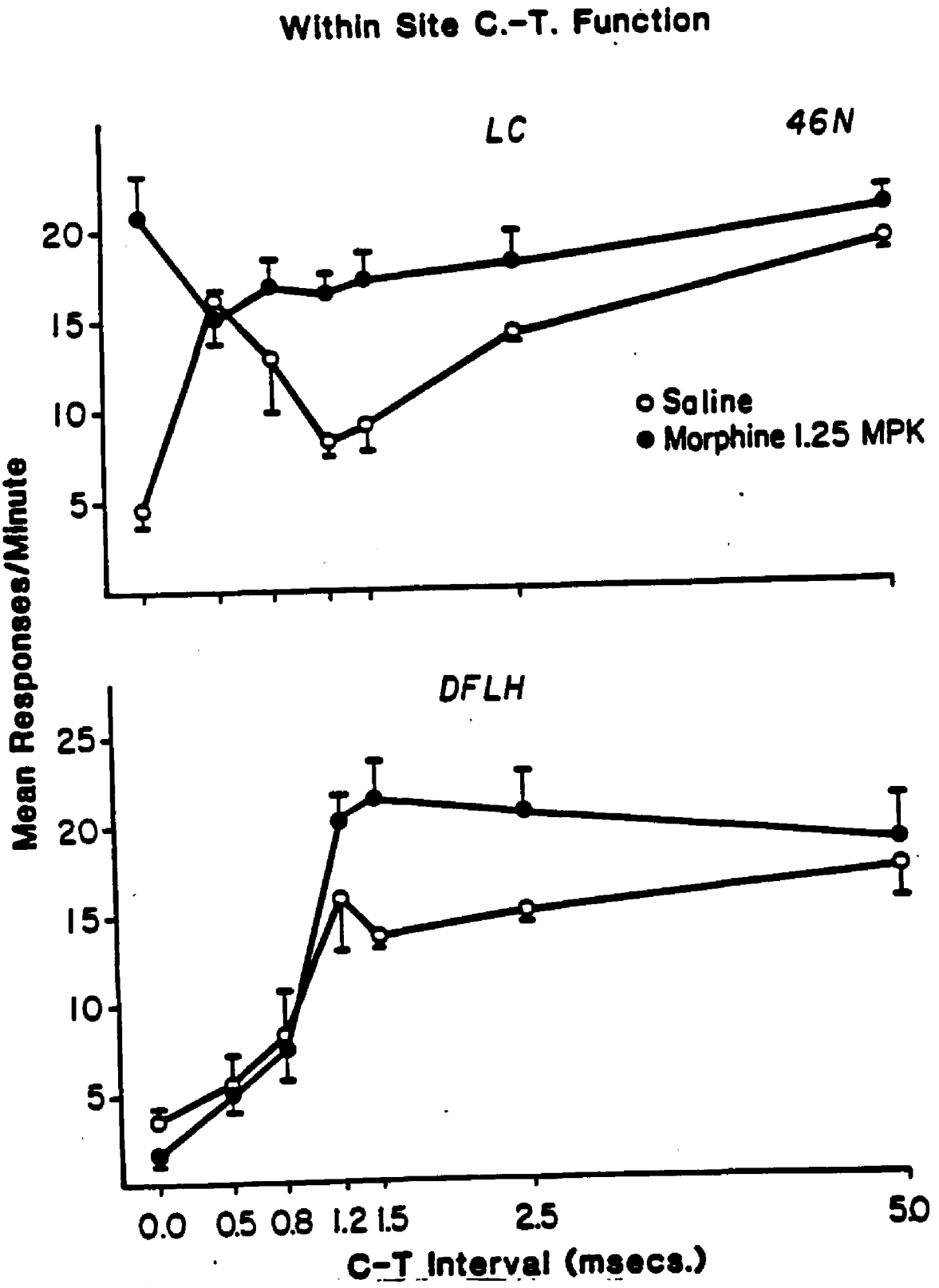


Figure 8. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 46N from the LC-FF group

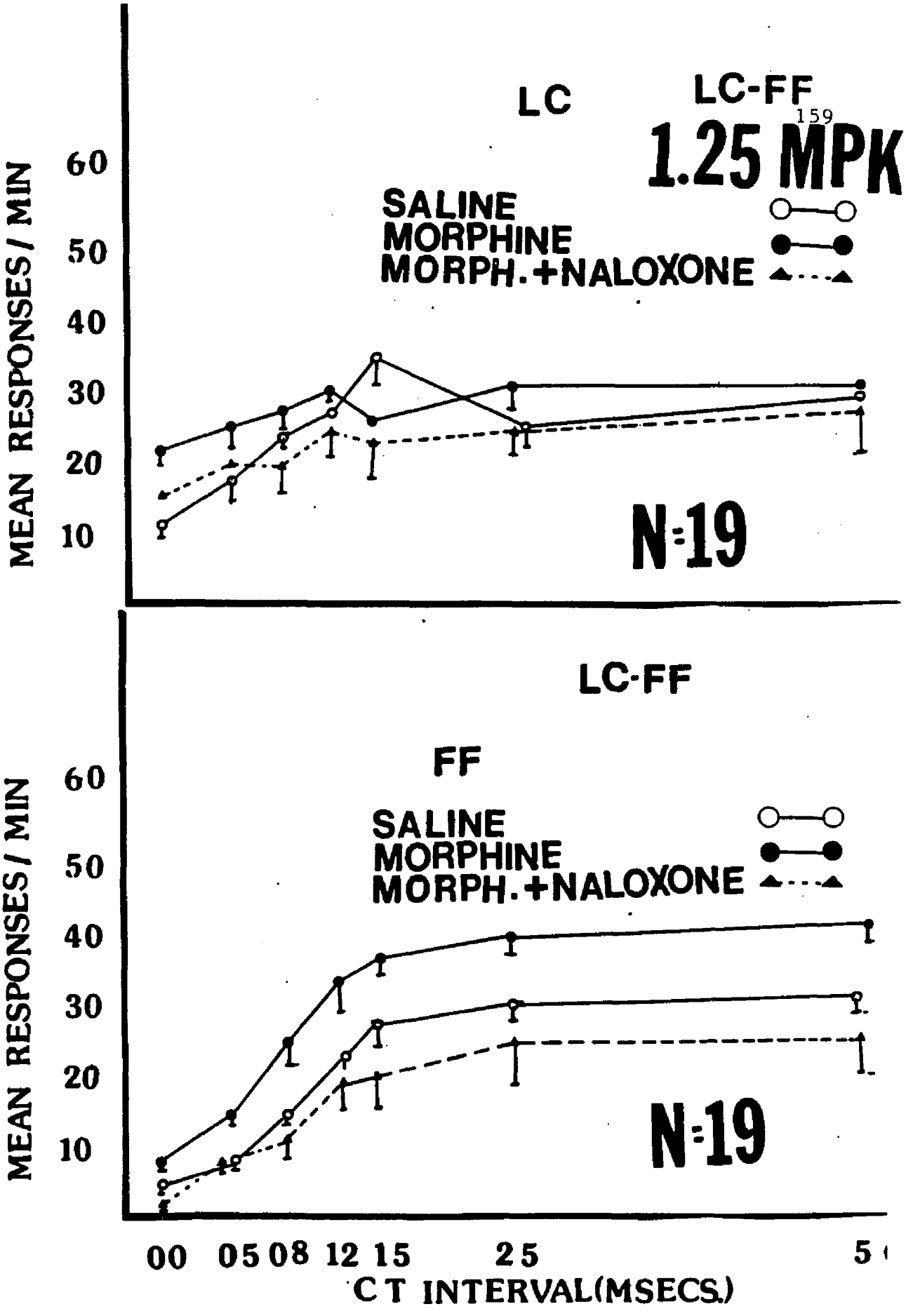


Figure 9. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-FF placements in the morphine and naloxone Within-Site C-T condition.

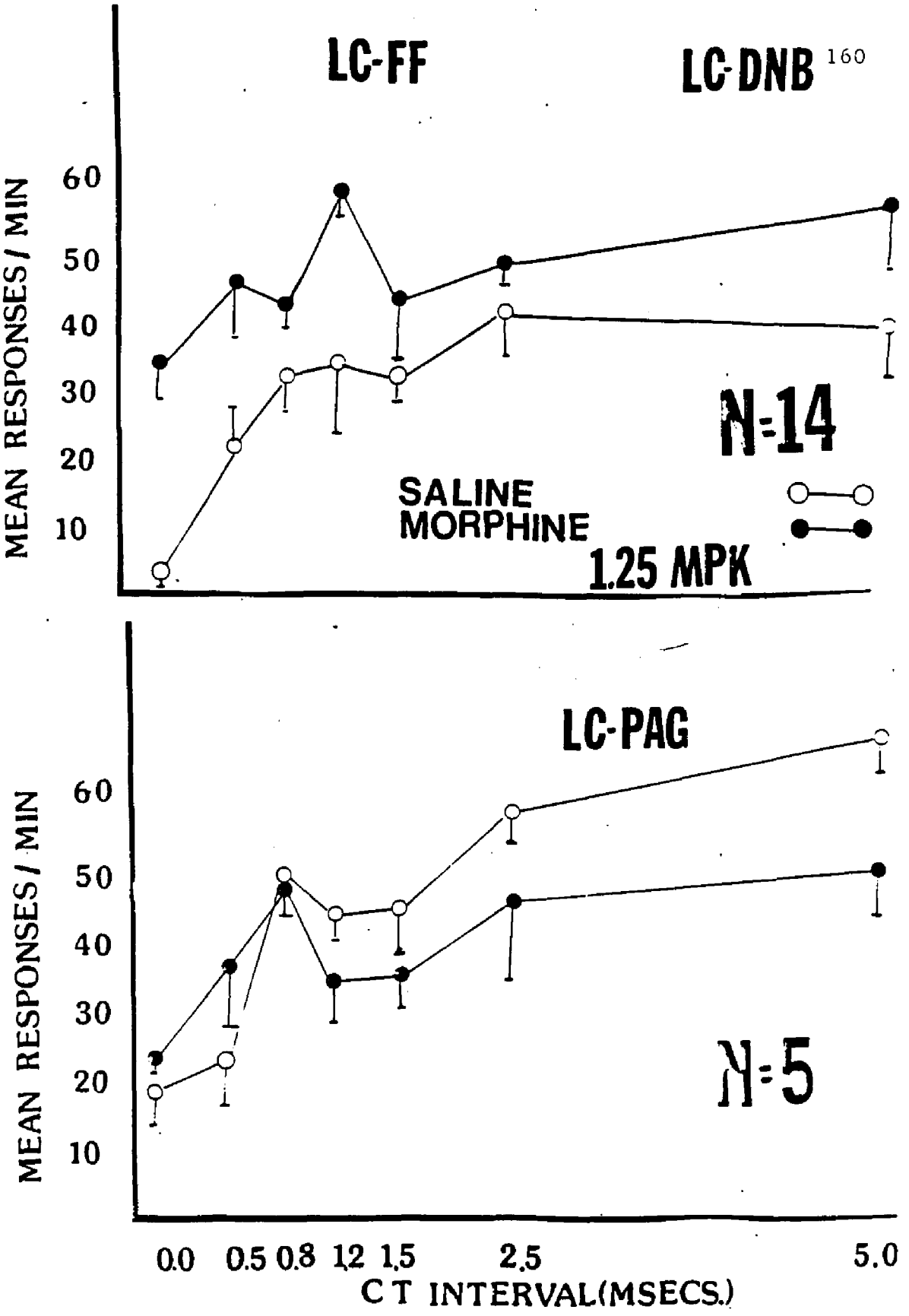
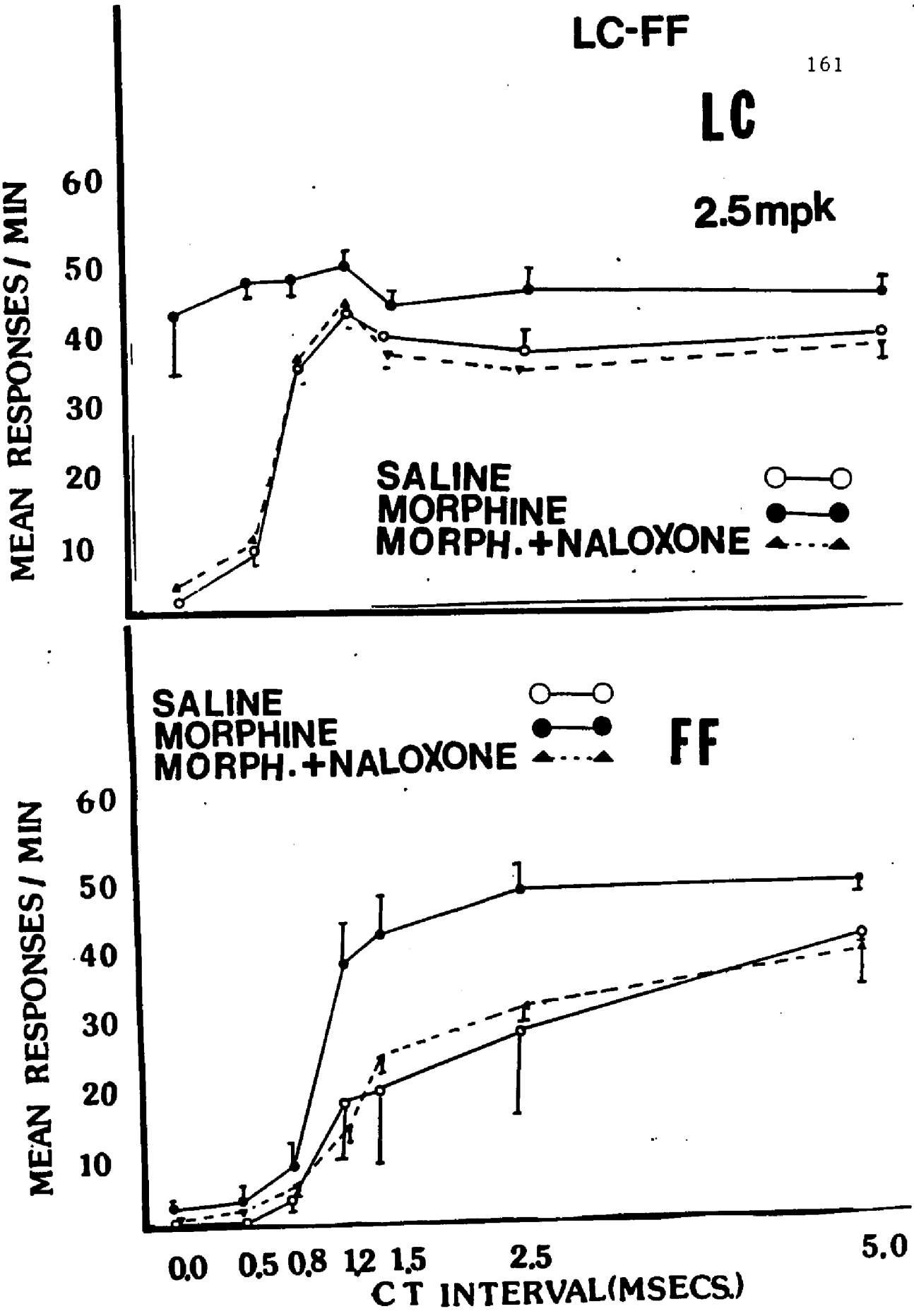


Figure 12. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-PAG placements in the morphine and naloxone Within-Site condition.


LC-FF
LC
2.5mpk
MEAN RESPONSES / MIN
60
50
40
30
20
10
SALINE
MORPHINE
MORPH.+NALOXONE
SALINE
MORPHINE
MORPH.+NALOXONE
FF
MEAN RESPONSES / MIN
60
50
40
30
20
10
0.0
0.5
0.8
1.2
1.5
2.5
5.0
CT INTERVAL(MSECS.)

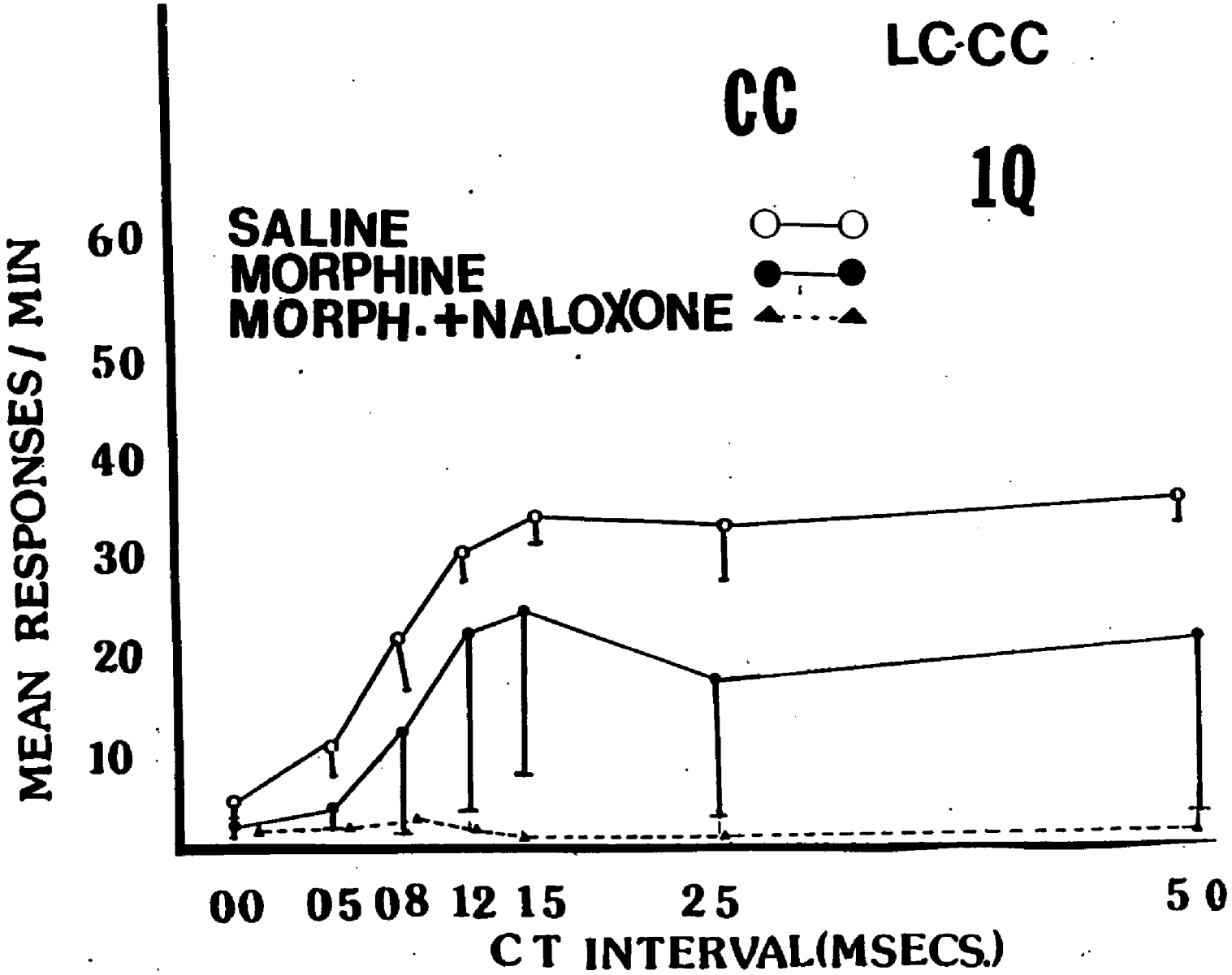


Figure 10. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 1Q from the LC-CC group of placements in the morphine and naloxone Within-Site C-T condition.

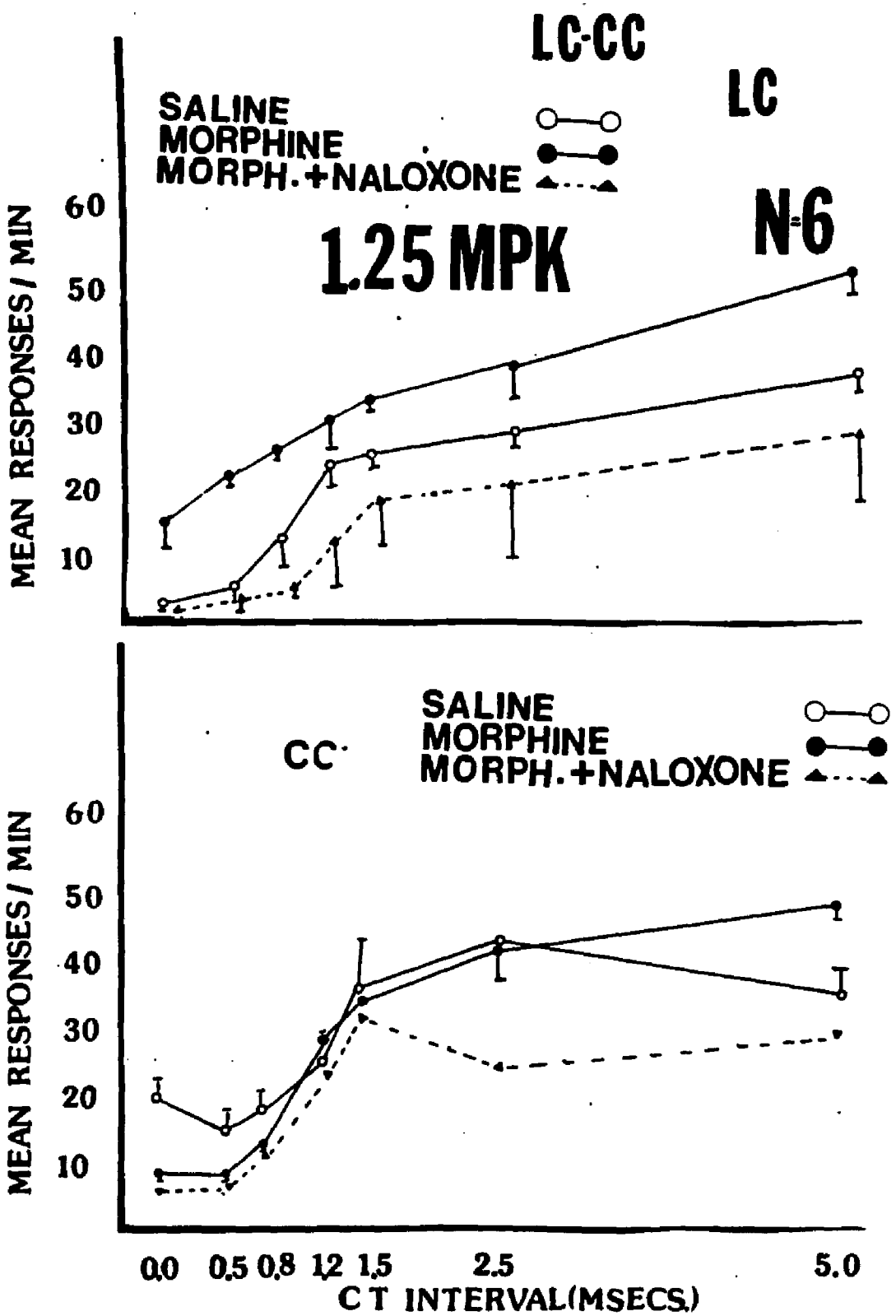
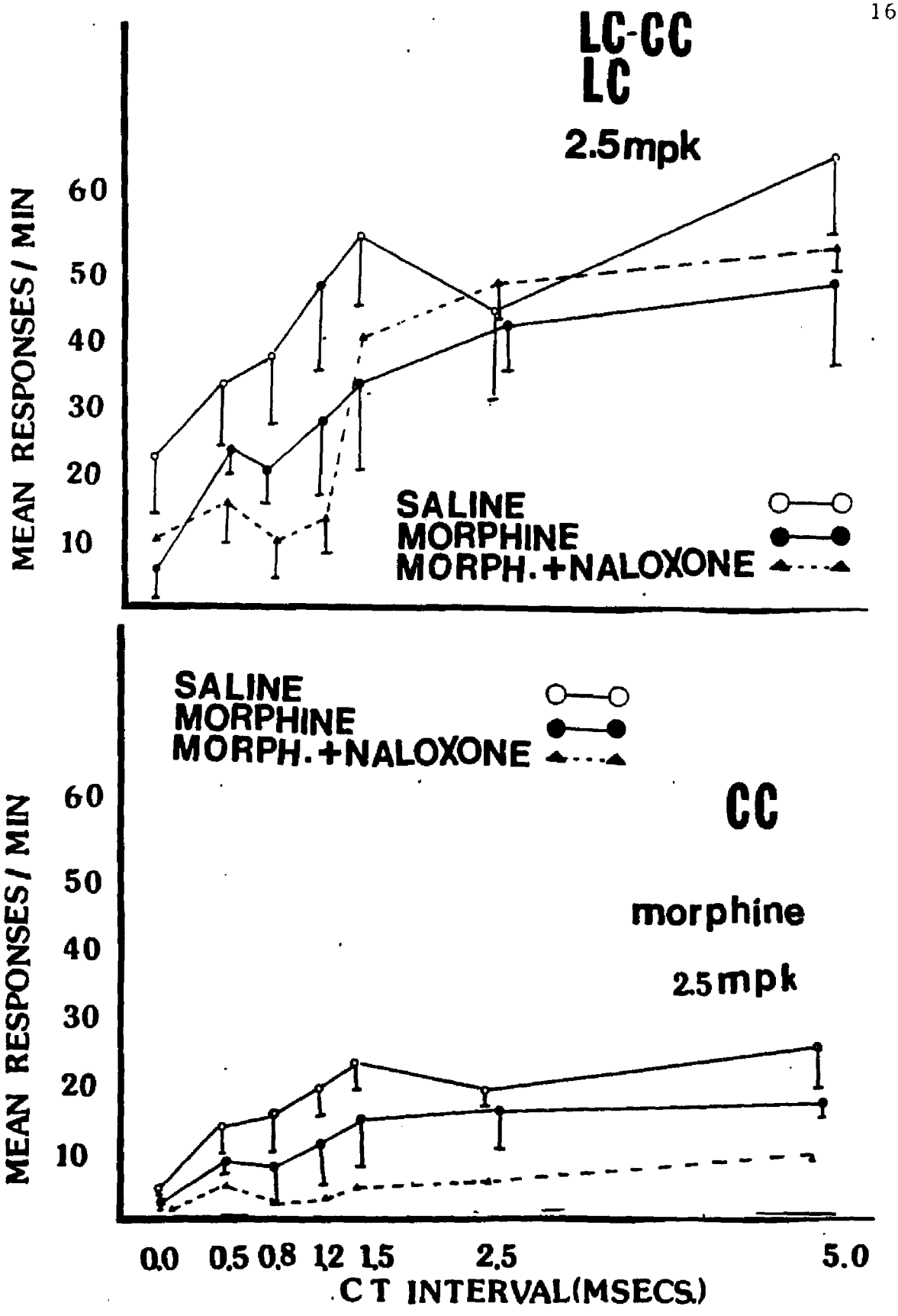


Figure 11. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-CC placements in the morphine and naloxone Within-Site condition.


LC-CC
LC
2.5mpk
MEAN RESPONSES / MIN
60
50
40
30
20
10
SALINE
MORPHINE
MORPH.+NALOXONE
SALINE
MORPHINE
MORPH.+NALOXONE
CC
morphine
2.5mpk
MEAN RESPONSES / MIN
60
50
40
30
20
10
0.0
0.5
0.8
1.2
1.5
2.5
5.0
CT INTERVAL(MSECS.)

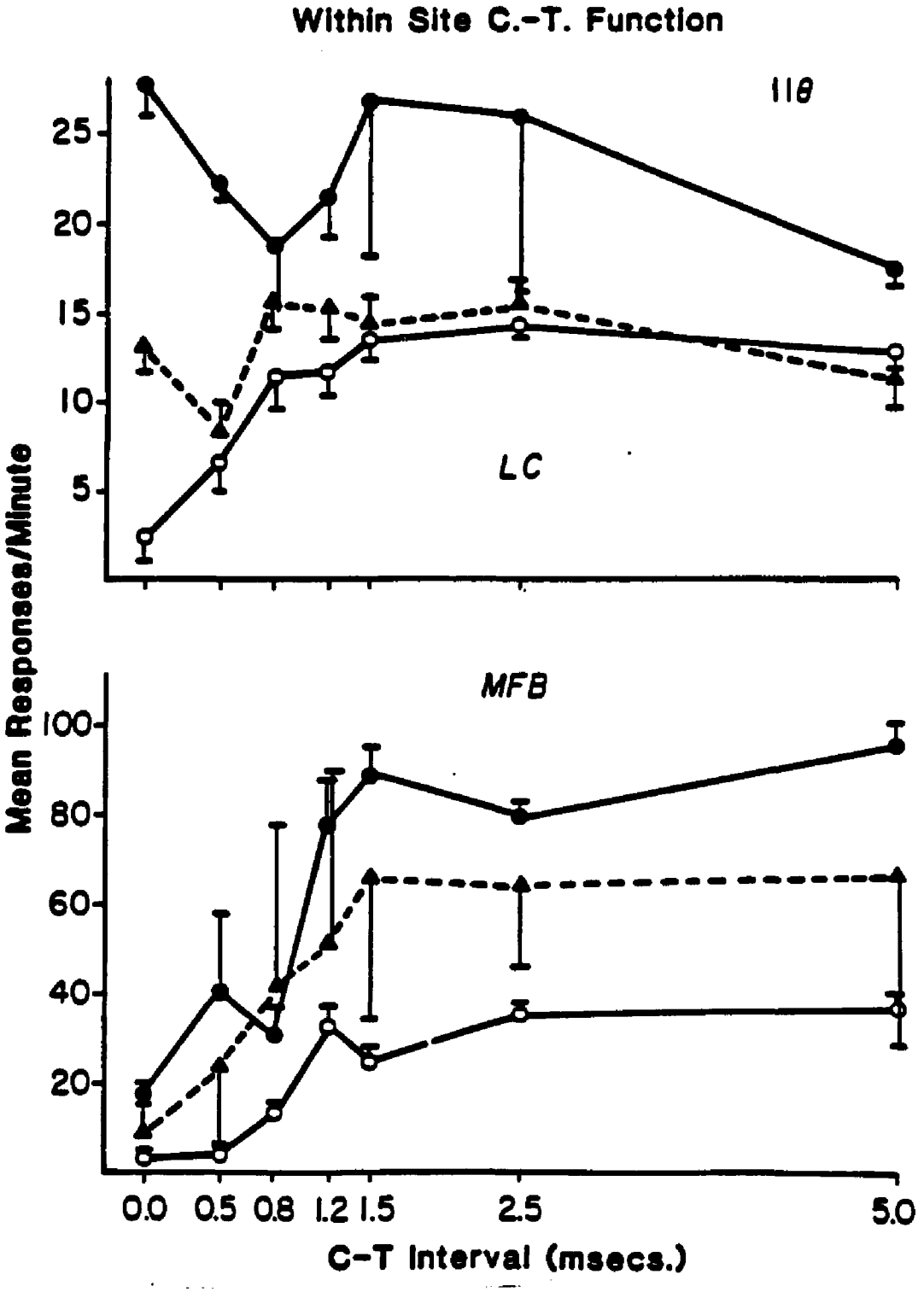


Figure 13. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 110 from the LC-MFB group of placements in the d- and l- amphetamine Within-Site condition at the 1.0 mpk dose.

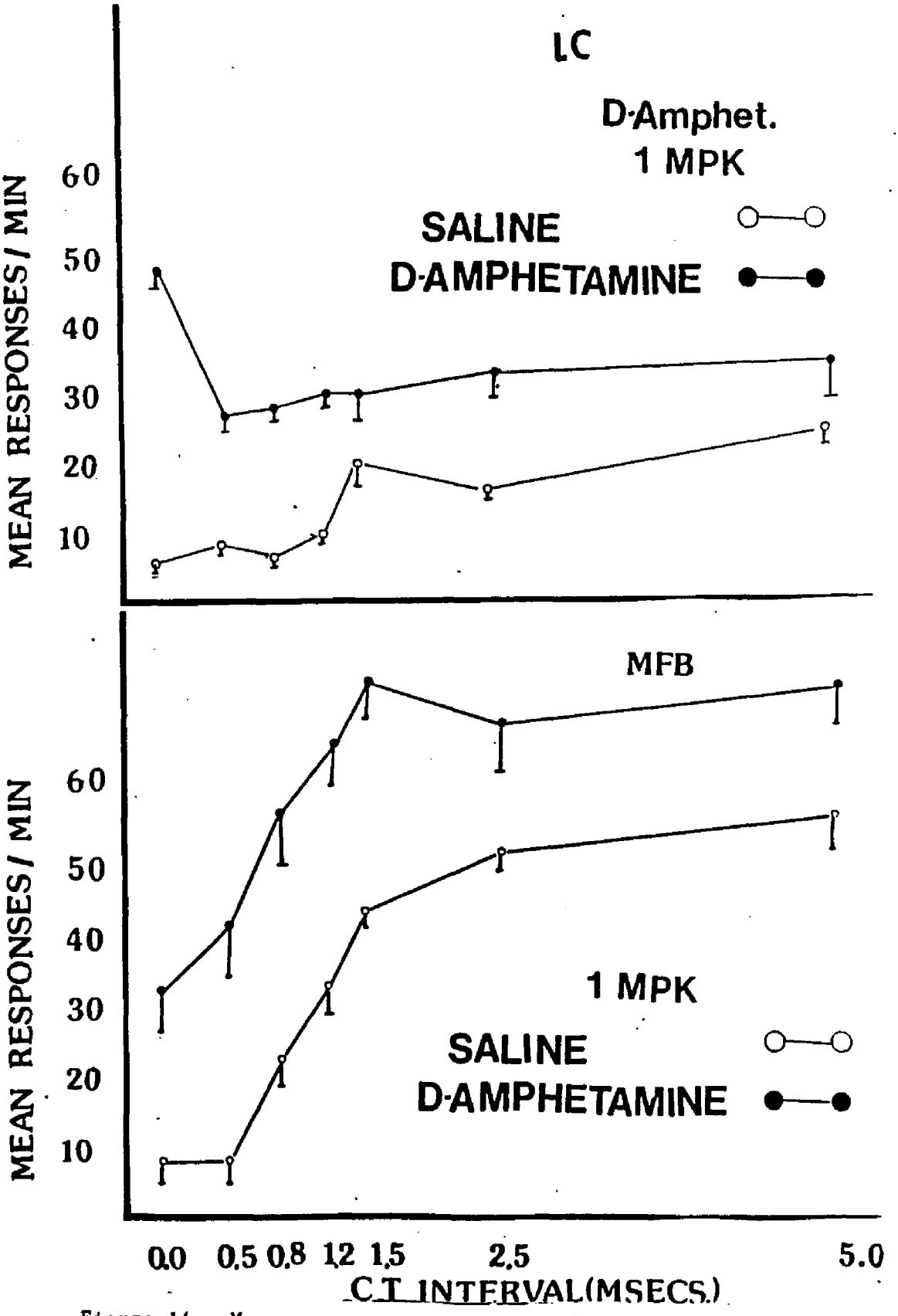
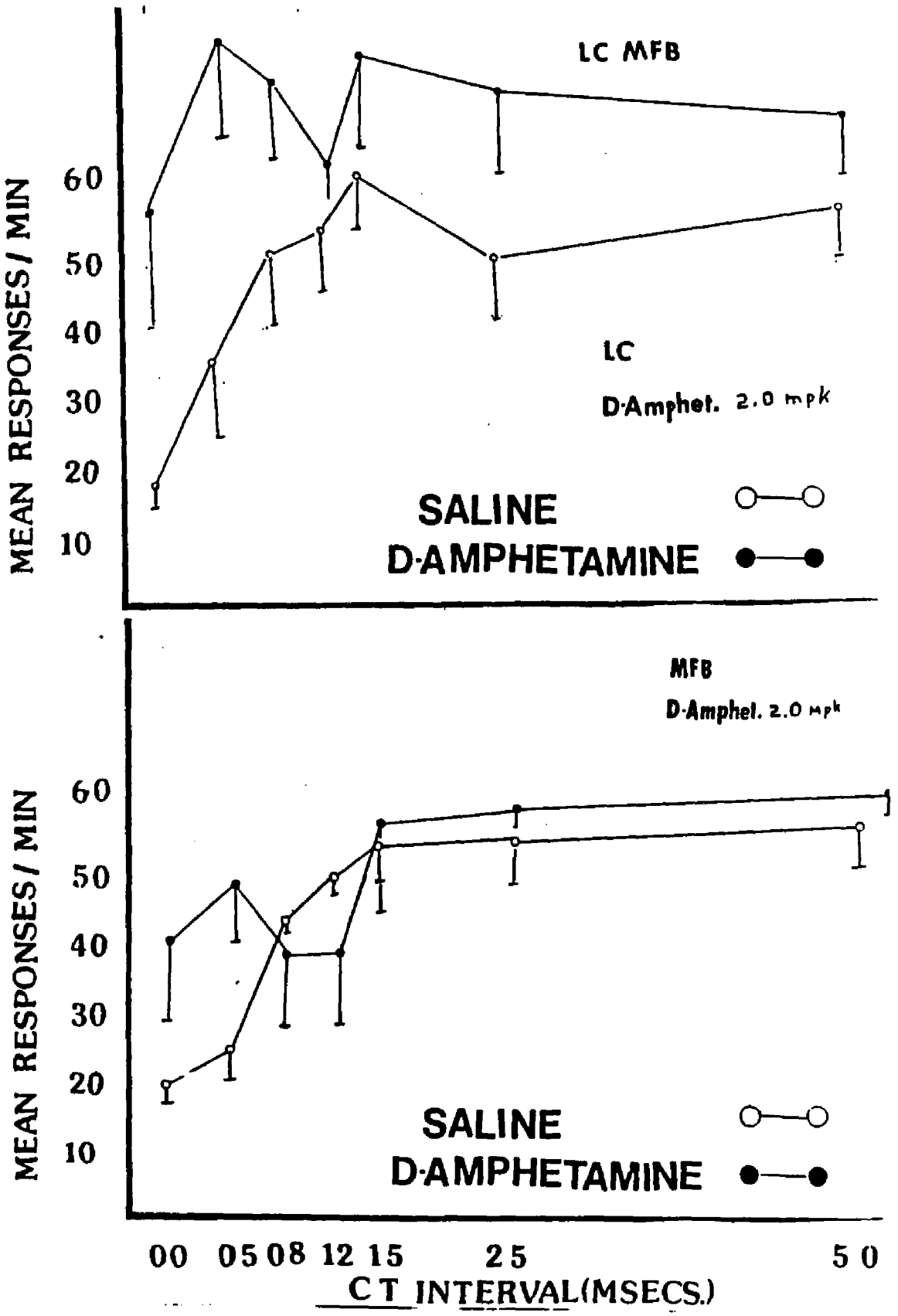
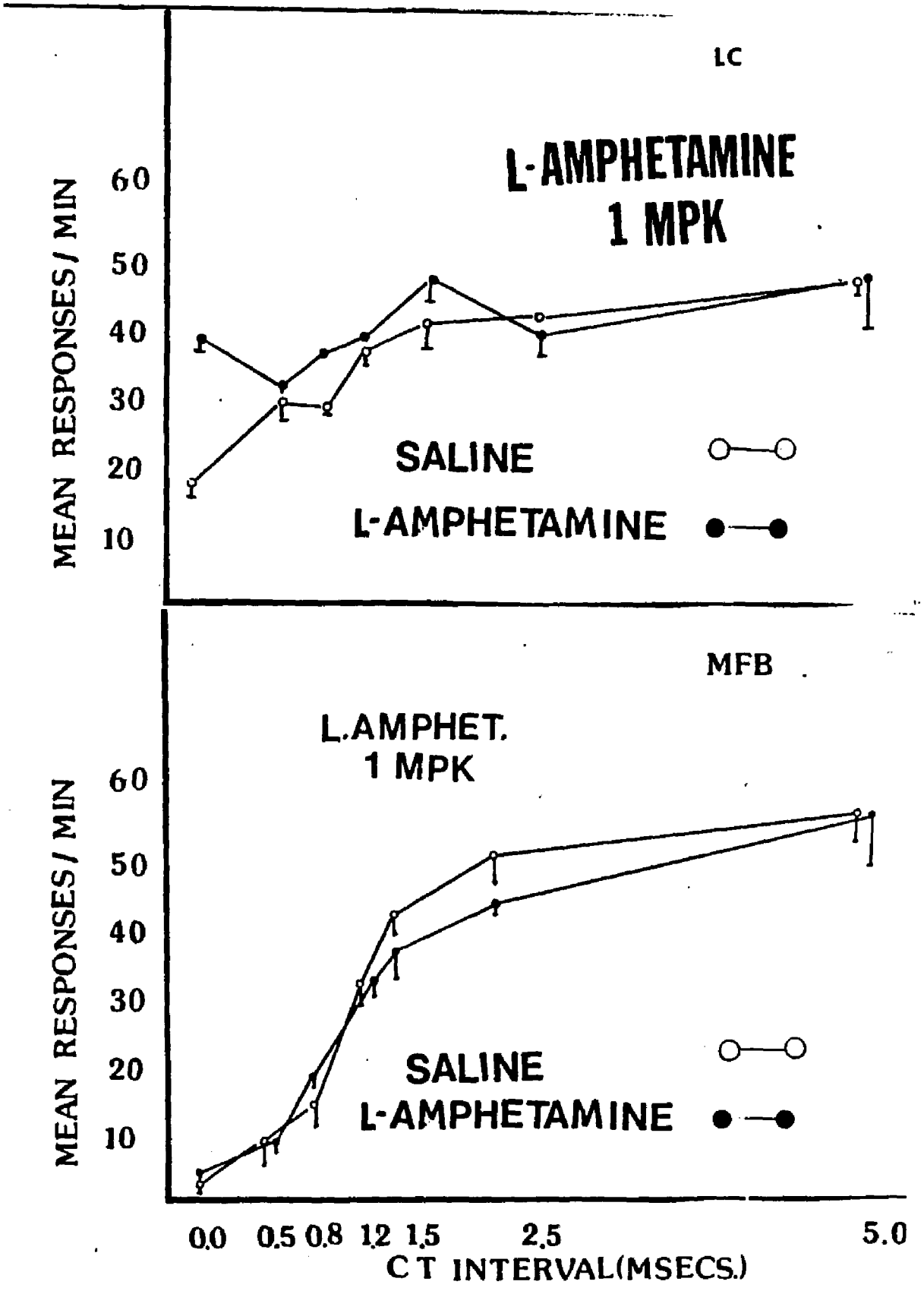
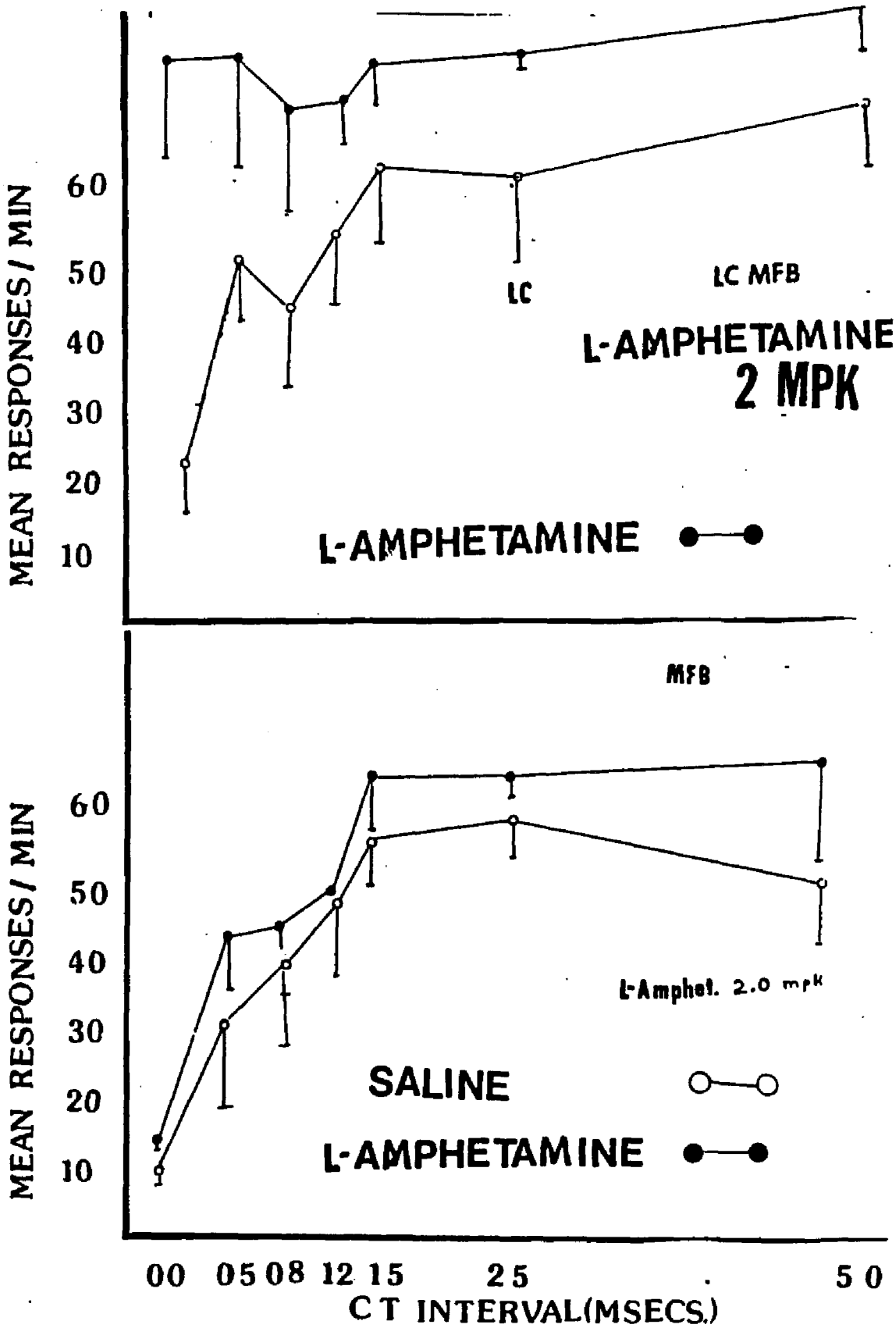


Figure 14. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-MFB placements in the d- and l- amphetamine Within-Site condition at the 1.0 and 2.0 mpk doses.


LC MFB
LC
D-Amphet. 2.0 mpk
SALINE
D-AMPHETAMINE
MEAN RESPONSES / MIN
60
50
40
30
20
10
MFB
D-Amphet. 2.0 mpk
SALINE
D-AMPHETAMINE
MEAN RESPONSES / MIN
60
50
40
30
20
10
00 05 08 12 15 25 50
CT INTERVAL (MSECS.)


LC
L-AMPHETAMINE
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
SALINE
L-AMPHETAMINE
MFB
L.AMPHET.
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
SALINE
L-AMPHETAMINE
0.0
0.5
0.8
1.2
1.5
2.5
5.0
C T INTERVAL(MSECS.)


MEAN RESPONSES / MIN
60
50
40
30
20
10
LC MFB
LC
L-AMPHETAMINE
2 MPK
L-AMPHETAMINE
MFB
L-Amphet. 2.0 mpk
SALINE
L-AMPHETAMINE
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)

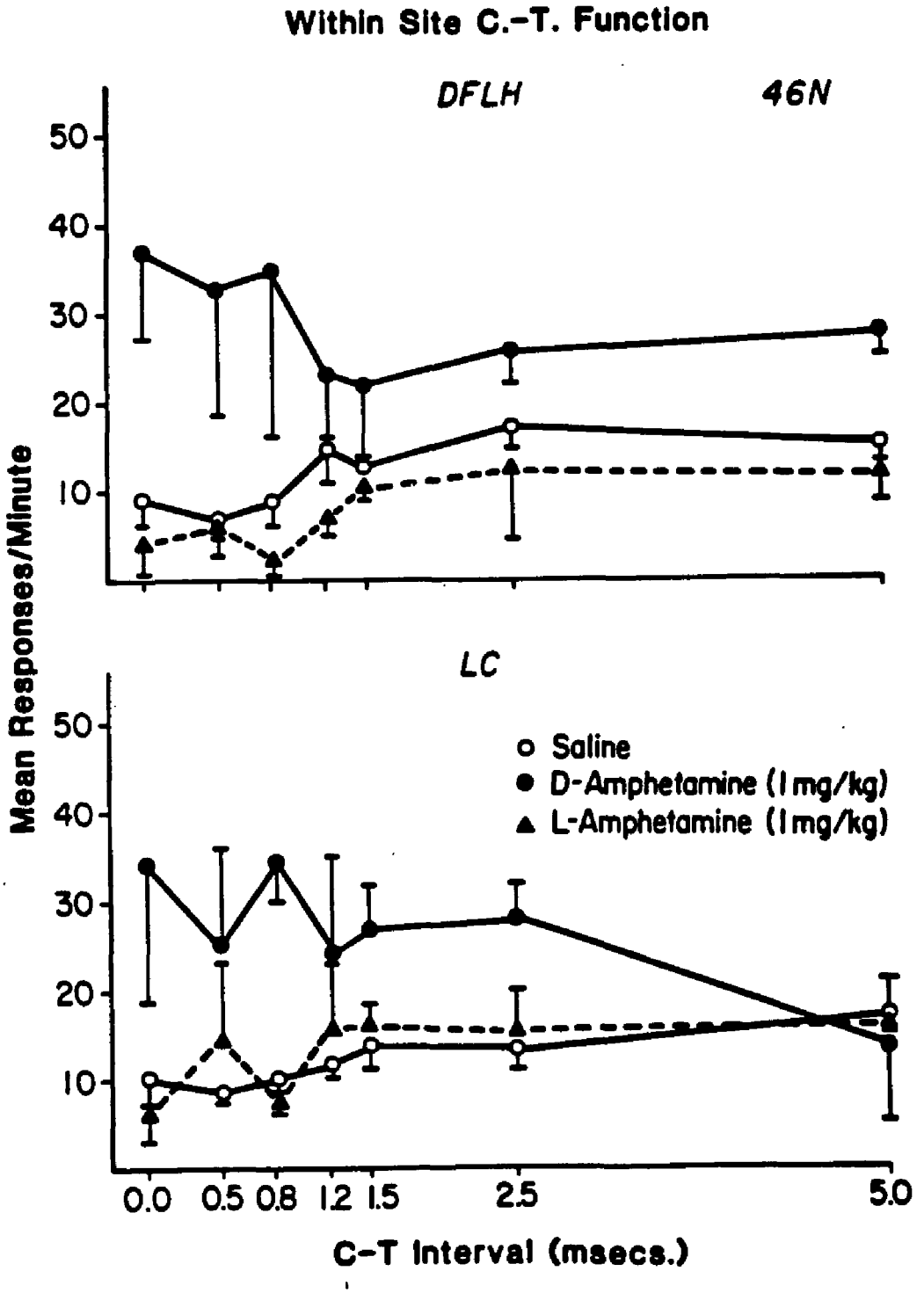
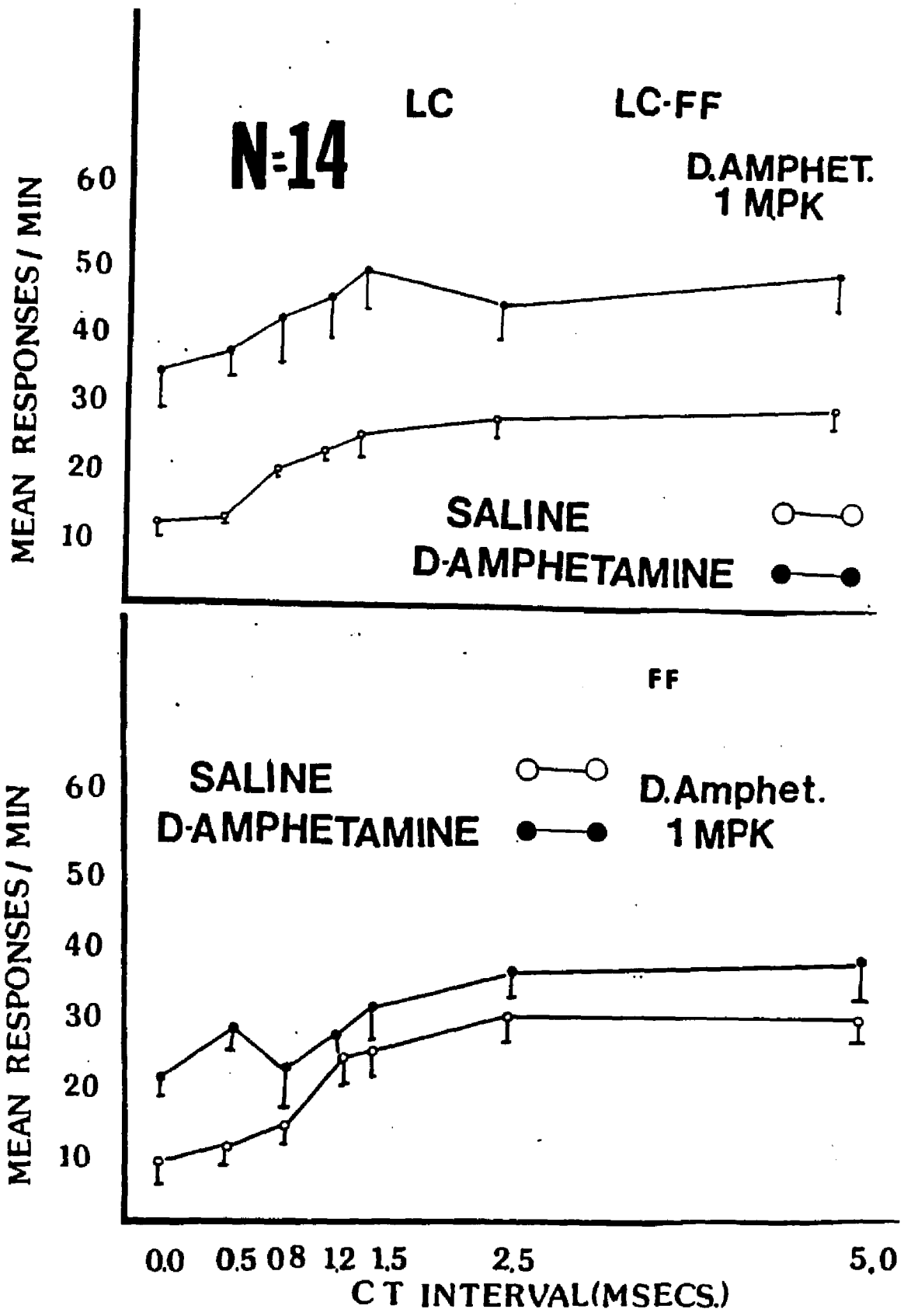
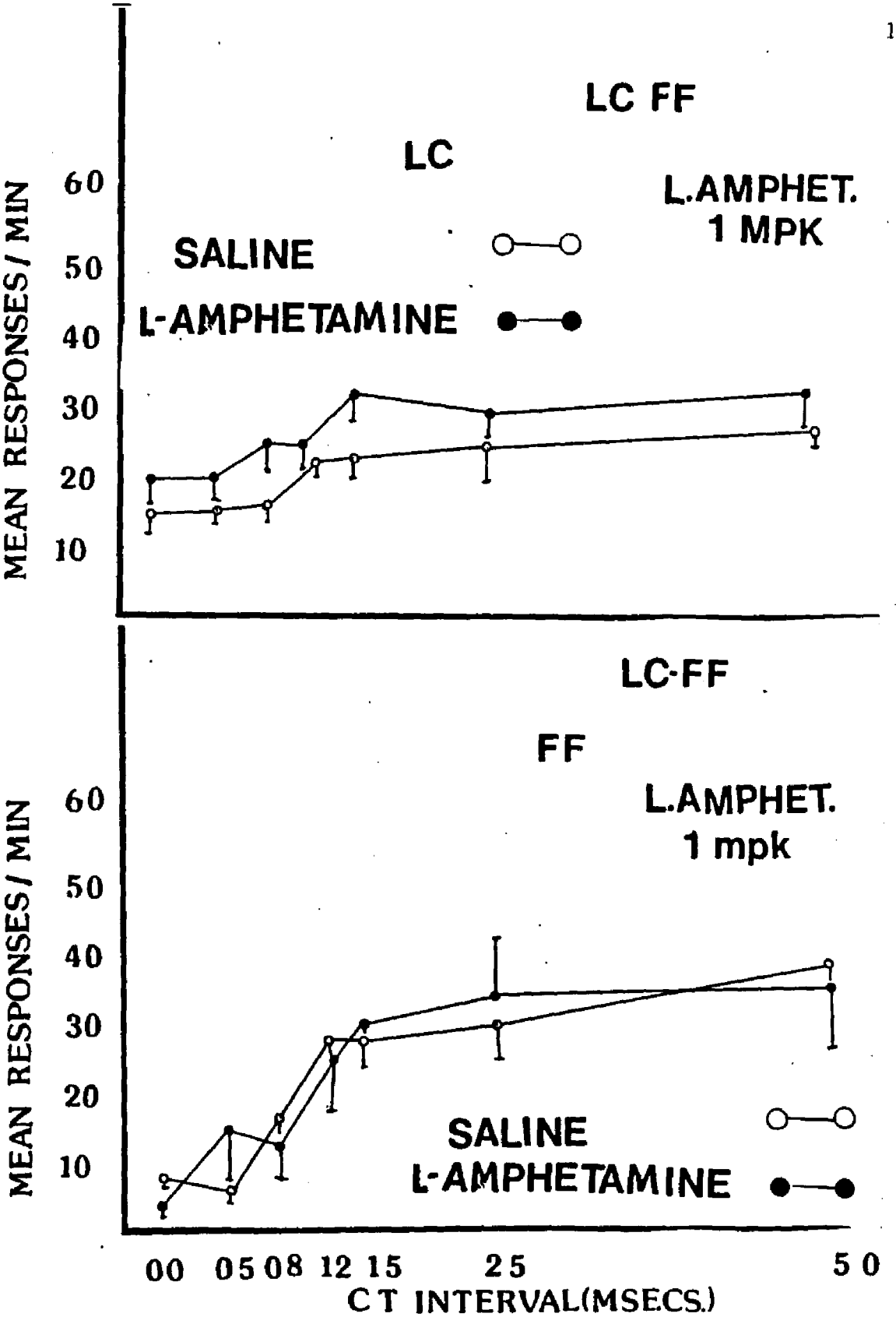
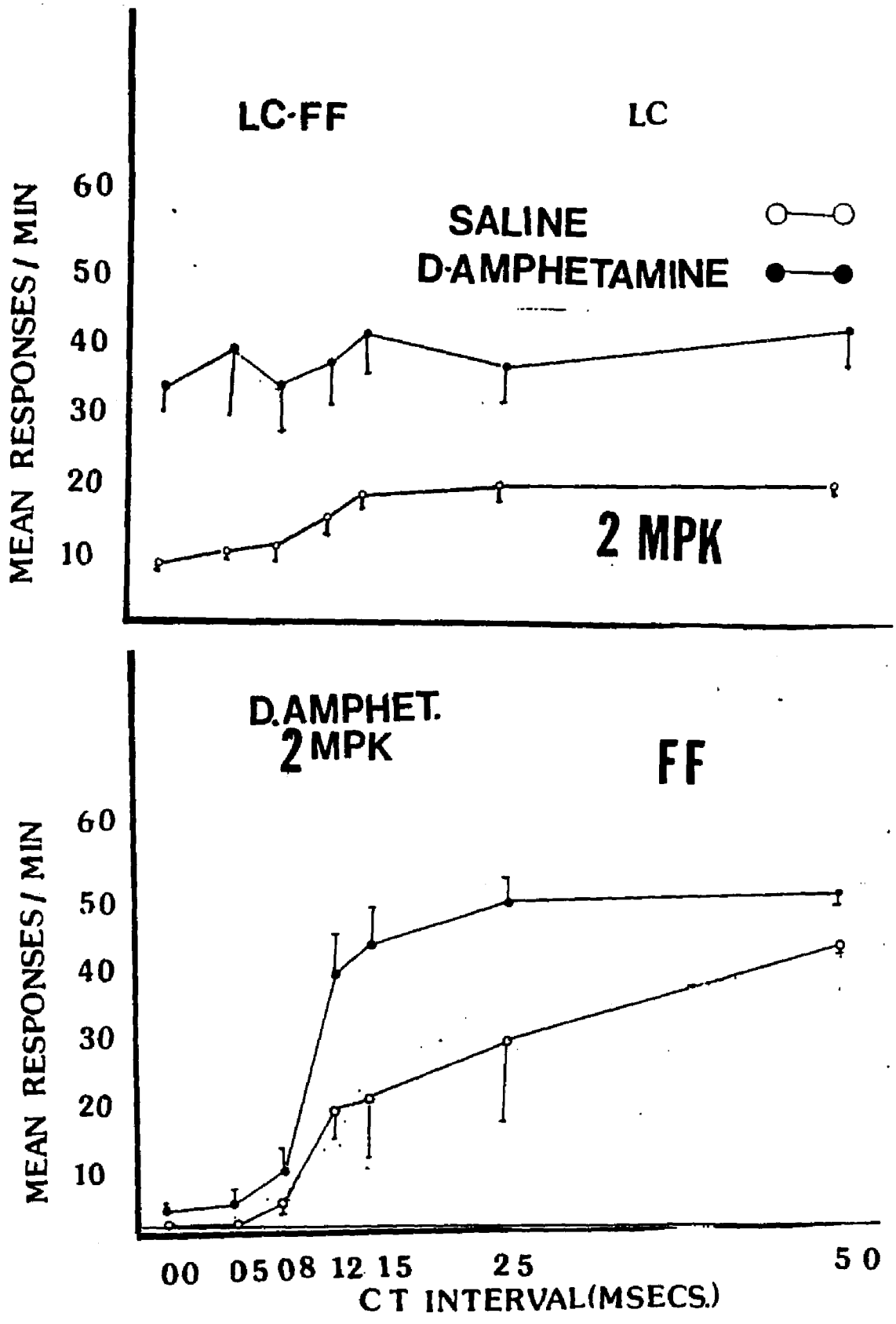


Figure 15. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 46N from the LC-FF group of placements in the d- and l- amphetamine Within-Site condition at the 1.0 mpk dose.


LC
LC-FF
N=14
D.AMPHET.
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
SALINE
D-AMPHETAMINE
FF
SALINE
D-AMPHETAMINE
D.Amphet.
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
0.0
0.5
0.8
1.2
1.5
2.5
5.0
C T INTERVAL(MSECS.)


LC FF
LC
L.AMPHET.
1 MPK
SALINE
L-AMPHETAMINE
MEAN RESPONSES / MIN
60
50
40
30
20
10
LC-FF
FF
L.AMPHET.
1 mpk
SALINE
L-AMPHETAMINE
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)


LC-FF
LC
SALINE
D-AMPHETAMINE
2 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
D.AMPHET.
2 MPK
FF
MEAN RESPONSES / MIN
60
50
40
30
20
10
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)

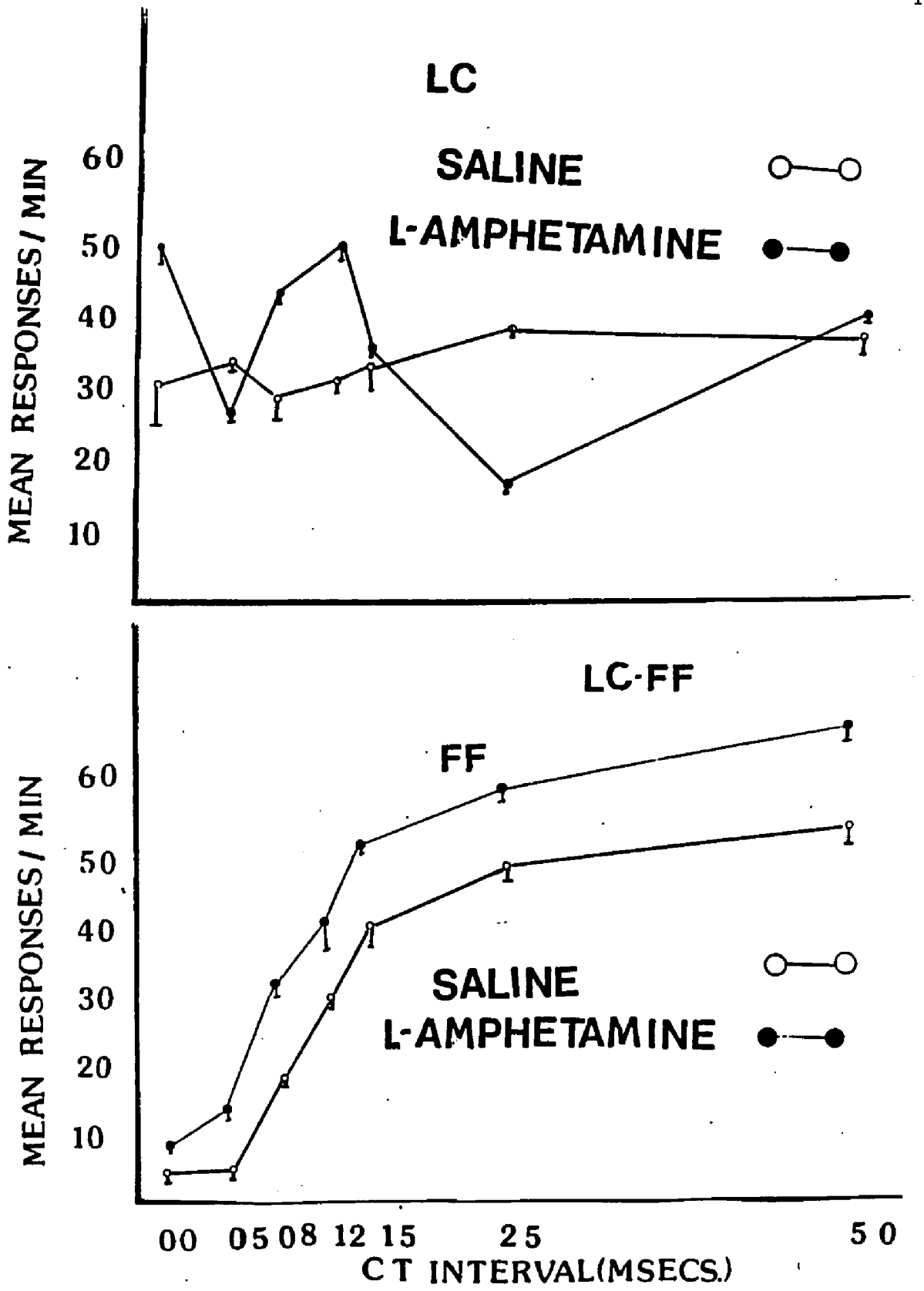


Figure 34. Mean responses per minute plotted as a function of the C-T interval in msec. for the LC-MFB group of placements in the clonidine Between-Site condition at the .1 mpk dose of clonidine and 1.25 mpk dose of morphine.

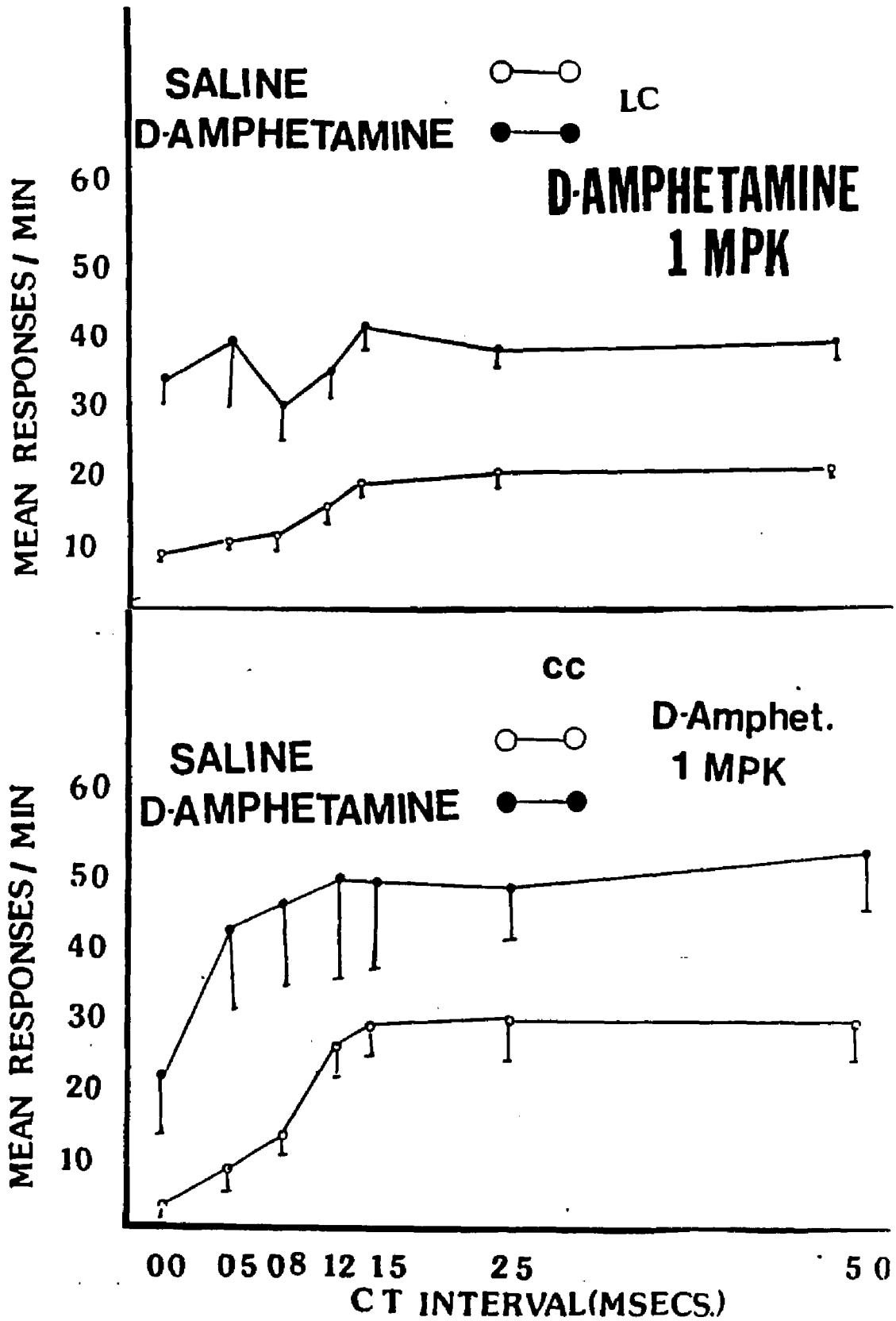
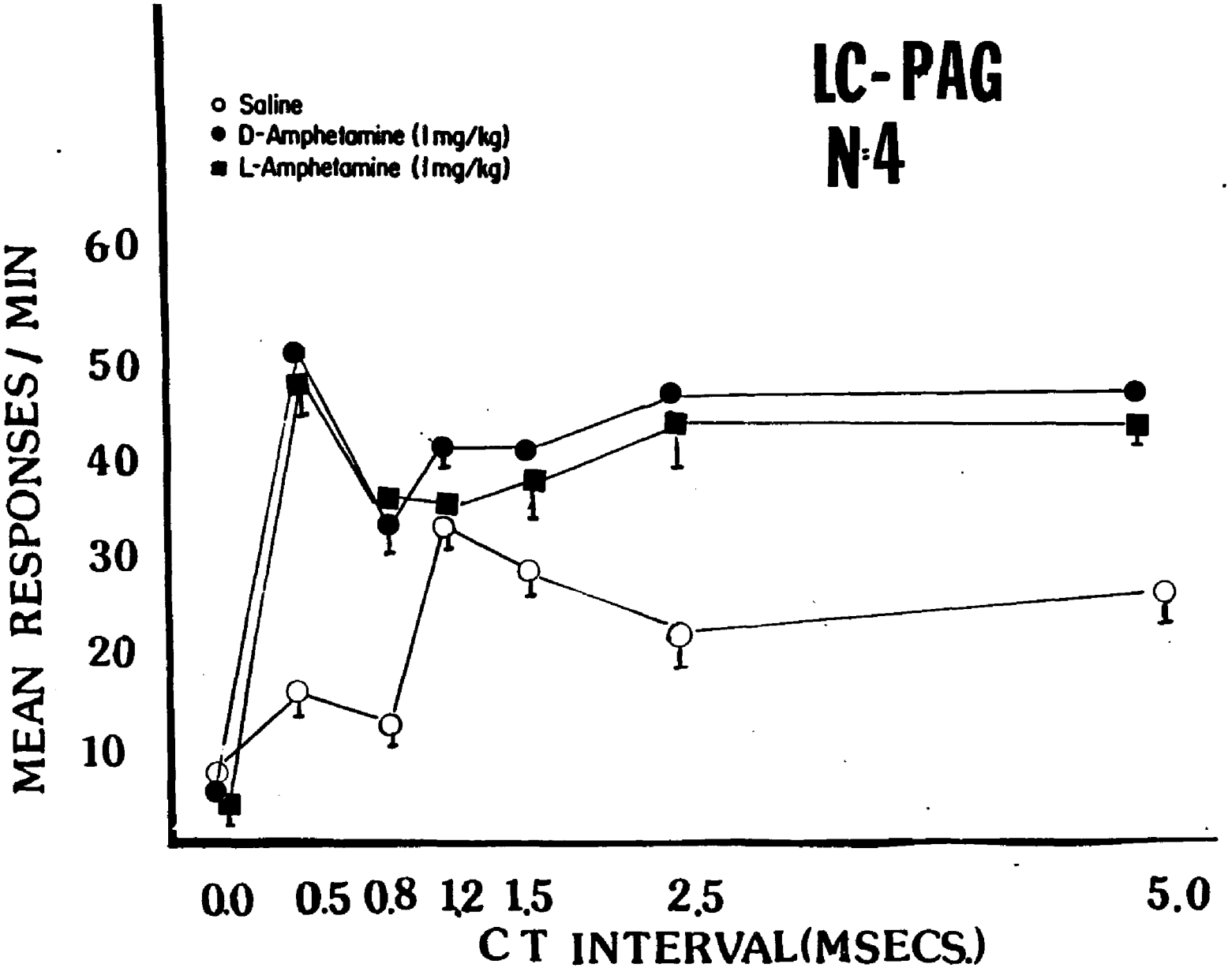
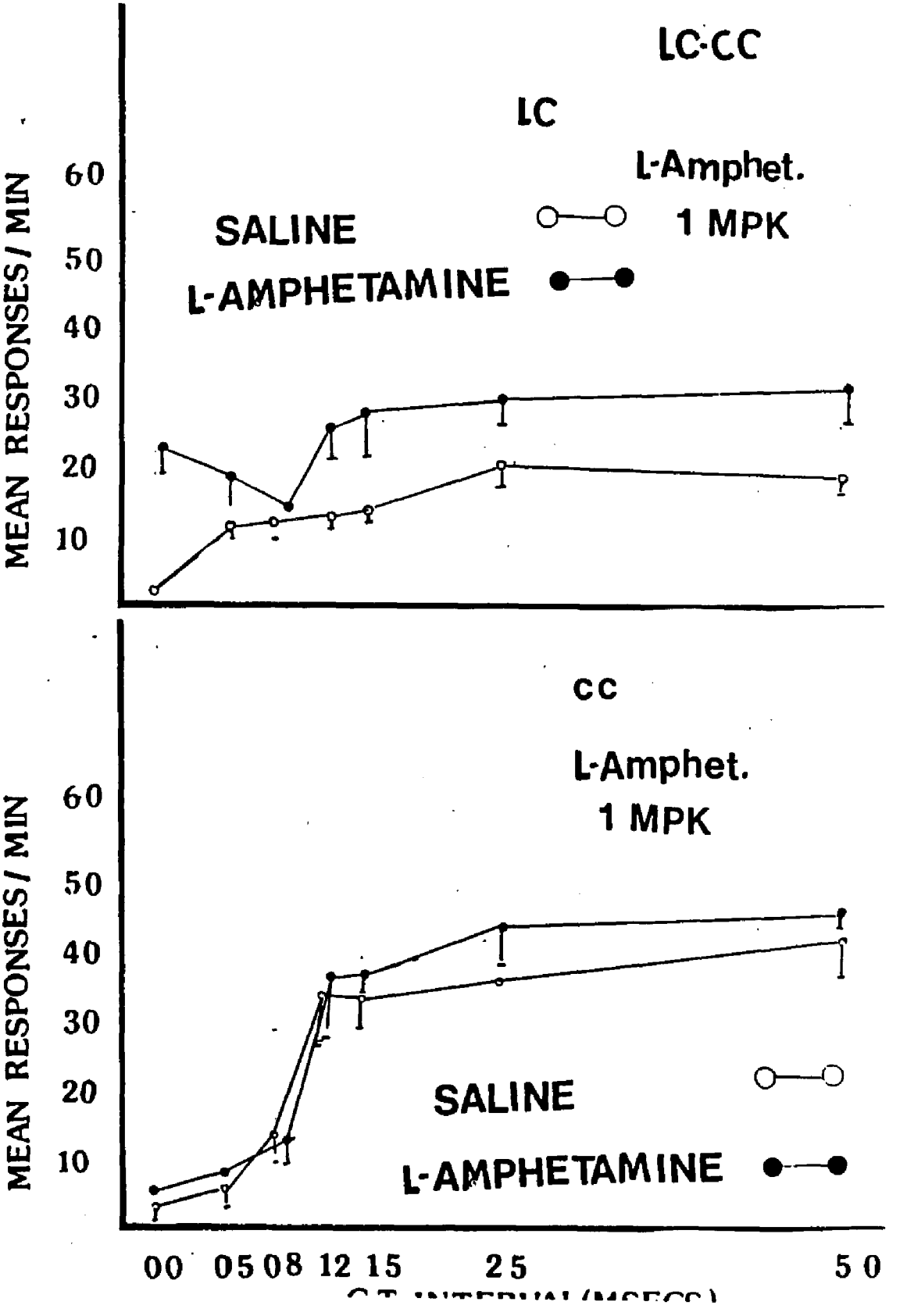
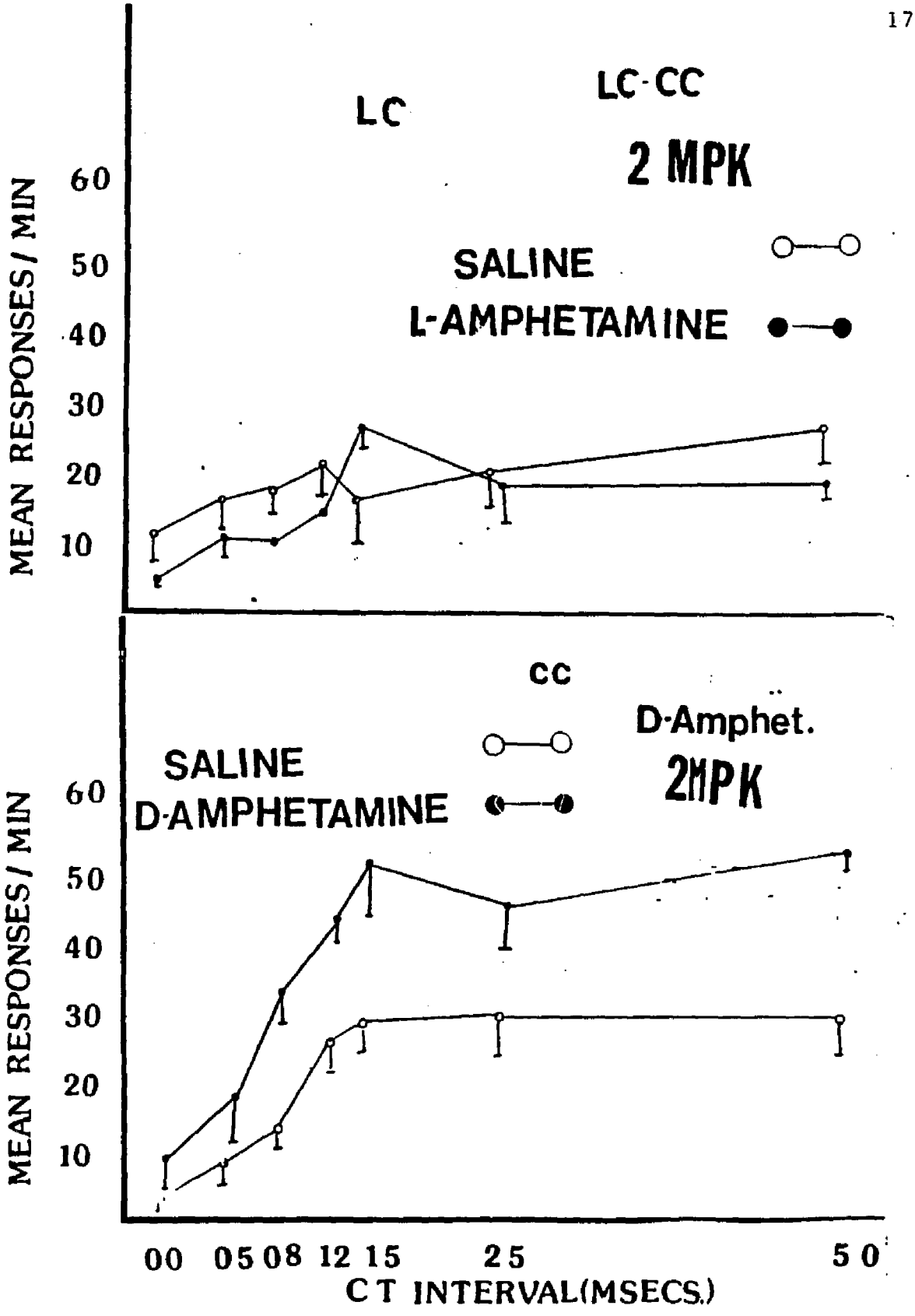


Figure 17. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-CC placements in the d- and l- amphetamine Within-Site condition at the 1.0 and 2.0 mpk dose.


LC-PAG
N=4
Saline
D-Amphetamine (1mg/kg)
L-Amphetamine (1mg/kg)
MEAN RESPONSES / MIN
60
50
40
30
20
10
0.0
0.5
0.8
1.2
1.5
2.5
5.0
CT INTERVAL(MSECS.)


LC-CC
LC
L-Amphet.
1 MPK
SALINE
L-AMPHETAMINE
MEAN RESPONSES/ MIN
60
50
40
30
20
10
CC
L-Amphet.
1 MPK
MEAN RESPONSES/ MIN
60
50
40
30
20
10
SALINE
L-AMPHETAMINE
00 05 08 12 15 25 50


LC
LC-CC
2 MPK
SALINE
L-AMPHETAMINE
MEAN RESPONSES / MIN
60
50
40
30
20
10
CC
D-Amphet.
2MPK
SALINE
D-AMPHETAMINE
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)

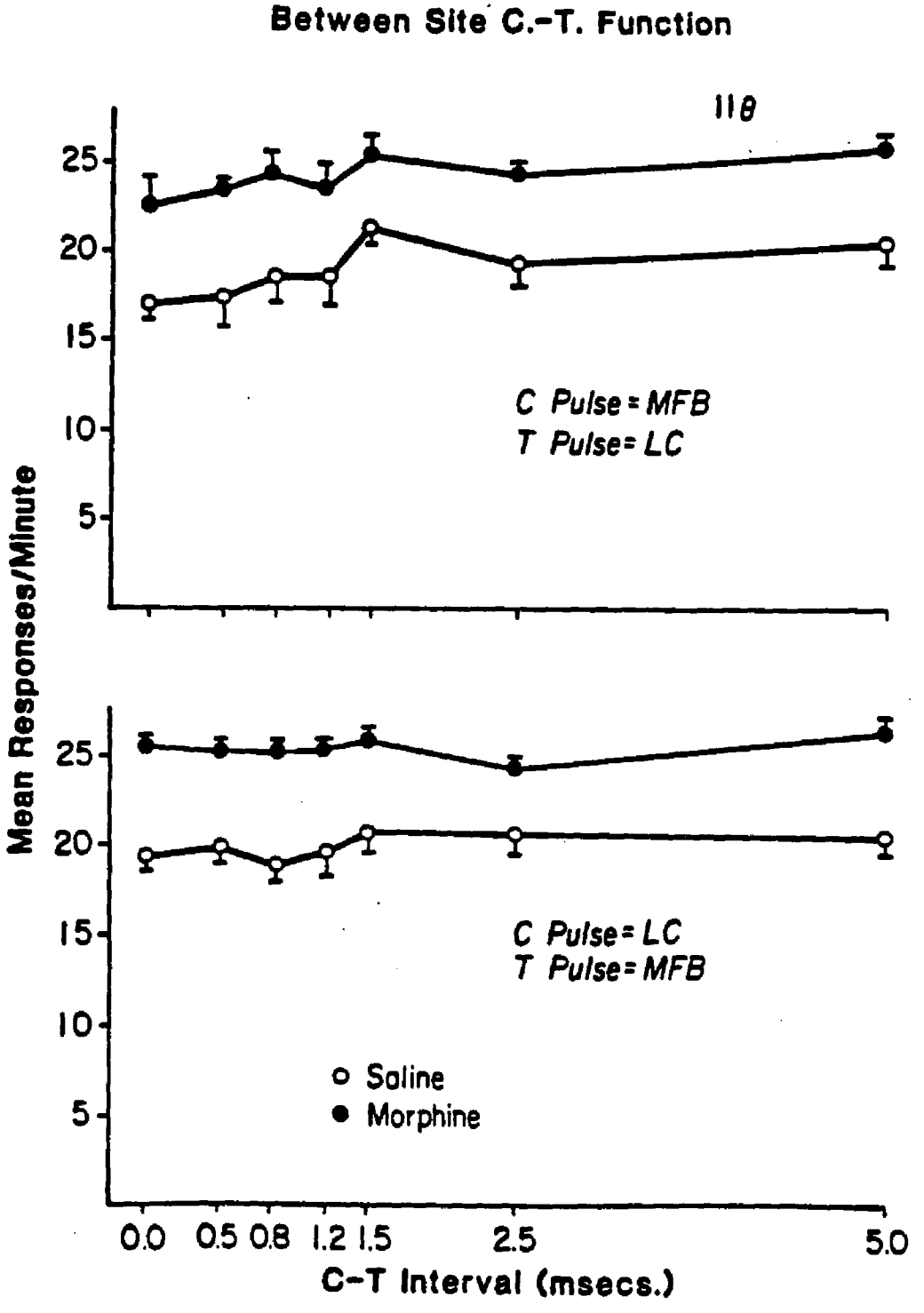


Figure 18. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 110 from the LC-MFB group of placements in the morphine and naloxone Between-Site condition.

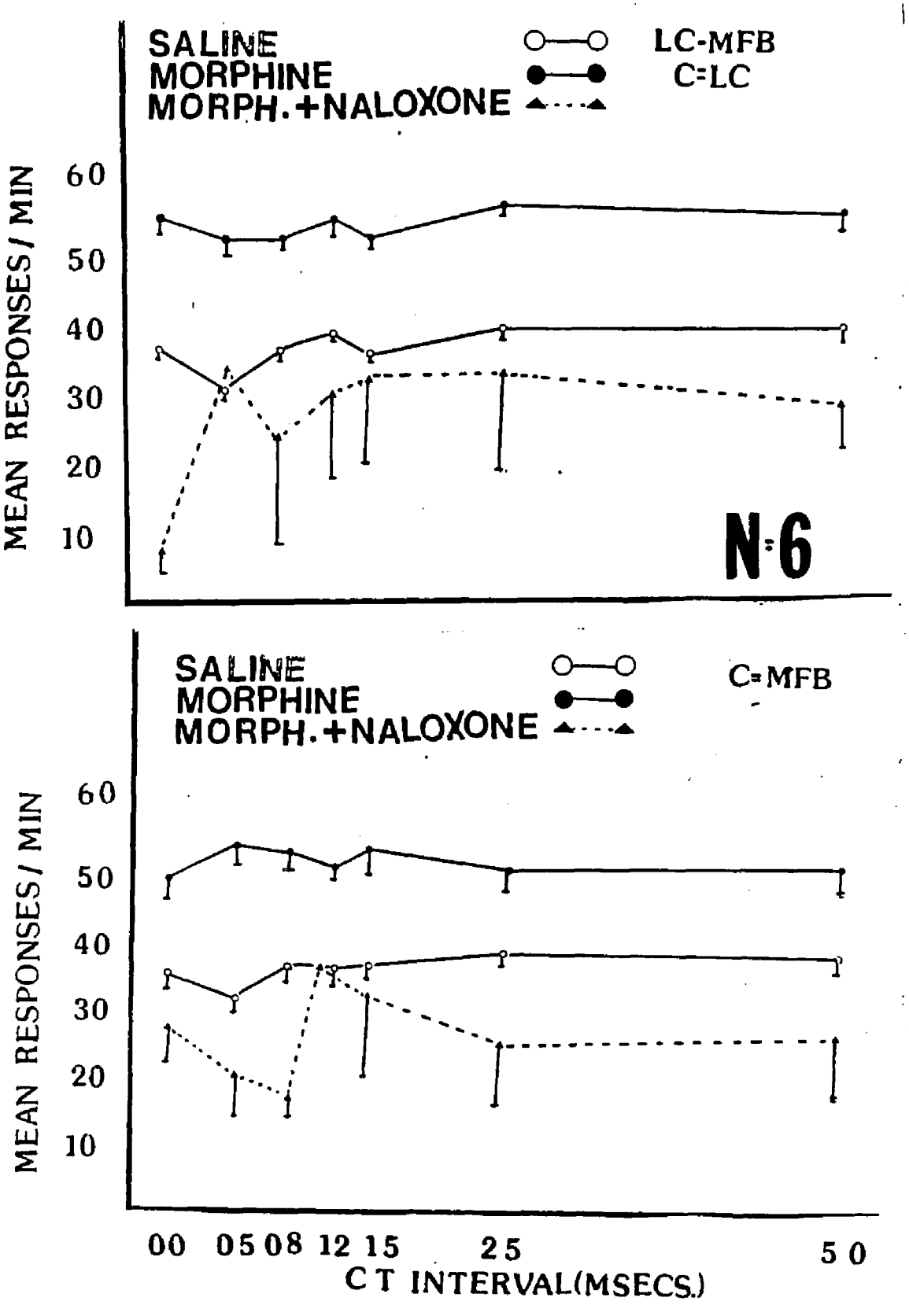
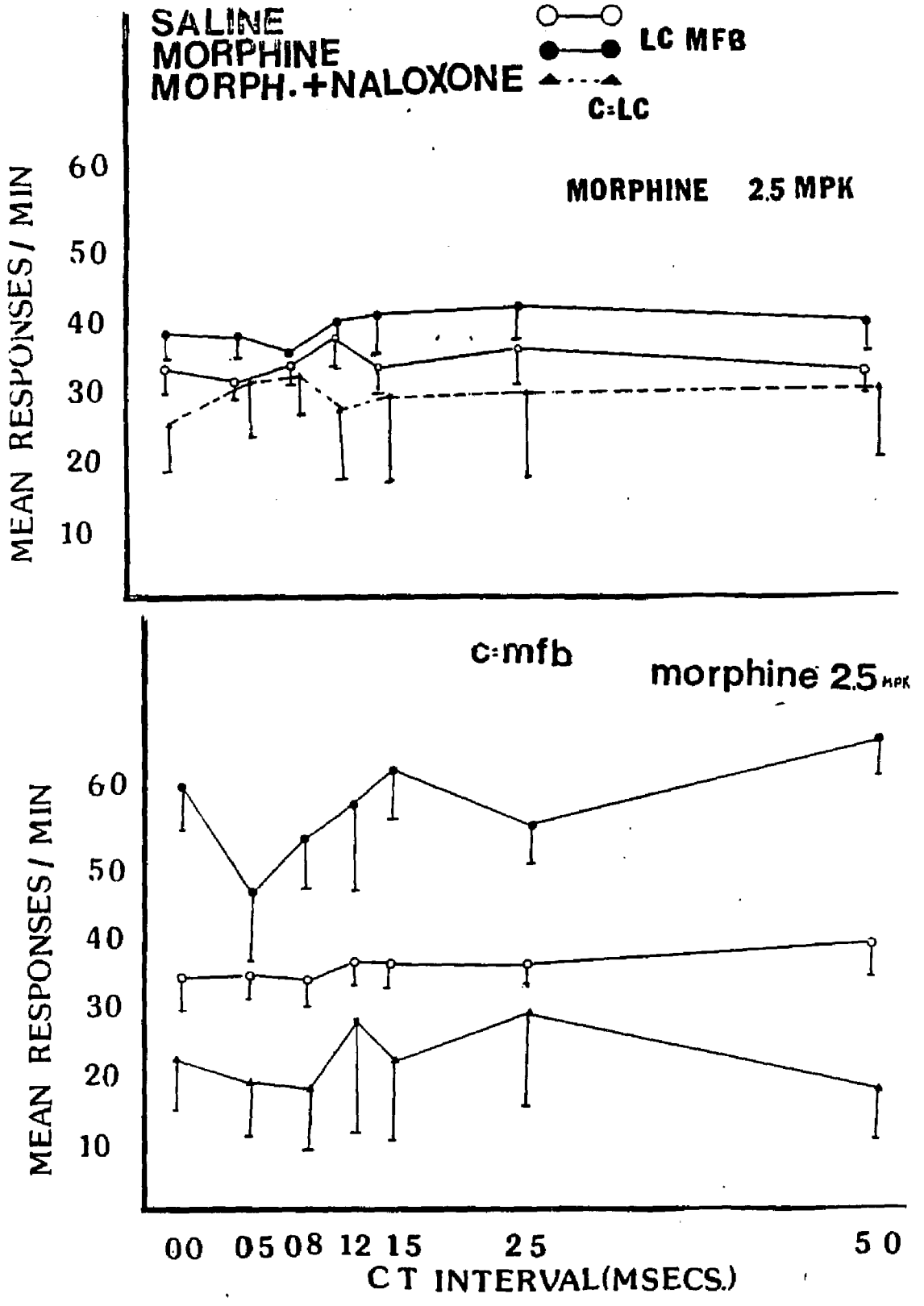


Figure 19. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-MFB group of placements in the morphine and naloxone Between-Site condition.


SALINE
MORPHINE
MORPH.+NALOXONE
LC MFB
C=LC
MORPHINE 2.5 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
c=mfb
morphine 2.5 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)

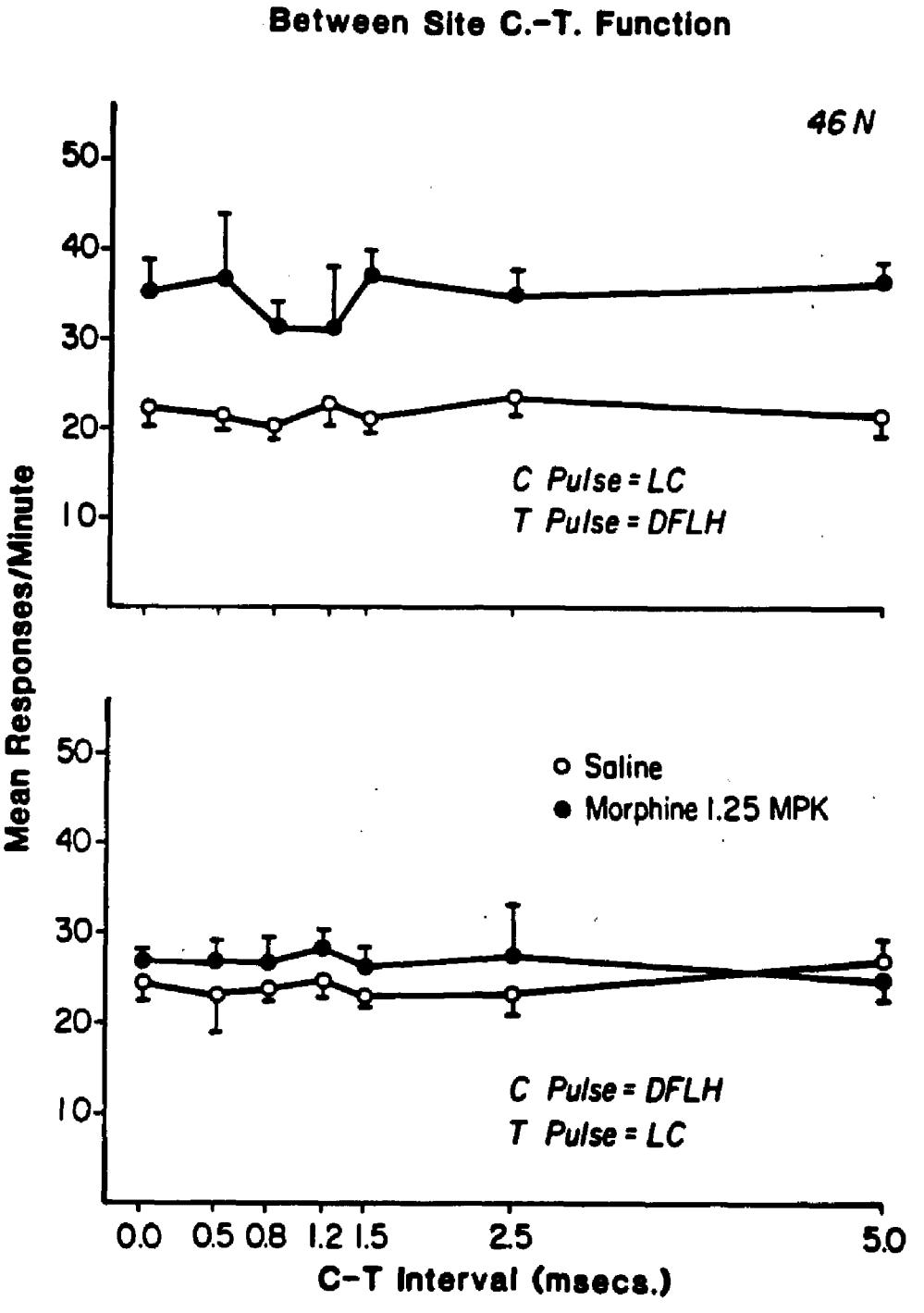


Figure 20. Mean responses per minute plotted as a function of the C-T interval in msec. for representative single subject 46N from the LC-FF group of placements in the morphine and naloxone Between-Site condition.

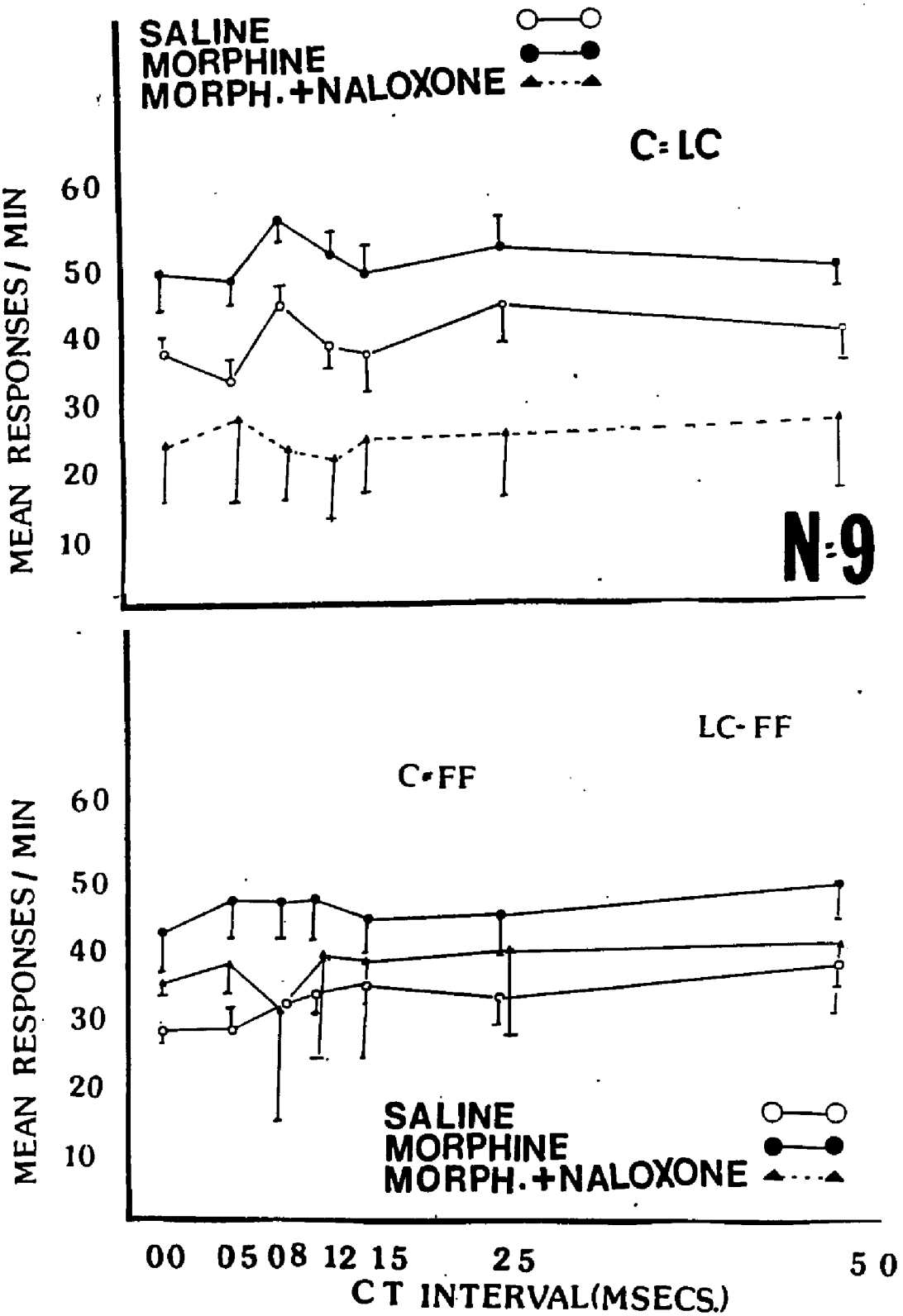
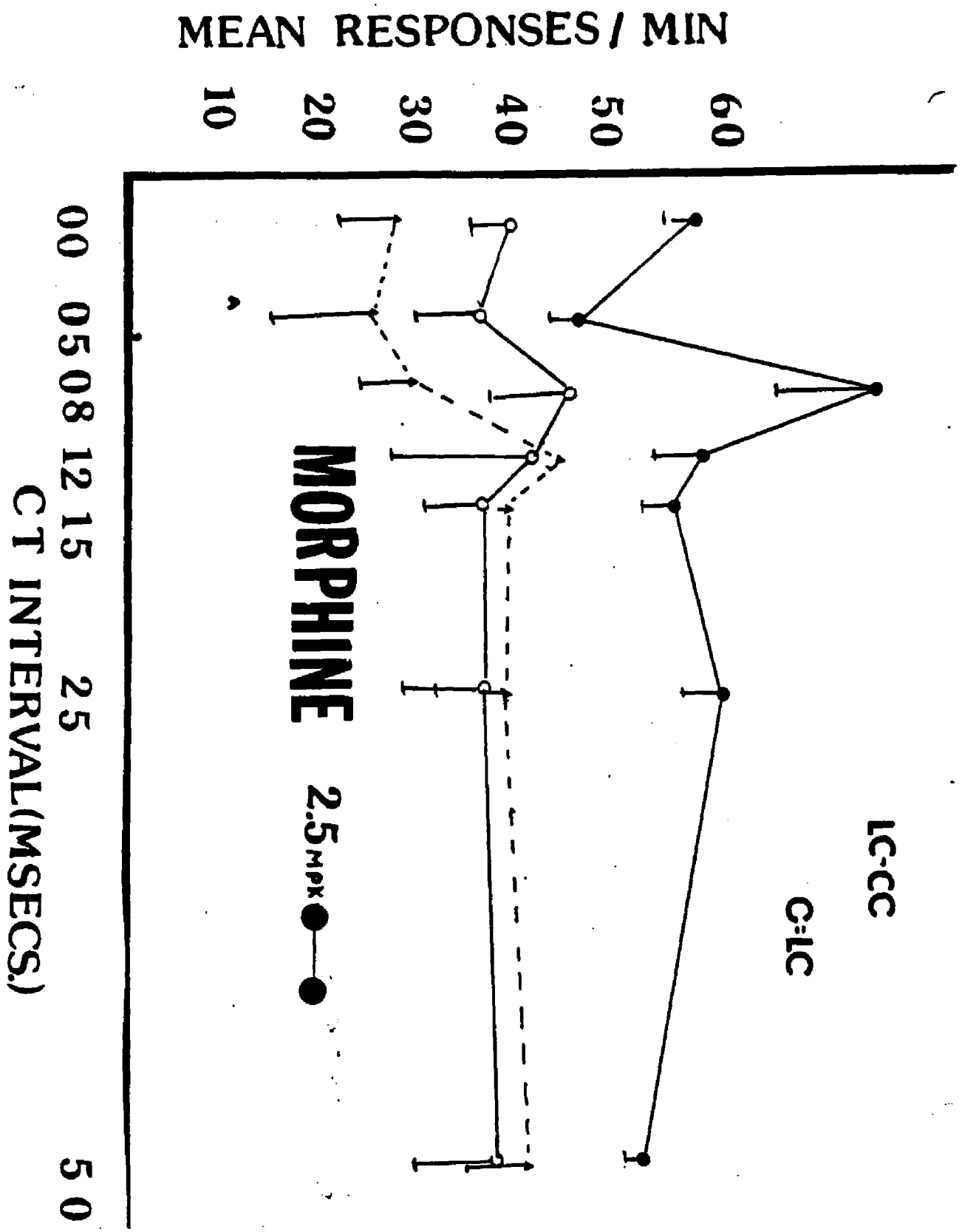


Figure 21. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-FF placements in the morphine and naloxone Between-Site condition.


MEAN RESPONSES / MIN
10
20
30
40
50
60
00
05
08
12
15
25
50
CT INTERVAL(MSECS.)
MORPHINE
2.5MPK
LC-CC
C-LC

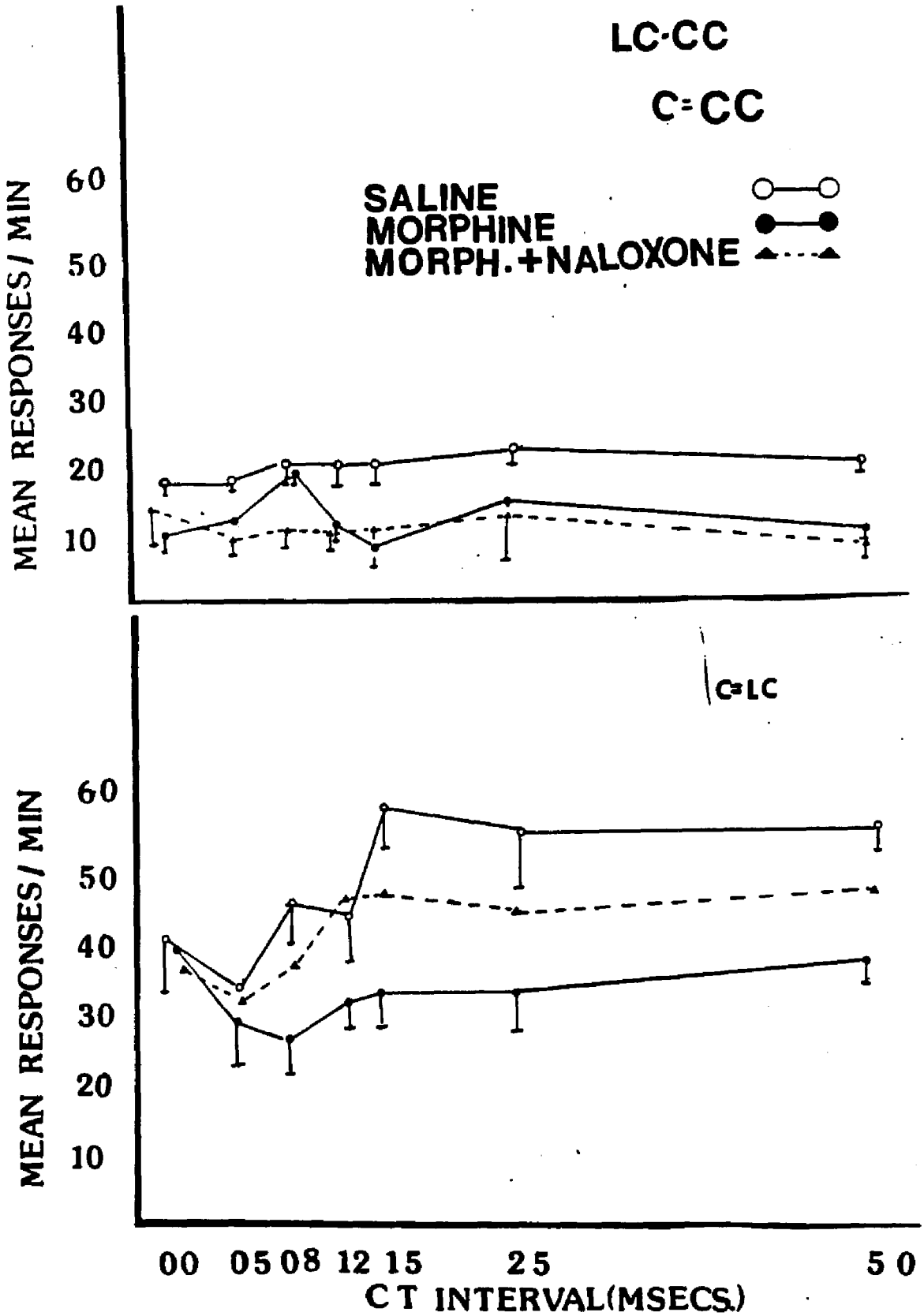
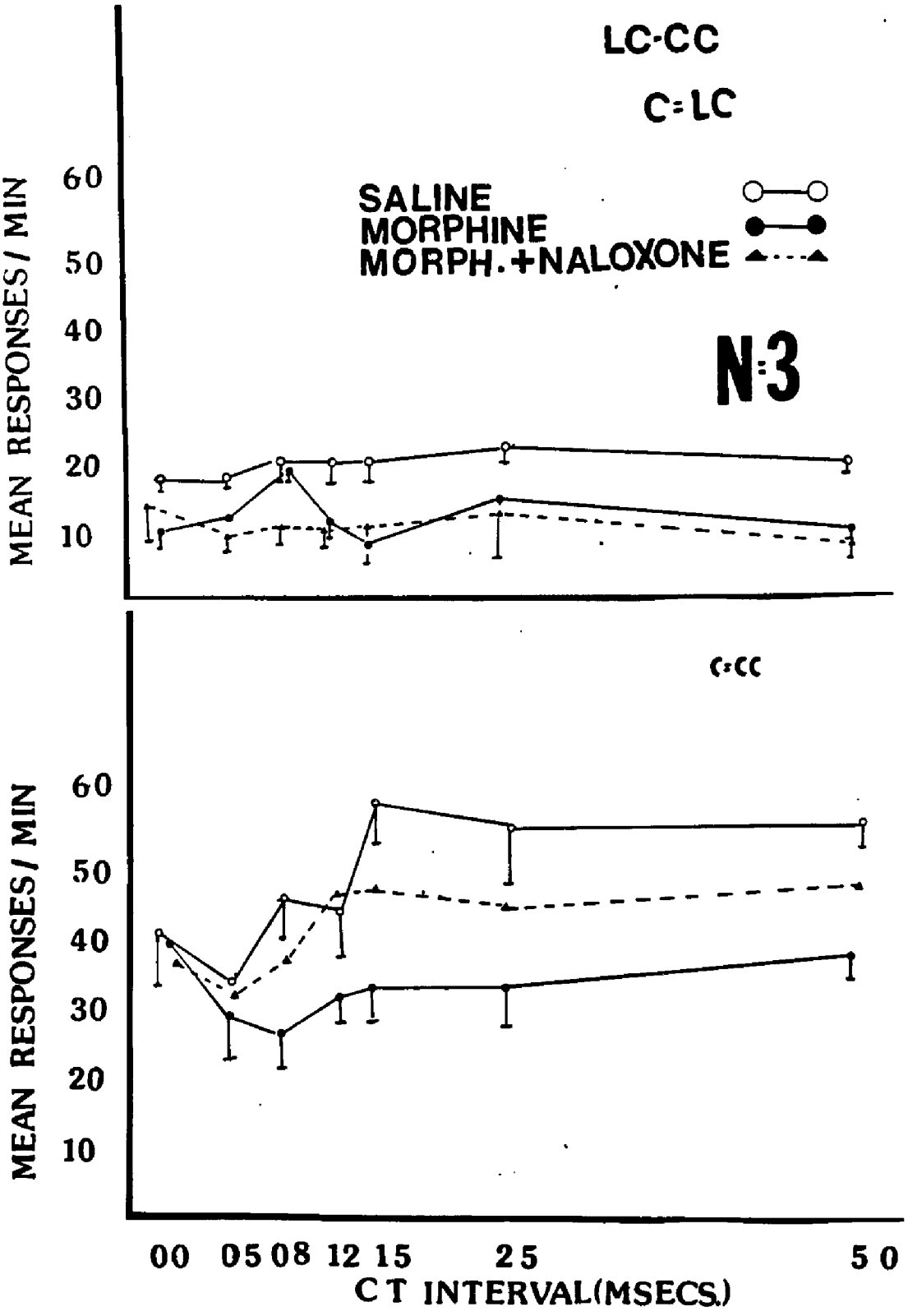
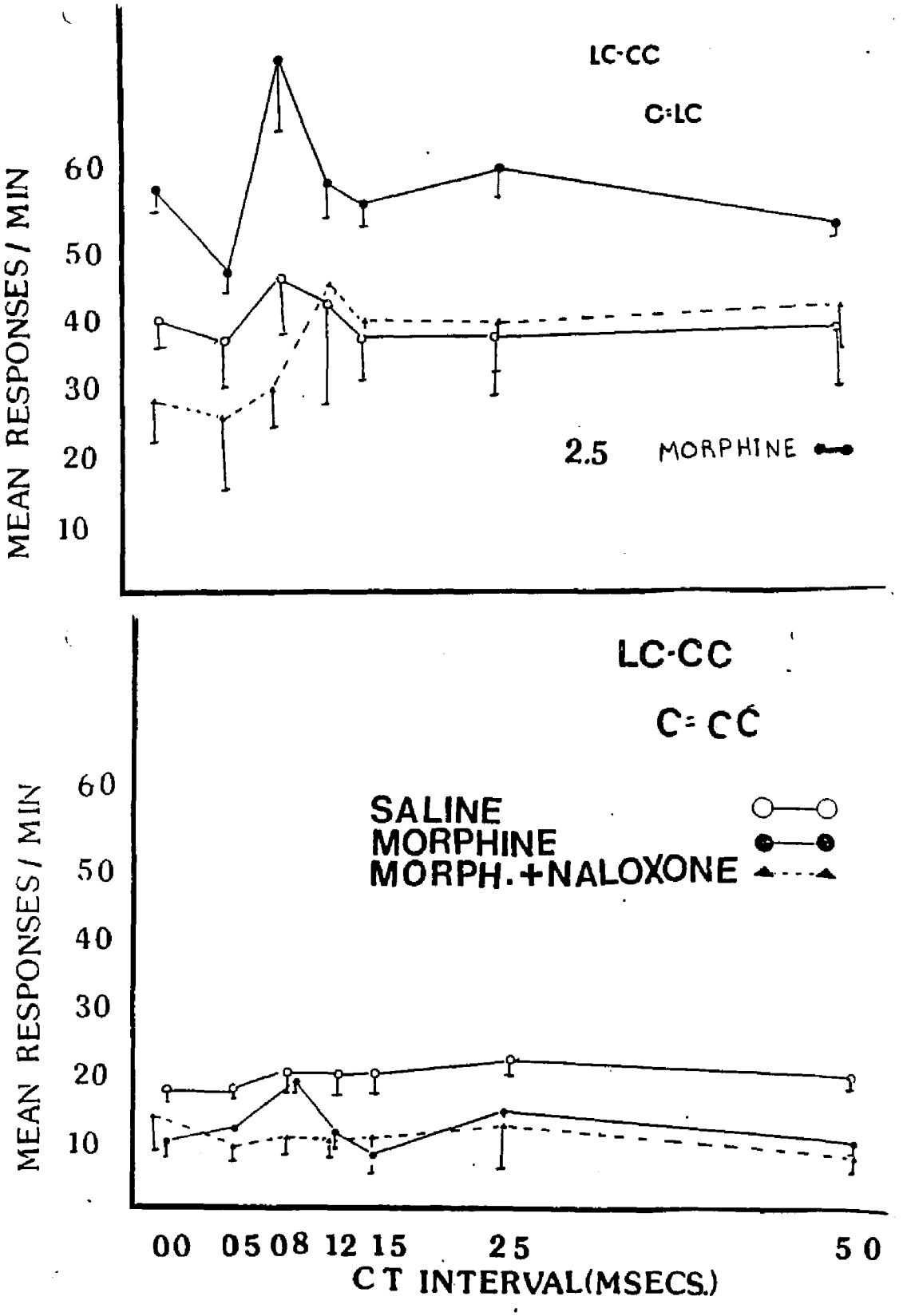


Figure 23. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-CC placements in the morphine and naloxone Between-Site condition.


LC-CC
C=LC
SALINE
MORPHINE
MORPH.+NALOXONE
N=3
MEAN RESPONSES/ MIN
60
50
40
30
20
10
C=CC
MEAN RESPONSES/ MIN
60
50
40
30
20
10
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)


LC-CC
C-LC
MEAN RESPONSES / MIN
60
50
40
30
20
10
2.5 MORPHINE
LC-CC
C-CC
SALINE
MORPHINE
MORPH.+NALOXONE
MEAN RESPONSES / MIN
60
50
40
30
20
10
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)

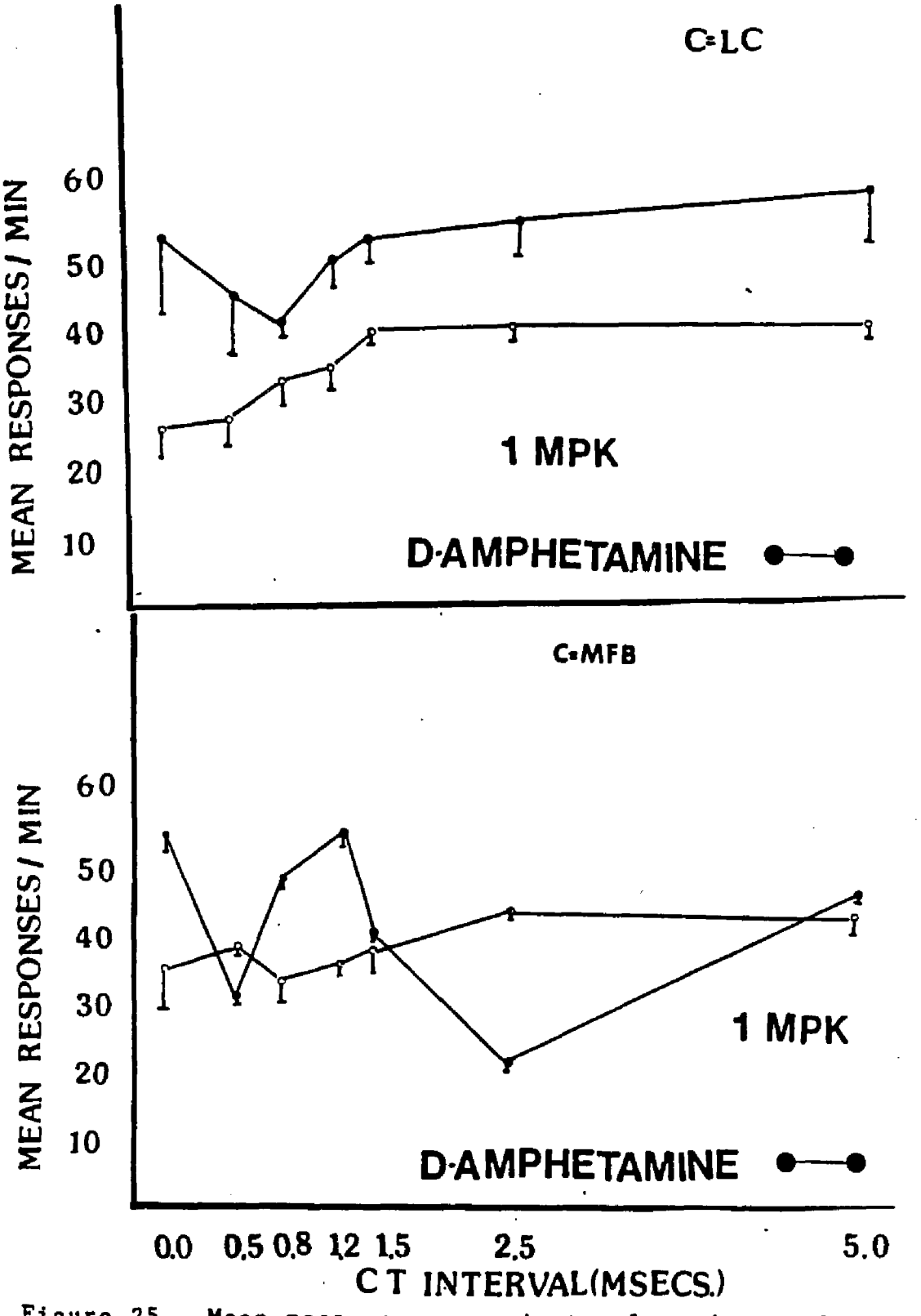
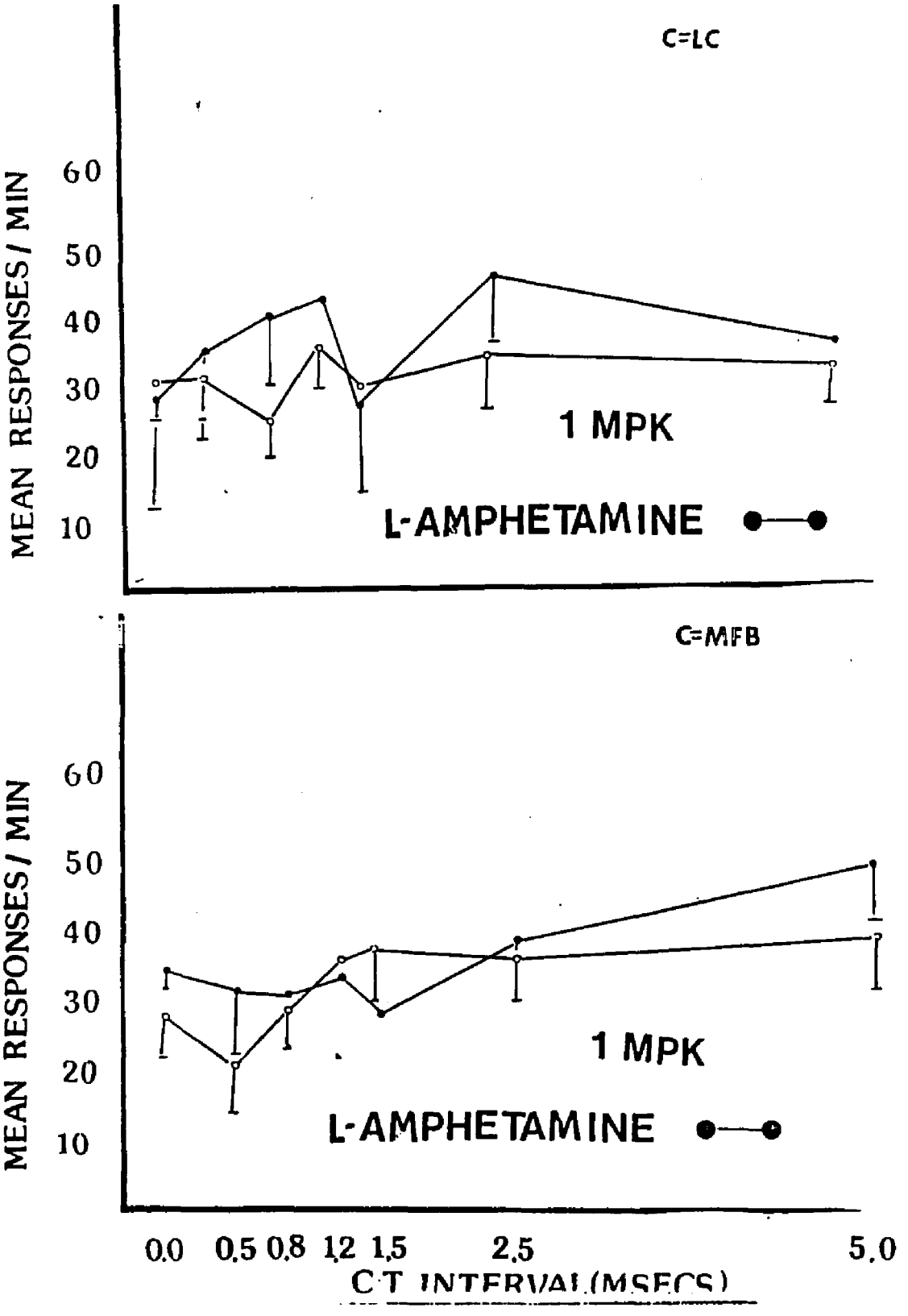
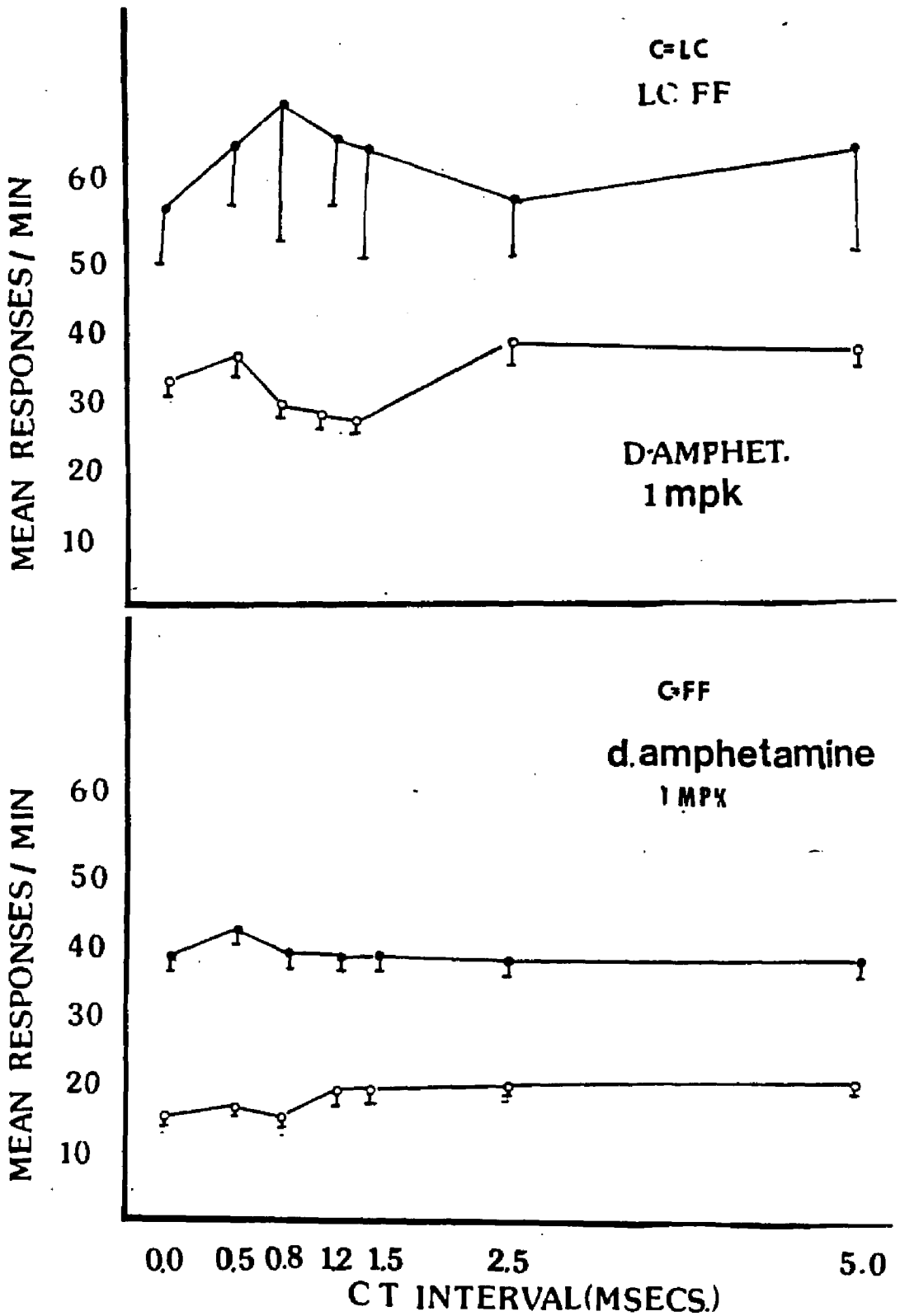
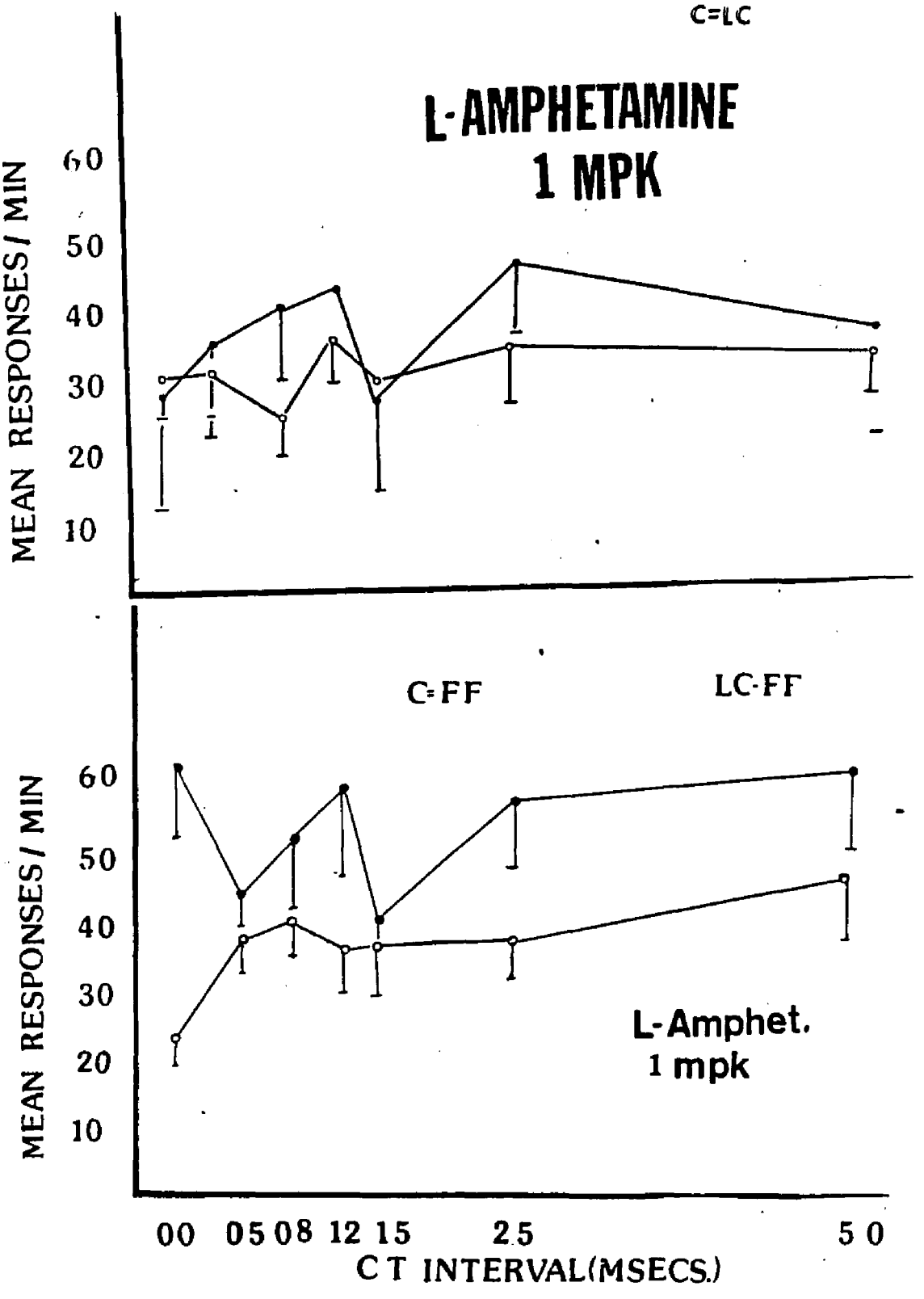


Figure 25. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of LC-MFB placements in the d- and l- amphetamine Between-Site condition at the 1.0 and 2.0 mpk dose.


C=LC
MEAN RESPONSES / MIN
60
50
40
30
20
10
1 MPK
L-AMPHETAMINE
C=MFB
MEAN RESPONSES / MIN
60
50
40
30
20
10
1 MPK
L-AMPHETAMINE
0.0 0.5 0.8 1.2 1.5 2.5 5.0
C-T INTERVAL (MSECS)


C=LC
LC FF
D-AMPHET.
1 mpk
MEAN RESPONSES / MIN
60
50
40
30
20
10
C=FF
d.amphetamine
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
0.0
0.5
0.8
1.2
1.5
2.5
5.0
C T INTERVAL(MSECS.)


C=LC
L-AMPHETAMINE
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
C=FF
LC-FF
L-Amphet.
1 mpk
00 05 08 12 15 25 50
CT INTERVAL(MSECS.)

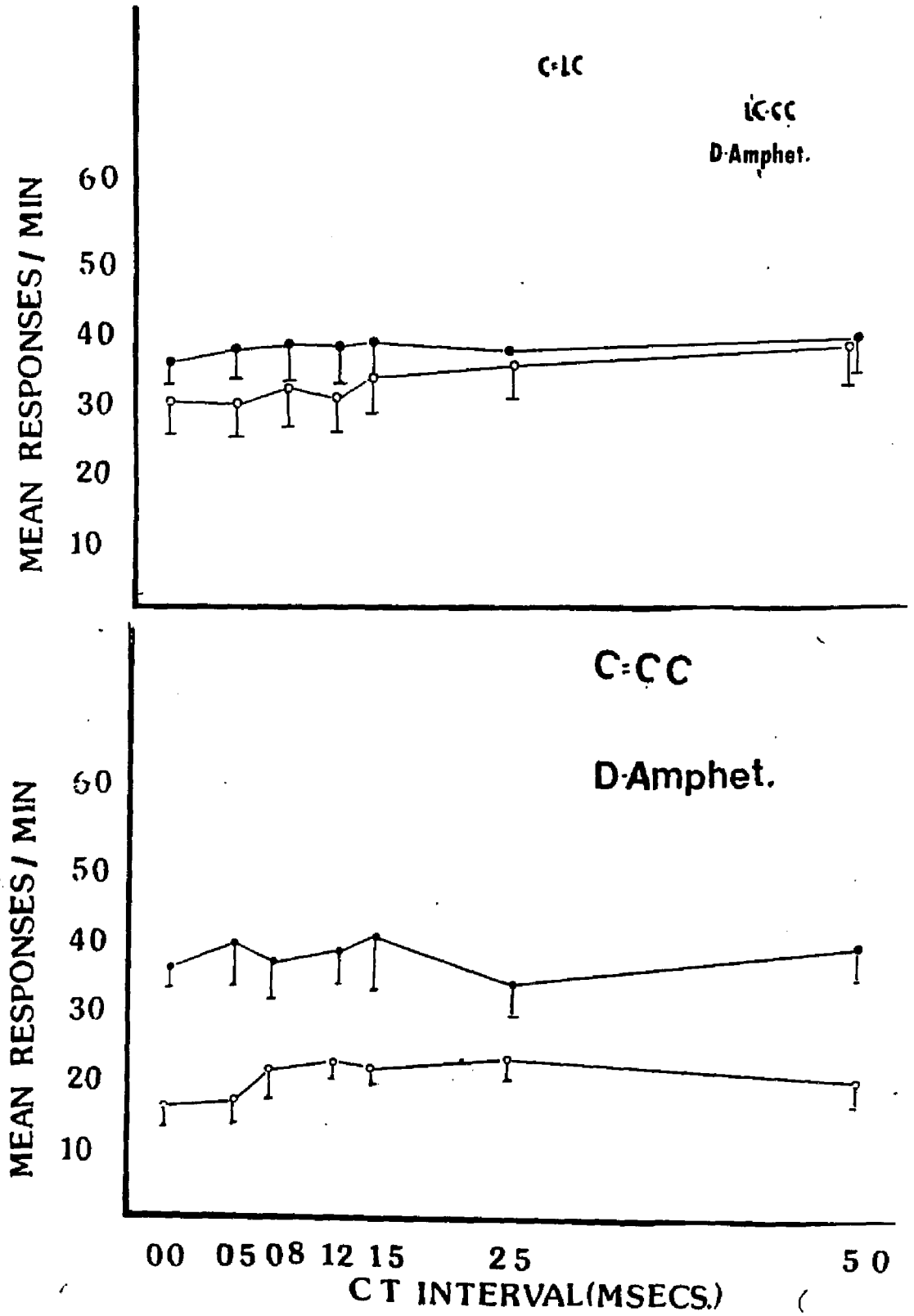
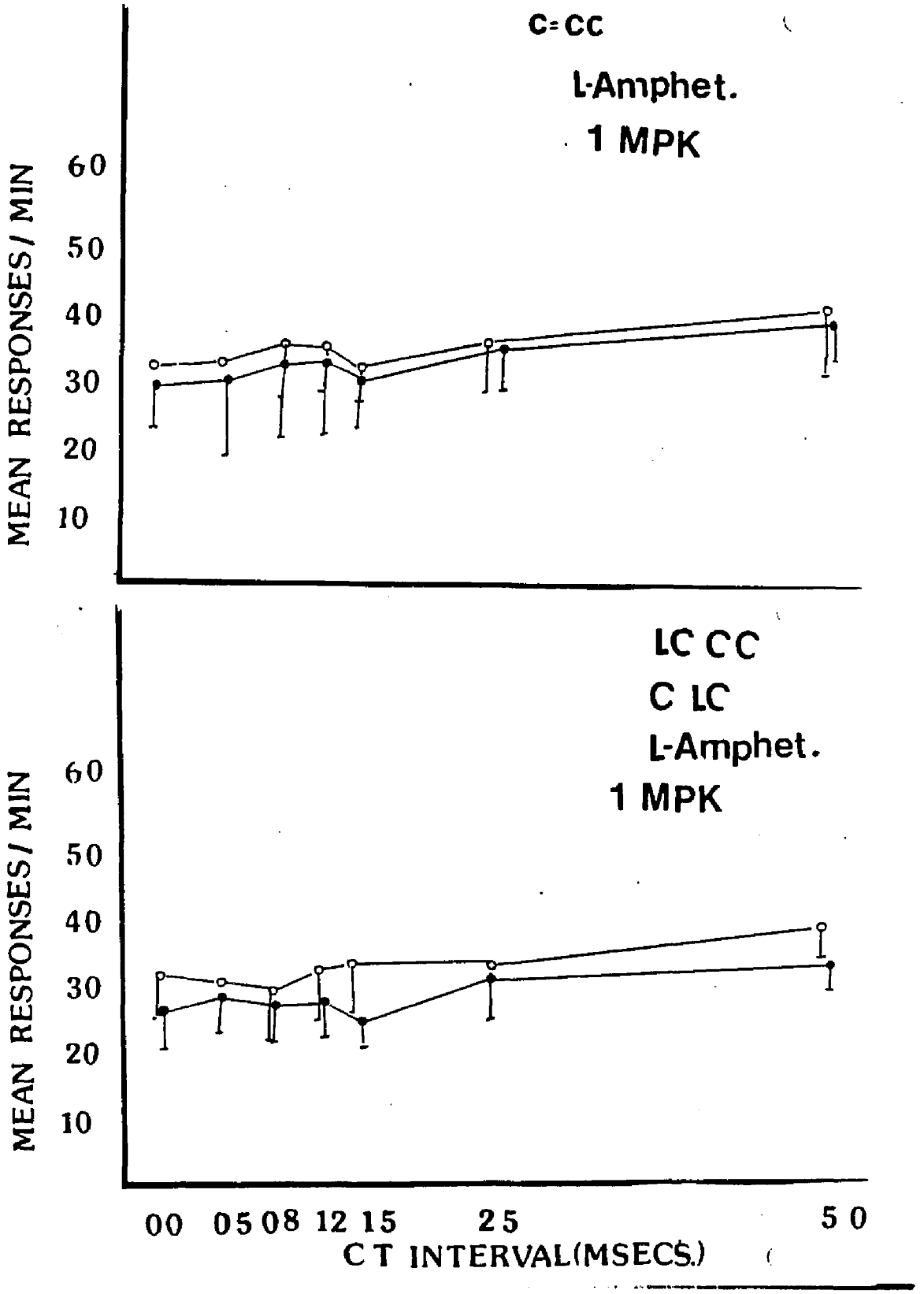


Figure 26. Mean responses per minute plotted as a function of the C-T interval in msec. for the LC-CC group of placements in the d- and l- amphetamine Between-Site condition at the 1.0 and 2.0 mpk dose.


C=CC
L-Amphet.
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
LC CC
C LC
L-Amphet.
1 MPK
MEAN RESPONSES / MIN
60
50
40
30
20
10
00 05 08 12 15 25 50
C T INTERVAL(MSECS.)

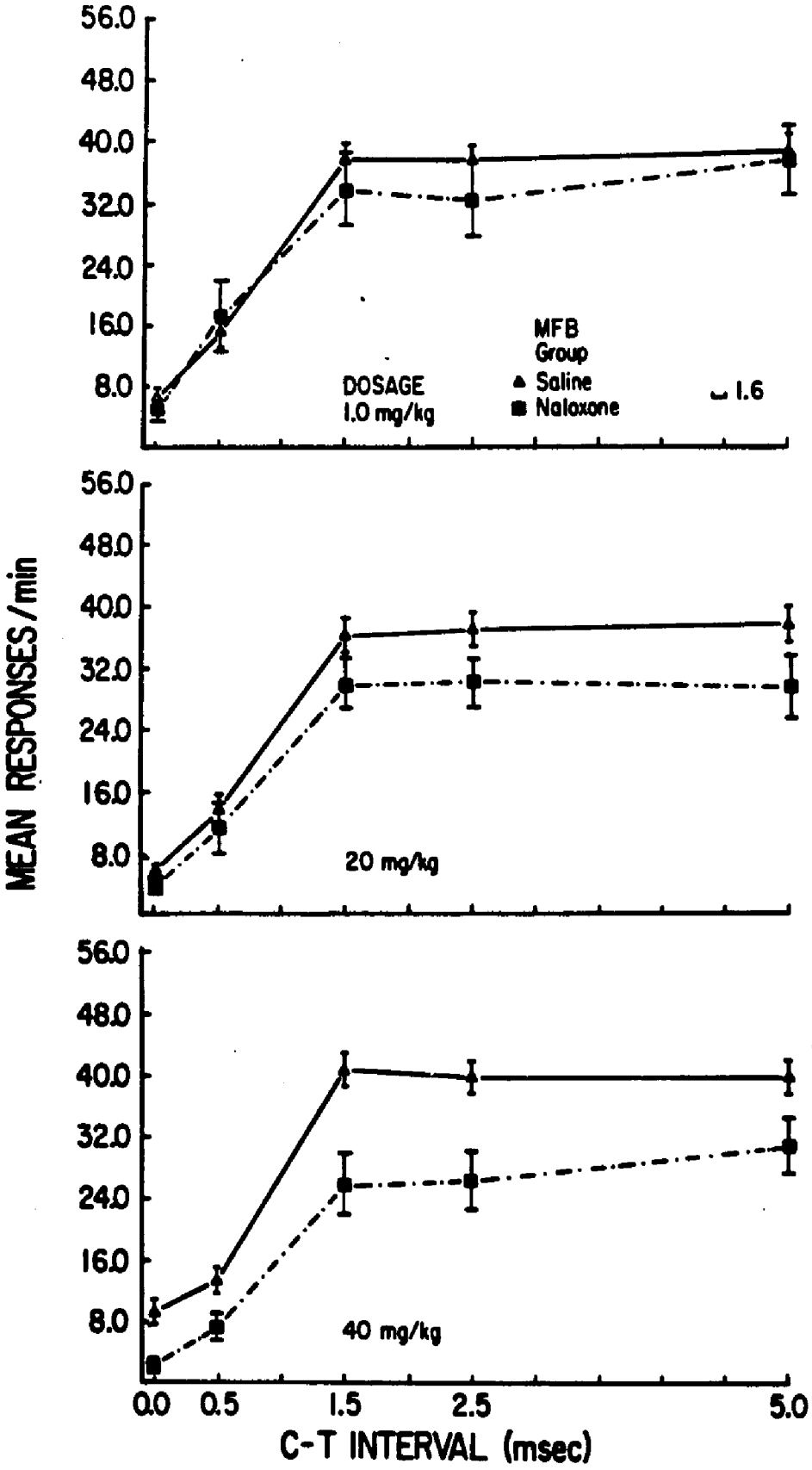


Figure 28. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of medial forebrain bundle placements in the Within-Site C-T condition under three doses of naloxone (1.0, 10.0 and 40.0 mpk).

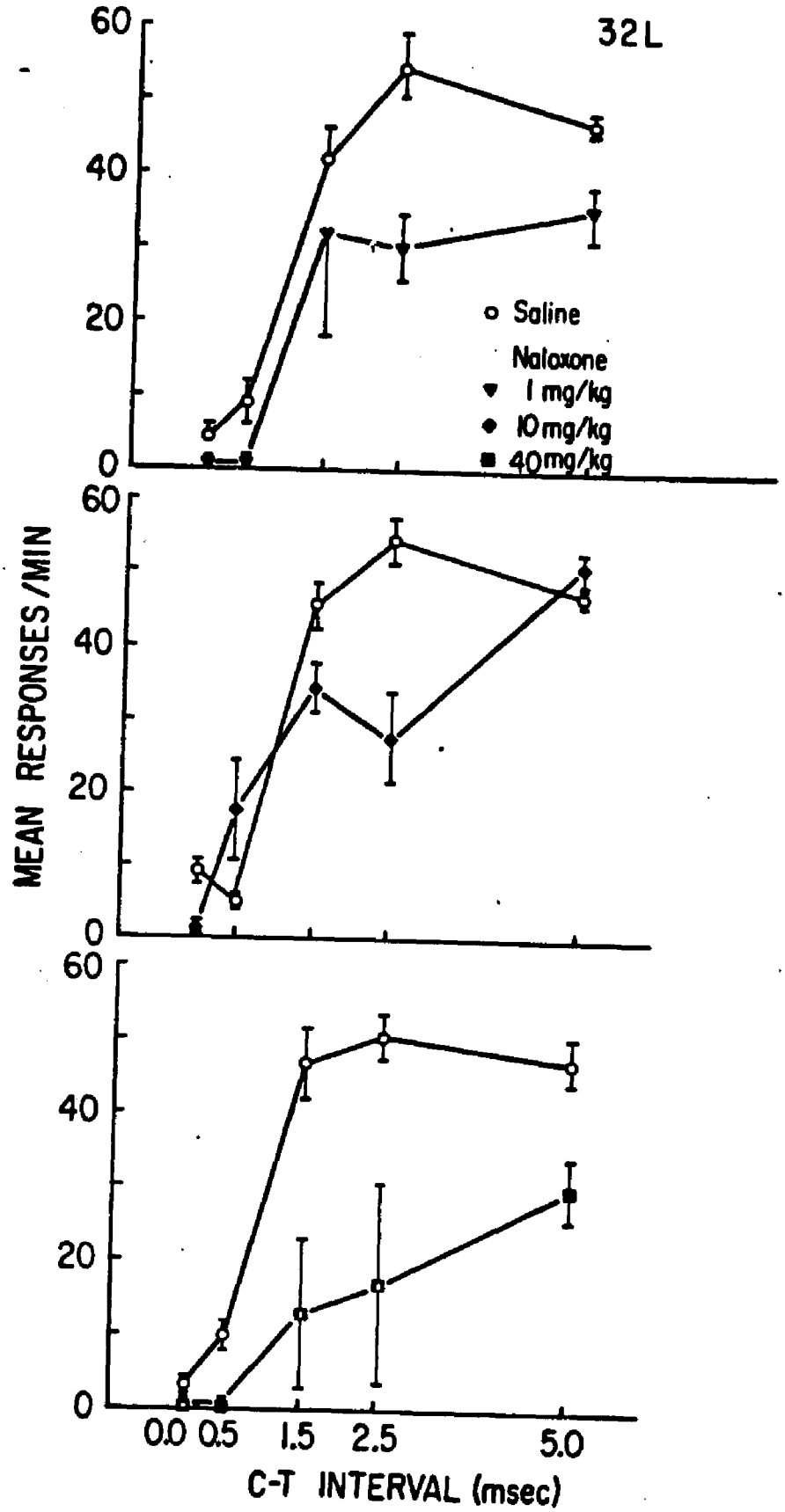


Figure 29. Mean responses per minute plotted as a function of the C-T interval in msec. for representative MFB animal 32L under the three doses of naloxone (1.0, 10.0 and 40.0 mpk).

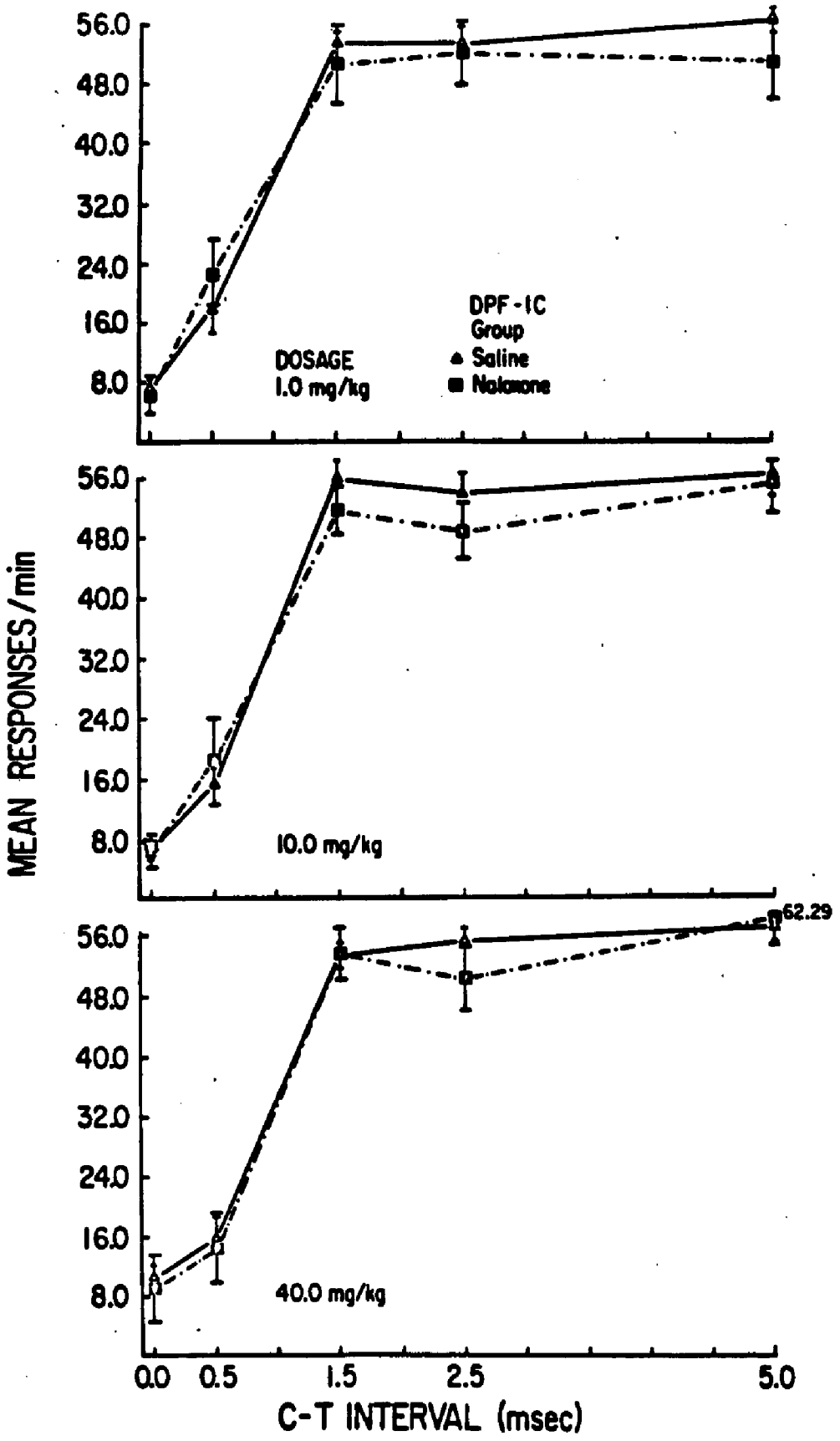


Figure 30. Mean responses per minute plotted as a function of the C-T interval in msec. for the group of ventral mammothalamic (dorsal perifornical) placements in the Within-Site condition under three doses of naloxone (1.0, 10.0 and 40.0 mpk).

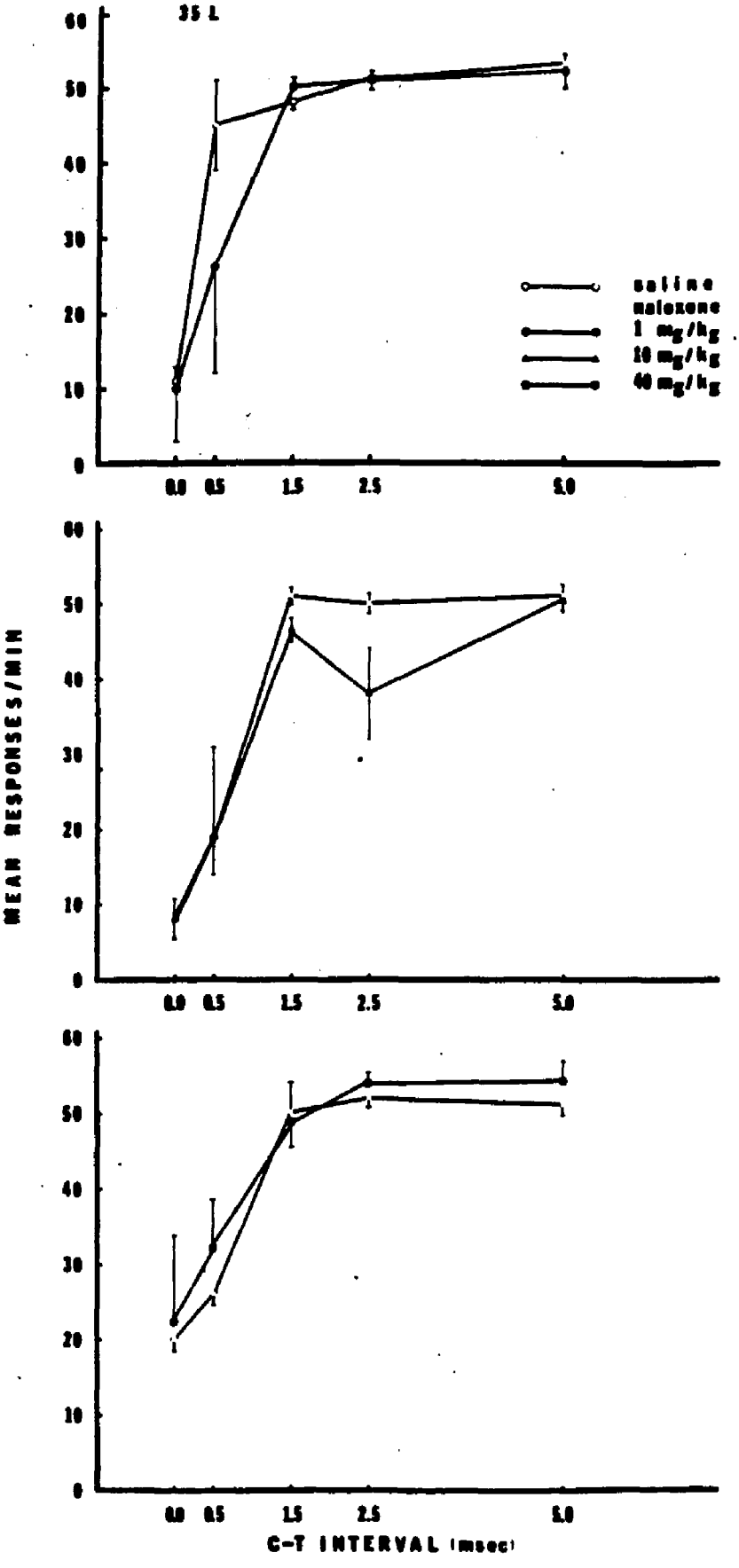


Figure 31. Mean responses per minute plotted as a function of the C-T interval in msec. for representative VMT animal 35L under the three doses of naloxone (1.0, 10.0 and 40.0 mpk).

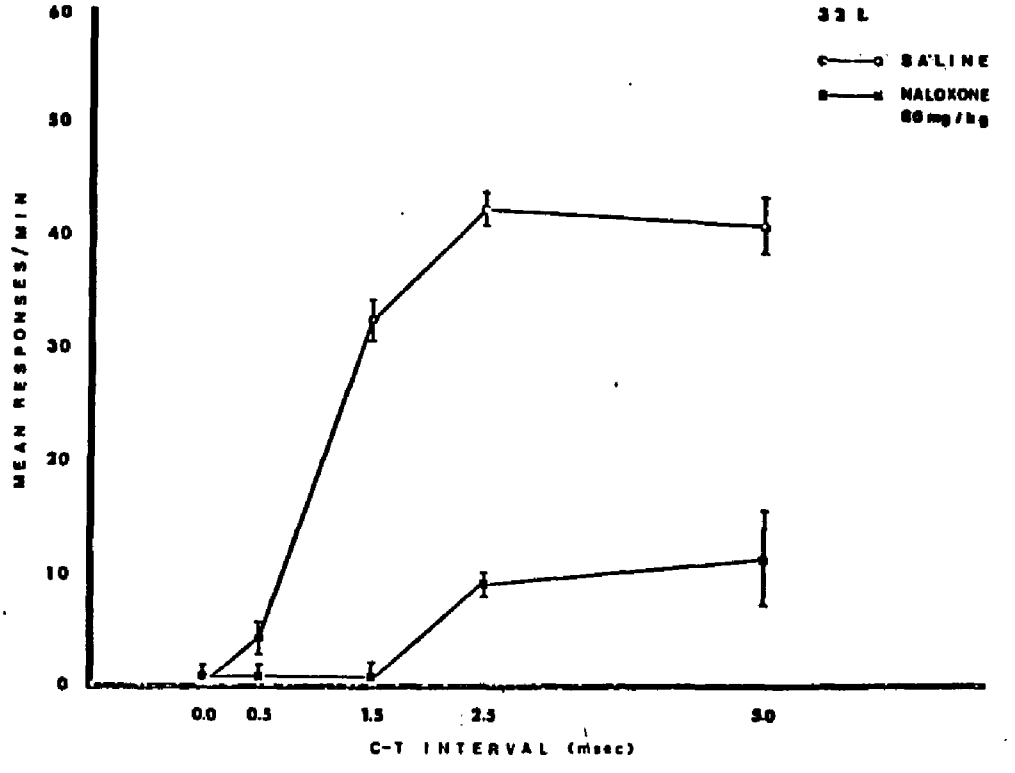
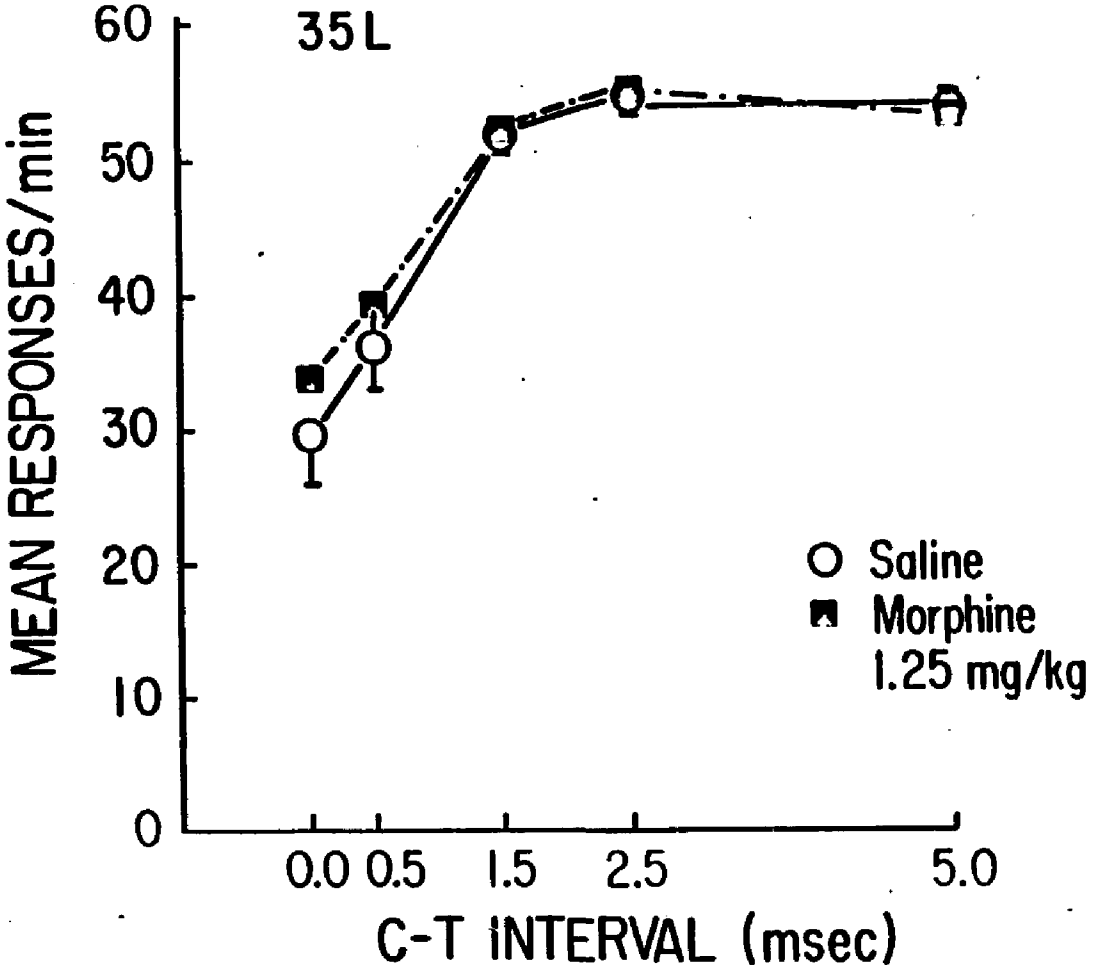


Figure 32. Mean responses per minute plotted as a function of the C-T interval in msec. for representative VMT animal 35L and MFB placement 32L under the 80 mpk dose of naloxone.


35L
60
50
40
30
20
10
0
MEAN RESPONSES/min
0.0 0.5 1.5 2.5 5.0
C-T INTERVAL (msec)
Saline
Morphine
1.25 mg/kg

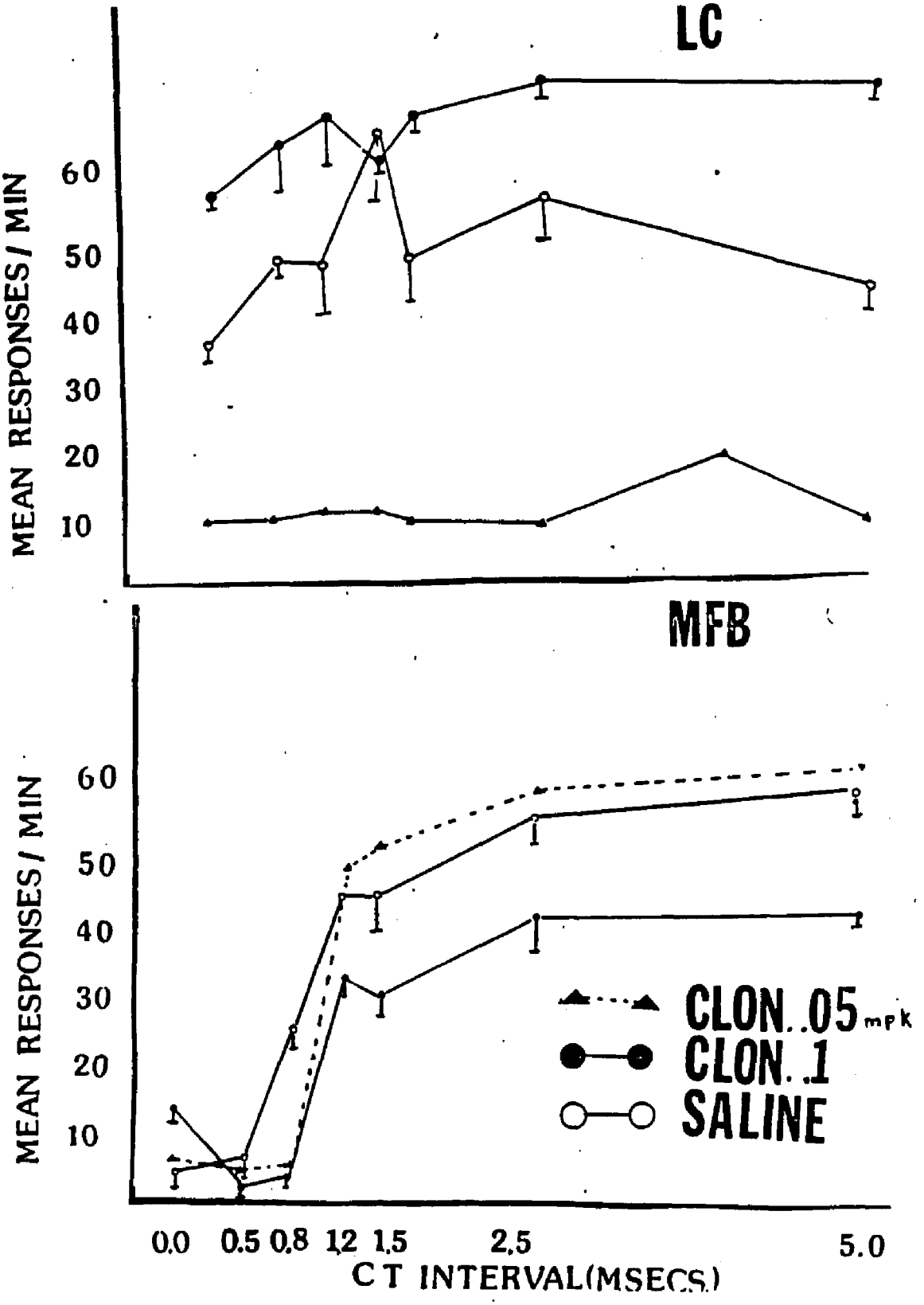
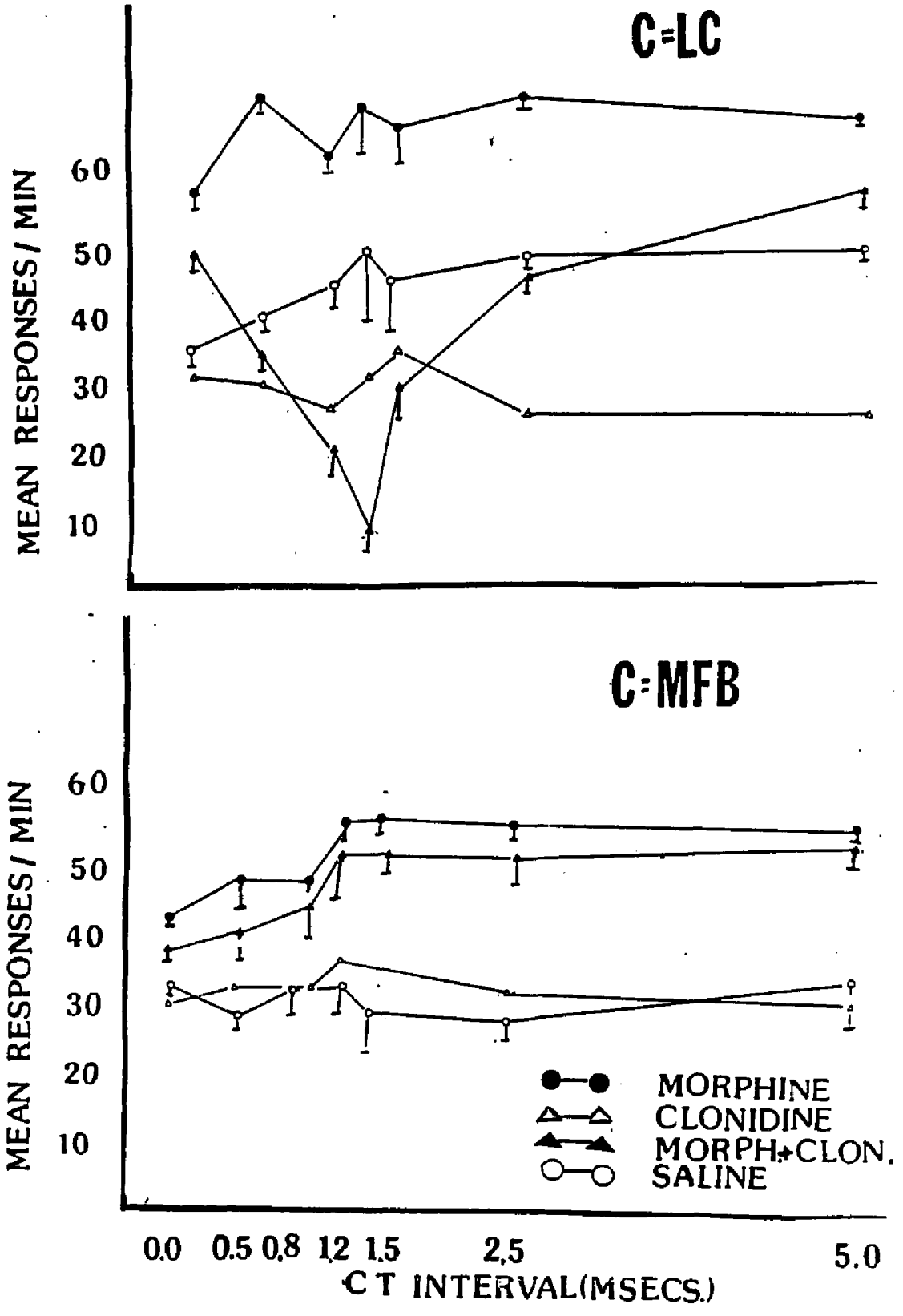


Figure 33. Mean responses per minute plotted as a function of the C-T interval in msec. for the LC-MFB group of placements in the clonidine Within-Site condition at the .05, .1 and .5 mpk dose.


C=LC
C=MFB
MEAN RESPONSES / MIN
60
50
40
30
20
10
MORPHINE
CLONIDINE
MORPH+CLON.
SALINE
0.0 0.5 0.8 1.2 1.5 2.5 5.0
CT INTERVAL(MSECS.)

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