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MONOPHASIC PULSE PAIR ANALYSIS OF INTRACRANIAL SELF-STIMULATION LOCI AND INTERACTIONS

by

Richard J. Bodnar

A dissertation submitted to the Graduate
Faculty in Psychology in partial fulfill-
ment of the requirements for the degree
of Doctor of Philosophy, The City Univer-
sity of New York.

1976

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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

MONOPHASIC PULSE PAIR ANALYSIS OF INTRACRANIAL SELF-STIMULATION LOCI AND INTERACTIONS

by

Richard J. Bodnar

Adivsers: Profs. Steven J. Ellman and Solomon S. Steiner

Intracranial self-stimulation (ICSS) is elicited from discrete sub-cortical brain loci; these ICSS loci neurophysiologically interact, and lesions of one ICSS locus can alter other ICSS behavior, suggesting an interconnected and interactive ICSS system. To further test such a notion, rats were stereotaxically implanted with pairs of bipolar electrodes aimed at one of the following combinations: a) locus coeruleus (LC)-hypothalamus (HYP); b) midbrain central gray (MCG)-HYP; c) substantia nigra (SN)-HYP; and d) septum-LC. Animals were shaped to lever-press for ICSS on a crf schedule, using 700 msec. trains of monophasic rectangular-wave stimulation delivered in pulse pairs. The initial pulses were designated the C pulses, while the trailing pulses were the T pulses; all were .1 msec. in duration. The interval between succeeding C pulses (C-C interval) was usually set at 30 msec. After shaping, each pole of each bipolar electrode was tested to determine the site which elicited the highest rates at the lowest current. The preferred ICSS pole was located in the LC for pontine placements, the MCG and SN for mesencephalic placements, and the medial forebrain bundle/perifornical area (MFB/PA) for HYP placements. The preferred pole within each site was then tested for differential reactivity of ICSS rates to temporal patterning. At a constant current and C-C interval, the interval between C and T pulses (C-T interval) was varied between 0 and 5 msec. In this single-site condition, ICSS

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rates at C-T intervals between .5 and 1.2 msec. were generally similar to rates elicited when the T pulses were omitted, and significantly lower than rates elicited at longer C-T intervals. The C-T interval which initially supported higher ICSS rates was significantly shorter for MCG, SN, and HYP placements than for LC or septal placements. After single-site functions were determined, the C and T pulses were divided between each animal's ICSS loci at their respective current intensities. ICSS rates in this split-site condition were similar across all C-T intervals. The two pulses delivered in the divided condition were significantly higher than the sum of rates elicited when each site was stimulated singly with C pulses alone, suggesting neurophysiological interactions. Differential responding occurred in some site combinations dependent upon which site of the combination received the C pulse. In LC-internal capsule/Fields of Forel (IC/FF) combinations, response rates were always and significantly higher for the C-LC, T-IC/FF condition than for the reverse condition, suggesting the interaction occurs through an ascending excitatory influence. ICSS response rates were always and significantly higher for the C-SN, T-IC/FF and the C-MCG, T-MFB/PA conditions than for their respective reverse conditions, also suggesting ascending excitatory interactions for these combinations. In contrast, ICSS response rates were usually similar regardless of interaction condition for LC-MFB/PA, SN-MFB/PA, MCG-IC/FF and LC-septal combinations, suggesting bidirectional interactive influences. In some cases, the C-MFB/PA, T-LC and the C-IC/FFT, T-MCG conditions yielded higher rates than their

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respective reverse conditions, suggesting descending excitatory influences. Directional interactions were not altered by either strychnine, a glycine inhibitor, or picrotoxin, a GABA inhibitor, suggesting that the differential ICSS interaction rates were not the result of inhibitory influences. The directional nature of the interactions also served as a predictor of LC lesions attenuating IC/FF ICSS or facilitating MFB/PA ICSS. These results suggest the existence of an interconnected interactive ICSS system with subsystems which are sensitive to temporally-distributed and directionally-oriented influences.

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A central theme in psychological theory has been the role of both rewarding and aversive stimuli in determining behavior. Physiological psychologists, in particular, have been interested in the neural mechanisms which determine whether a given stimulus will be rewarding or aversive. The search for these mechanisms became intensified when Olds and Milner (1954) discovered that rats would learn to perform tasks which would result in electrical stimulation delivered to selective brain areas; this phenomenon became known as intracranial self-stimulation (ICSS). This discovery suggested that hedonistic theories of reinforcement may have some validity, as opposed to a pure tension-reduction notion; that is, rats were behaving to acquire the effects of the stimulation and not to relieve some internal deprivation state. Furthermore, the behavior had no apparent pre-existing drive state for the stimulation and no apparent satiation effects. Hence, the behavior could be controlled almost entirely by stimulus parameter manipulation. In addition, ICSS behavior was not ubiquitous; that is, some brain areas supported ICSS at high rates, while others didn't support it at all. Hence, some brain areas were thought to be specifically organized to transmit information about the reward value of stimulation which gave rise to several neuronal models of drive and reinforcement (Deutsch, 1964; Gallistel, 1973; Crow, 1972a). Further models attempted to delineate the brain areas which subserved ICSS by delineating the neurohumoral transmitter substances responsible for the behavior. The most prevalent of these were hypotheses which implicated monoamines, particularly catecholamines(Stein, 1964; Poschel & Ninteman,

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1964). Other studies (Ellman, Ackermann, Bodnar, Jackler, & Steiner, 1975; Ackermann, Bodnar, Jackler, Steiner, & Ellman, in preparation,a) have investigated the interaction characteristics of ICSS loci in order to determine whether an ICSS neurophysiological system exists. The present studies are extensions of the investigation into the interactive properties of the ICSS system.

In order to provide a cohesive background into these studies, this section is broken into six areas which will include a review of relevant literature as well as an overall rationale. First, a review of the histofluorescence literature will be done in order to provide a neuroanatomical and neurochemical background for the brain loci presented later. Second, a systematic review of sites which sustain ICSS will be done, paralleling the histofluorescence data. Third, several ICSS hypotheses, including the noradrenergic, dopaminergic, and mixed hypotheses will be discussed concerning the role of specific neurochemicals in subserving ICSS behavior. Fourth, evidence for an ICSS system will be presented by a review of lesion studies and interaction studies. Review of the lesion studies will show the effects upon ICSS behavior from one brain locus by lesioning another ICSS site. The behavioral evidence for neurophysiological interactions between brain loci will be discussed. Fifth, as an introduction to the present experiments, a description of the monophasic pulse pair technique will be presented as well as a review of the ICSS literature using this technique. Specific studies which bore directly upon the methodologies utilized in the present experiments will be reviewed in detail. Sixth, a brief outline of the ra-

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tionales and purposes of the five experiments will be presented. Detailed rationales of each experiment are presented separately, with methods, results, and discussion.

A. Histofluorescence Studies

As stated above, early attempts to describe ICSS behavior centered upon the identification of the neurohumoral transmitter substance(s) responsible for the behavior; several early hypotheses implicated the monoamines, particularly the catecholamines (Stein, 1964; Poschel & Ninteman, 1964). Correspondingly, development of the histofluorescence method (Anden, Dahlstrom, Fuxe, Larsson, Olson, & Ungerstedt, 1966; Fuxe, 1965; Dahlstrom & Fuxe, 1964) provided a means by which monoamine-containing cell bodies, fibers, and terminals could be mapped through the brain. Dahlstrom and Fuxe (1964) mapped ascending monoaminergic cell groups through the sub-cortex; cell groups identified as catecholaminergic were labelled "A", while cell groups identified as indolaminergic (serotoninergic) were labelled "B". Within each letter designation, cell groups were numerically ordered according to their posterior-to-anterior position. The catecholaminergic-containing cell groups will be reviewed in detail here, beginning in the hindbrain and proceeding anteriorally. Included in the description will be corresponding neuroanatomical location, emanating fibers, and terminal areas.

Hindbrain cell groups. Seven hindbrain cell groups (A-1, A-2, A-3, A-4, A-5, A-6, and A-7) were identified by Dahlstrom and Fuxe (1964). Cell groups A-2 and A-4 are located in the dorsal medulla, while cell groups A-1, A-3 and A-5 are located in the

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ventral medulla. All of these catecholaminergic cell groups contain exclusively norephinephrine, as demonstrated by Ungerstedt (1971) pharmacologically. Ascending fibers from cell groups A-1, A-3, and A-5 join and form the ascending ventral noradrenergic bundle (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971). Cell groups A-2 and A-4 send diffuse fibers rostrally towards the dorsal pontine nucleus, the locus coeruleus. There are two pontine catecholaminergic-containing cell groups; cell group A-7 is located in the ventrolateral pons, contains exclusively norepinephrine and projects its fibers into the ventral noradrenergic bundle (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971). In the dorsal pontine area, cell group A-6, which corresponds with the nucleus locus coeruleus, has the highest concentration of norephinephrine-containing cell bodies; fibers arising from the locus coeruleus contribute to the ventral noradrenergic bundle and forms, with axons from A-2 and A-4, both the dorsal noradrenergic bundle and periaqueductal noradrenergic bundle,traverses the mesencephalon ventro-lateral to the aqueduct, passes through the dorso-medial hypothalamic area, and terminates in the cortex, thalamus, hippocampus, and septum. The periaqueductal noradrenergic bundle is initially formed by A-6 cell bodies, but, as it traverses the mesencephalon, it receives projections from scattered cathecholamine-containing neurons in the periaqueductal midbrain central gray (Lindvall & Bjorklund, 1974). The periaqueductal noradrenergic bundle is located lateral to the cerebral aqueduct in the mesencephalon and lateral to the III ventricle in the diencephalon, terminating in the preoptic hypo-

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thalamic area and amygdala. The ventral noradrenergic bundle, comprised of fibers from cell groups A-1, A-3, A-5, A-6, and A-7, traverses the ventral mesencephalon and ventro-lateral hypothalamic area, terminating diffusely in forebrain areas (Lindvall & Bjorklund, 1974).

Mesencephalic cell groups. Dahlstrom and Fuxe (1964) identified three catecholaminergic cell groups (A-8, A-9, and A-10) in the mesencephalon, all of which were clustered in the ventro-lateral and ventro-medial tegmentum. Cell group A-8 corresponds with the nucleus substantia nigra, pars lateralis, while cell group A-9 corresponds with the nucleus substantia nigra, pars compacta (Dahlstrom & Fuxe, 1964). Cell group A-10 corresponds with the nuclei in the medial ventral tegmental area (Dahlstrom & Fuxe, 1964). Ungerstedt (1971) demonstrated pharmacologically through 6-hydroxydopamine (6-OHDA) lesions that these three cell groups contain exclusively dopamine. Ascending dopaminergic fibers projecting from the substantia nigra, pars compacta (A-9) form the nigro-striatal pathway which traverses the ventro-lateral hypothalamus and terminates predominantly in the caudate nucleus and putamen (Lindvall & Bjorklund, 1974). Cell group A-10 projects fibers rostrally to form the meso-limbic pathway which traverses the lateral hypothalamic area and terminates in the amygdala, olfactory tubercule, nucleus accumbens, and medial septal nucleus (Hokfelt, Ljungdahl, Fuxe, & Johnson, 1974; Lindvall & Bjorklund, 1974; Lindvall, Bjorklund, Moore, & Stenevi, 1974; Ungerstedt, 1971; Anden et al., 1966; Fuxe, 1965; Lindvall, 1975; Brownstein, Saavedra, & Palkovits, 1974).

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Diencephalic cell groups. In addition to fibers of passage and terminals from posterior catecholaminergic cell groups originating in the hindbrain (dorsal noradrenergic bundle, periaqueductal noradrenergic bundle, and ventral noradrenergic bundle) and mesencephalon (nigro-striatal pathway and meso-limbic pathway), the hypothalamus has intrinsic catecholaminergic cell groups which are designated A-11, A-12, A-13, and A-14, all of which are located near the III ventricle. Groups A-11 and A-13 are located rostro-caudally to one another between the dorso-lateral part of the hypothalamic dorso-medial nucleus and the zona incerta. Cell group A-11 also possess cell bodies as far caudal as the posterior midbrain cerebral aqueduct (Lindvall et al., 1974). Group A-12 surrounds the infindibular recess which joins the hypothalamic area with the pituitary gland; most of these cells are found in the area of the arcuate nucleus (Dahlstrom & Fuxe, 1964; Lindvall & Bjorklund, 1974). Group A-14 is found in the area of the anterior periventricular hypothalamic nuclei. Together, projections from groups A-11, A-13, and A-14 form the diffuse incerto-hypothalamic tract which runs laterally and longitudinally throughout the dorso-medial portions of the hypothalamus and terminates in forebrain areas (Bjorklund, Lindvall, & Nobin, 1975).

Telencephalon. No catecholaminergic cell groups have been identified in the telencephalon. However, the telencephalon is a major terminal area for caudally-situated catecholamine cell groups. Some telencephalic areas receive common terminals from different catecholaminergic cell groups, providing common summation points

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for different fiber systems. The nucleus accumbens, medial septum, olfactory tubercule, and amygdala possess terminals from both the three ascending noradrenergic bundles and from the dopaminergic meso-limbic system (Lindvall & Bjorklund, 1974; Lindvall, 1975; Brownstein et al.,1975). The caudate nucleus and putamen possess terminals exclusively from the nigro-striatal pathway, meso-limbic pathway, and the noradrenergic bundles (Lindvall & Bjorklund, 1974).

Before concluding this section, two points should be noted. First, the histofluorescence technique only identifies monoamines; the brain loci mentioned above most surely possess neurons with transmitters other than monoamines. Second, histofluorescence is only one means of identifying monoaminergic pathways; other means will be discussed throughout the dissertation where appropriate, particularly evidence which suggests hypothalamic terminals from posterior catecholaminergic pathways. However, the histofluorescence method provides a useful map in identifying brain loci which support ICSS.

B. ICSS Loci

In the first section, a systematic identification of catecholamine-containing cell groups was accomplished. This mapping review was done because sites which sustain ICSS behavior are highly correlated with sites which contain catecholamines. ICSS behavior has been elicited through a variety of operant techniques from many brain loci ranging from the myelencephalon to the telencephalon. Sites in the myelencephalon and metencephalon supporting ICSS are cell groups A-2 (Carter & Phillips, 1975), A-7 (Ritter & Stein, 1974;

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Mattiace, in preparation), and A-6 (Farber, Steiner, & Ellman, 1972; Crow, Spear, & Arbuthnott, 1972; Ritter & Stein, 1973; Ellman, Ackermann, Farber, Mattiace, & Steiner, 1974; Ellman et al., 1975). The dorsal, periaqueductal, and ventral noradrenergic bundles which project from these hindbrain cell groups also support ICSS (Steiner, Bodnar, Ackermann, & Ellman, 1973; Ellman et al., 1974, 1975; Phillips, Brooke, & Fibiger, 1975; Thallman, 1975; Cooper & Taylor, 1967; Bodnar, Ellman, & Steiner, 1974; Ritter & Stein, 1974; Ackermann, Bodnar, Jackler, Steiner, & Ellman, in preparation, (b).)

Mesencephalic loci, including cell groups A-8, A-9, and A-10 support ICSS (Routtenberg & Malsbury, 1969; Crow, 1972(b); Phillips & Fibiger, 1973; Clavier, Phillips, & Fibiger, 1975; Ackermann et al., in preparation(b); Deutsch, 1964; Olds & Peretz, 1960), though a recent study (Belluzzi, Ritter, Wise, & Stein, 1975) suggests that substantia nigra, pars compacata ICSS is mediated by input from the ventral noradrenergic bundle. Mesencephalic ICSS has also been reported in the substantia nigra, pars reticulata (Ackermann et al., in preparation (a); Clavier et al., 1975) which has afferents distinct from the compacta cells (Rinvik, 1975), but contains dendritic fields from the dopamine cell bodies of the compacta (Bjorklund & Lindvall, 1975). Other sites supporting ICSS in the mesencephalon are the interpeduncular nucleus, the brachium conjunctivum, and the medial longitudinal fasciculus, including surrounding cell bodies along its length from dorsal raphe nucleus to the oculomotor nucleus (Crow, 1972; Routtenberg & Malsbury, 1969; Margules, 1969; Ellman et al., 1974; Liebman, Mayer, & Liebeskind, 1973; Olds,

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Travis, & Schwing, 1960). The medial longitudinal fasciculus, dorsal raphe nucleus, and oculomotor nucleus possess catecholamine cell bodies and fibers which may be the caudal extent of cell group A-11 (Felton, Laties, & Carpenter, 1974; Lindvall & Bjorklund, 1974; Lindvall, Bjorklund, Nobin, & Stenevi, 1974; Nobin & Bjorklund, 1973; Palkovits & Jacobowitz, 1974).

Diencephalic ICSS has been studied most extensively in the hypothalamus, particularly in the early ICSS parametric studies (Olds, 1958, Olds & Peretz, 1960; Deutsch, Howarth, Ball, & Deutsch, 1962; Howarth & Deutsch, 1962; Deutsch, Adams, & Metzner, 1964; Deutsch, 1964; Roberts, 1958; Bower & Miller, 1958; Keesey, 1962, 1964; Miller, 1958; Coons, 1964; Hoebel & Teitelbaum, 1962; Caggiula & Hoebel, 1966; Caggiula, 1969). Many structures in the hypothalamus elicit ICSS (see review Olds & Olds, 1962) including the medial forebrain bundle, crus cerebri, zona incerta, Fields of Forel, preoptic area, dorso-medial nucleus, fornix, and ventro-medial nucleus.

Telencephalic ICSS has been elicited from several limbic areas. In addition to Olds and Milner's (1954) initial study in the septum, early studies found ICSS in the caudate (Olds, 1958; Olds & Peretz, 1960; Olds & Olds, 1962) and the amygdala (Steiner & D'Amato, 1964). Recent studies have mapped ICSS sites throughout the caudate and putamen, nucleus accumbens, and frontal sulcus (Carter & Phillips, 1975; Phillips et al., 1975; Rolls & Cooper, 1974; Phillips, Carter, & Fibiger, 1976).

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Thus, ICSS behavior can be elicited from many brain loci in the subcortex. However, the behavior is not ubiquitous throughout the brain, but rather concentrated in discrete areas. Many of the studies described above have control animals which did not self-stimulate and had electrodes which were adjacent to, but not impinging upon, the loci mentioned in this section. It is apparent from this review that sites which support ICSS behavior and sites which contain catecholamines are highly correlated. Therefore, it is not surprising that catecholaminergic theories of ICSS prevail in the literature (Poschel & Ninteman, 1964; Stein, 1962, 1967, 1968; Stein & Wise,1969, 1971; Crow, 1972; Snyder, Banerjee, Yamamura, & Greenberg, 1974; German & Bowden, 1974).

C. Catecholaminergic Hypotheses

The catecholaminergic hypotheses suggest that the integrity of catecholamine systems is necessary for ICSS behavior in either a given site or through all brain loci, depending upon the extent of catecholaminergic manipulation. The most common technique utilized by various investigators has been the introduction of pharmacological neuroleptics which specifically affect catecholamine levels. For example, d-amphetamine, a potent psycho-mimetic and releaser of catecholamines, increases ICSS behavior (Stein, 1964; Stein & Wise, 1971; Phillips & Fibiger, 1973; Ellman et al., 1975; Steiner & Stokely, 1973), as do monoamine-oxidase inhibitors (Poschel & Ninteman, 1964). Alpha-methyl paratyrosine, which blocks the enzyme tyrosine hydroxylase and therefore the synthesis of dopamine and norepinephrine, reduces ICSS (Black & Cooper, 1971; Stinus & Thierry,

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1973) as does diethyldithiocarbonate, which blocks dopamine beta hydroxylase, the enzyme which converts dopamine to norepinephrine (Ritter & Stein, 1973; Ritter, Belluzi, Wise, & Stein, in preparation). Pimozide, chloropromazine, and haloperidol, all potent postsynaptic blockers of dopamine, reduce ICSS (Stein, 1962; Phillips, Brooke, & Fibiger, 1975; Dresse, 1966; Liebman & Butcher, 1973; Lippa, Antelman, Fisher, & Canfield, 1973). 6-hydroxydopamine, a toxic agent which selectively destroys catecholaminergic terminals, reduces or abolishes ICSS either through interventricular injections (Cooper, Cott, & Breese, 1974), through intercisternal injections to the sites themselves (Stinus, Thierry, & Cardo, 1975), or through intercisternal injections to areas connected to ICSS sites (Belluzi et al., 1975; Clavier et al., 1975). Finally, increased amounts of dopamine metabolites were found after mesencephalic ICSS (Arbuthnott, Crow, Fuxe, Olson, & Ungerstedt, 1970), as were increased amounts of norepinephrine metabolites found after hypothalamic, amygdala (Stein & Wise, 1969) and locus coeruleus ICSS (Anlezark, Walter, Arbuthnott, Crow, & Eccleston, 1975). Thus, high correlations between catecholamine levels and propensity for ICSS behavior has been suggested through histochemical, anatomical, pharmacological, and metabolic techniques.

Since the catecholamine group contains two transmitters, several theories of ICSS have attempted to explain the roles of norepinephrine and dopamine in ICSS behavior. These theories can be broken into three main groups: a) ICSS behavior is mediated solely by norepinephrine (Stein, 1964, 1967, 1968); b) ICSS is me-

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diated solely by dopamine (Crow, 1972; Lippa et al., 1973; Liebman & Butcher, 1973; Cooper et al., 1974; Cooper & Breese, 1975); and c) ICSS is mediated by both dopamine and norepinephrine, and furthermore, other transmitters may be involved; this is a so-called "mixed" hypothesis (Phillips & Fibiger, 1973; Ellman et al., 1976; Clavier & Routtenberg, 1974, 1976a,b; Rolls & Cooper, 1974; Coons, Smith, & Ungerleider, in press).

The first specific catecholaminergic theory of ICSS suggested that ICSS behavior is mediated by norepinephrine; the main proponent of this position was Stein and his co-workers (Stein, 1964, 1967, 1968). Stein bases his theory on five findings. First, d-amphetamine increases both ICSS behavior and norepinephrine release (Stein, 1964). Second, ICSS behavior elicited from the hypothalamus and amygdala produces increased amounts of norepinephrine metabolites (Stein & Wise, 1969). Third, interference with dopamine-beta hydroxylase synthesis reduces hypothalamic ICSS levels because norepinephrine is prevented from synthesis. This result is reversed by intraventricular administration of l-norepinephrine, the active stereotypic isomer (Wise & Stein, 1971). Fourth, lesions of the ventral noradrenergic bundle reduced substantia nigra, pars compacta ICSS behavior. Hence, lesions of a noradrenergic system reduced ICSS behavior in a dopaminergic area, suggesting that ICSS is dependent upon noradrenergic integrity (Belluzzi et al., 1975). Fifth, substantia nigra ICSS is reduced after subcutaneous administration of diethyldithiocarbamate, which blocks the synthesis of dopamine into norepinephrine (Ritter et al., in preparation).

The second specific catecholaminergic hypothesis postulates

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that ICSS is mediated by dopamine (Crow, 1972; Lippa et al., 1973; Liebman & Butcher, 1973; Cooper et al., 1974). This hypothesis is based upon three findings: a) antipsychotic agents reduce ICSS behavior (Phillips et al., 1975; Lippa et al., 1973; Liebman & Butcher, 1973); b) antipsychotic agents selectively affect dopamine receptors (Snyder et al., 1974; Iversen, 1975); and c) 6-hydroxydopamine reduces ICSS in hypothalamic and locus coeruleus ICSS areas after pretreatment with desimiprimine which protects noradrnergic terminals from destruction (Cooper et al., 1974; Cooper, 1974; Cooper & Breese, 1975).

The third hypothesis involves roles for both norepinephrine and dopamine in the elicitation of ICSS behavior, dependent upon the brain locus tested (Phillips, 1975; Ellman, 1975). A series of experiments involving the optical isomers of amphetamine reinforce this third view and raises serious questions for the views that a single transmitter substance can mediate all ICSS. These experiments are based on findings (Coyle &Snyder, 1969, 1970; Snyder et al., 1974) that d-amphetamine is eight to ten times as potent as l-amphetamine in preventing re-uptake from noradrenergic synaptosomes, while nearly equipotent for other synaptosomes. Taylor and Snyder (1970) provided behavioral evidence for this differentiation by demonstrating that locomotor activity is enhanced by d-amphetamine ten times as potently as by l-amphetamine, while compulsive gnawing behavior is potentiated by d-amphetamine only twice as well as by l-amphetamine. From this, it was implied that reactivity to d-amphetamine, but not l-amphetamine indicated noradrenergic mediation, while nearly equipotent

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reactivity for the two isomers indicated dopaminergic mediation. The biochemical distinctions made by Coyle and Snyder (1969) have been challenged (Ferris, Tang, & Maxwell, 1972; Harris & Baldessarini, 1973), but the behavioral distinctions elucidated by the d- and l-amphetamine screening procedure have been extended, particularly in ICSS studies. The experiments demonstrated that ICSS is greatly enhanced by d-amphetamine, but not l-amphetamine in the locus coeruleus (Bodnar et al., 1974; Ellman et al., 1975, 1976; Phillips et al., 1975), the periaqueductal noradrenergic bundle (Bodnar et al., 1974; Ackermann et al., in preparation, b), and the hypothalamus (Phillips & Fibiger, 1973; Bodnar et al., 1974; Ellman et al., 1975; Stephens & Herberg, 1975). All of these areas have high concentrations of norephinephrine-containing cell bodies or fibers. Conversely, ICSS behavior was nearly equipotentally enhanced by both isomers in the substantia nigra, pars compacta (Phillips & Fibiger, 1973), nucleus accumbens (Phillips et al., 1975), caudate-putamen (Carter et al., 1975), the far-lateral hypothalamus (Stephens & Herberg, 1975), and the dorsal midbrain medial and ventral to the aqueduct, including the oculomotor nucleus and dorsal raphe (Bodnar et al., 1974; Ellman et al., 1976). All of these areas have large concentrations of dopaminergic cell bodies or fibers. Therefore, whether or not the biochemical data of the d- and l-amphetamine screening procedure in inferring that differential reactivity indicates noradrenergic or dopaminergic mediation, remains valid, it is clear that this pharmacological screen reliably distinguishes ICSS loci into two classes, dependent upon their relative reactivity to l-amphetamine. This differentiation implies that the mechanisms for ICSS, presently un-

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explained, are different across loci and thus cannot be explained by a single transmitter model. The co-existence of differentially reactive ICSS sites with parallel fiber bundles and common terminal areas raised the question as to whether these two transmitter systems interacted.

It is obvious from all of these studies that any specific catecholaminergic hypothesis, be it noradrenergic or dopaminergic, cannot account for all of the ICSS behavioral manipulations. A more fruitful way in determining the characteristic properties of individual ICSS sites would be to determine how these sites comprise a part of an overall ICSS system. Two main tests of ICSS loci working as a system appear in lesion studies and interaction studies.

D. ICSS Loci as a System: Lesion and Interaction Studies

Lesion studies in the ICSS literature have been largely inconclusive; that is, the size of lesions have varied, the ICSS placements have varied, and hence overall results have varied. Most of these studies have centered around the effects of lesions upon hypothalamic medial forebrain bundle ICSS. Medial forebrain bundle ICSS was impervious to a) lesions of the mesencephalic raphe (Lorens, 1966), b) lesions of the ventral tegmental area of Tsai (Valenstein & Campbell, 1966; Boyd & Gardner, 1967), and c) rostral and/or caudal lesions of the medial forebrain bundle itself (Olds & Olds, 1969). These early studies seemed to indicate that despite the number of ICSS sites, the ICSS phenomena was discrete and intrinsic to each particular area. However, several recent studies with more discrete lesions and more uniform electrode placements suggest that

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ICSS behavior in one site is mediated by the integrity of a second ICSS site. As previously stated, Belluzzi et al. (1975) found that lesions of the ventral noradrenergic bundle reduced ICSS behavior in the substantia nigra, pars compacta, although Clavier et al. (1975) failed to replicate this data. A recent study (Farber, Ellman, Mattiace, Ippolito, Holtzman, Halperin, & Steiner, in preparation) demonstrated that unilateral locus coeruleus lesions selectively abolished or greatly reduced ICSS behavior in the ipsilateral crus cerebri and Fields of Forel regions of the hypothalamus, while the same lesions spared ICSS behavior in the medial forebrain bundle and perifornical areas of the hypothalamus. In accordance with this, Koob et al. (1976) found that locus coeruleus lesions facilitated posterior medial forebrain bundle ICSS. Moreover, Clavier and Routtenberg (1976a) demonstrated that medial forebrain bundle lesions attenuated dorsal noradrenergic bundle ICSS. Thus, in these studies, a) lesions of predominant noradrenergic areas affected ICSS elicited from areas in which the greatest concentration of ascending noradrenergic and dopaminergic fibers are situated; b) the same type of lesion spared or facilitated ICSS behavior in areas of the hypothalamus (medial forebrain bundle) where extrinsic ascending fibers are few and where intrinsic tracts are diffusely organized into reticulum, in which direction of travel is largely undertermined (Lindvall & Bjorklund, 1974; Ungerstedt, 1971; Dahlstrom & Fuxe, 1964; Nauta, 1958; Millhouse, 1969); and c) that medial forebrain bundle lesions in turn attenuate ICSS from posterior noradrenergic projections.

A second technique in which individual ICSS sites can be tested

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as a system is interaction studies, by investigating the relationship of two or more brain loci by parametric manipulation of variables.

Physiological interaction studies are rare in the literature, considering the profusion of ICSS sites and the anatomical and histochemical evidence suggesting interrelationships among ICSS sites. Most work (Olds & Peretz, 1960; Routtenberg & Olds, 1966; Porrino, 1975) dealt with interactions between an ICSS site and an aversive site, and studied the relative inhibition of aversive stimulation upon ICSS performance. Studies involving interactions between two ICSS sites included substantia nigra/septum combinations (Albino & Lucas, 1962); contralateral hypothalamic combinations (Ungerleider & Coons, 1970; German & Holloway, 1973; Ackermann, Bodnar, Jackler, Ellman, & Steiner, in preparation, a); amygdala/hypothalamus combinations (Ellman et al., 1975; Ackermann et al., in preparation, a); substantia nigra/hypothalamus combinations and cell group A-10/hypothalamus (Ackermann et al., in preparation, a). In all of the latter studies, when two ICSS sites were tested for interaction, a facilitation in responding occurred. On the other hand, if one of the two sites did not elicit ICSS behavior, no interactive facilitation took place (Ungerleider & Coons, 1970; Coons et al., in press; Ellman et al., 1975; Ackermann et al., in preparation, a). Ackermann's et al. (in preparation, a) technique involved the determination of an intensity which elicited threshold responding for each site tested singly of less than ten reponses per minute. After this, the sites were stimulated simultaneously at those intensities, and if response rates were enhanced over the sum of the rates eli-

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cited by the sites stimulated singly, an interaction was said to occur. If an interaction occurred, the current intensity at one site was reduced repeatedly in fixed steps and retested until no interaction occurred. Ackermann et al. (in preparation, a) found that the magnitude of threshold reductions differed with differing site combinations. If both ICSS sites were reactive to d-, but not l-amphetamine, as determined by the amphetamine screen described previously, the magnitude of threshold reduction was generally small. On the other hand, if one of the ICSS sites in the combination was reactive to l-amphetamine, while the other was insensitive as determined by the amphetamine screen, the magnitude of threshold reduction was significantly larger. From this study, it seemed that simultaneous stimulation of differentially reacting inputs greatly enhanced each other, while simultaneous stimulation of similarly reacting inputs slightly enhanced each other, establishing a non-pharmacological validation of differences between ICSS loci.

It is apparent that the interaction data presented by Ackermann et al. (in preparation, a) and the lesion data presented by Farber et al. (in preparation) suggest the existence of an ICSS system in which one part of the system (e.g., locus coeruleus) is necessary for the integrity of ICSS behavior in a second part of the system (e.g., crus cerebri and Fields of Forel) and that simultaneous stimulation of the system results in potentiation. Thus, the fact that the locus coeruleus, a noradrenergic pontine nucleus (Ungerstedt, 1971; Lindvall & Bjorklund, 1974; Ross & Reis, 1974), abolishes ICSS in dopaminergic hypothalamic areas, is in accordance with the interaction data which demonstrated large threshold reductions when a nor-

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adrenergic site and a dopaminergic site were stimulated simultaneously. However, this data does not reveal the site of interaction because neither the locus coeruleus nor the nigro-striatal tract which traverses the hypothalamus where the locus coeruleus lesions are most effective, are known to have terminals in the hypothalamus (Lindvall & Bjorklund, 1974; Olson & Fuxe, 1972; Loizou, 1969). However, lesions of the locus coeruleus reduce dopamine beta hydroxylase activity in the hypothalamus by 50% (Ross & Reis, 1974), and hypothalamic lesions change locus coeruleus tyrosine hydroxylase and dopamine-beta hydroxylase activity in a way that characterizes a reversible retrograde reaction of central noradrenergic neurons (Ross, Joh, & Reis, 1975). In addition, histofluorescence studies (Ungerstedt, 1971; Lindvall & Bjorklund, 1974) demonstrate that the ascending fiber tracts have varicosities throughout the hypothalamus. Thus, the site of interaction in Ackermann's et al. (in preparation, a) study and in Farber's lesion study (in preparation) could indeed be in the hypothalamus, but direct neuroanatomical evidence is lacking.

These studies suggest that the source of the interaction is either at the anterior site itself, or at a placement anterior to both sites with common termination. The nature of both studies precluded the ability to discern the direction of the ICSS system because their methodologies employed sinusoidal stimulation. In sinusoidal stimulation, the current is delivered to the electroce site throughout the stimulus train, which precludes the insertion of delays into the stimulation parameters between the two sites. In order to determine directionality of interaction within a system, assymetrical components of the system should be differentially af-

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fected by manipulation of temporal delay. If, as in the postulated case of an ascending catecholamine system, the components of the system are arranged posterior-to-anterior, then initial stimulation of the posterior segment followed by stimulation of the anterior segment should yield more optimal results than the reverse order. If Ackermann et al. (in preparation, a) and Farber et al. (in preparation) are correct, initial stimulation of the locus coeruleus followed by stimulation of the crus cerebri should elicit greater ICSS behavior than the reverse. In order to test such a hypothesis, one must first be able to deliver temporally-spaced stimulation between sites and, second, preferably make the stimulation discrete and non-overlapping. Therefore, to establish directionality of interaction, very brief pulses of stimulation must be delivered to each electrode site and the pulses must be able to be temporally spaced. Such a technique existed in classical neurophysiology (Sherrington, 1906; Erlanger & Gasser, 1937) in which the speed and excitability cycle of peripheral nerves was studied.

E. Monophasic Pulse Pair Stimulation Technique: Within Site and Between Site

The monophasic pulse pair technique, also known as the C-T technique, allows discrete stimulation of electrode locus. A "C" or conditioning monophasic pulse, of sufficient current intensity to fire the neuron but of short duration (.1 msec.), was followed at various intervals by a "T", or test monophasic pulse of equal duration (C-T interval). The length of the C-T interval determined, for peripheral nerves, the temporal summation characteristics (Lloyd, 1946; Eccles, 1964), the refractory characteristics

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(Erlanger & Gasser, 1937), and the latent addition characteristics (Lucas, 1910) of individual neurons. This C-T technique was modified by Deutsch (1964) to study the characteristics of central ICSS neurons in the medial forebrain bundle. Deutsch (1964), through a variety of behavioral techniques, duplicated the latent addition, refractory, and temporal summation characteristics observed peripherally, though caution should be used in interpreting the data in this way. In the peripheral studies, single or small groups of neurons were tested, and stimulation usually occurred along the axon. In Deutsch's (1964) and all other studies using this technique centrally, a) many unidentified and possible heterogeneous neurons are stimulated; b) the portion (dendrite, soma, axon) of each neuron stimulated is unknown; c) different neurons are probably stimulated in different portions, given the structure of the medial forebrain bundle (Millhouse, 1969); and d) different groups of neurons are stimulated with different amounts of current, depending upon their distance from the source of stimulation. Because one pulse pair was not enough to sustain ICSS behavior, Deutsch utilized trains of stimulation in which successive pulse pairs were delivered at equally spaced intervals (C-C interval) within a train for each behavioral response. This is in contrast to the classic studies where only single C and T pulses were used.

In Deutsch's experiments, trains of C pulses alone were compared with C-T pulse pairs at various C-T intervals. Deutsch found that at short C-T intervals (.1 and .2 msec), response rates were greater than when C pulses alone were delivered. At C-T intervals

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between .3 and .8 msec., response rates were similar to those obtained by C pulses alone. Between C-T intervals of .5 and 1.2 msec., responding began to increase over that of C pulses alone. At C-T intervals of 1.5 msec. to longer C-T intervals such as 10 msec., responding steadily increases to peak rates. Deutsch suggested that axonal excitability cycles are the main causes of the limitation of central reward neurons to fire for every delivered pulse. Again, these inferences could be made only if the stimulated neuronal pool was homogenous and standardly stimulated. Szabo, in a series of papers (1973; Szabo, Nad, & Szabo, 1972, b; Szabo, Lenard, & Kosaras, 1974) challenged Deutsch's (1964) notion that axonal events are the sole reason for the changes in response rate as C-T interval is manipulated. Szabo maintained that synaptic activation of neurons on the periphery of the stimulation field was the mechanism by which ICSS behavior increased markedly between 0.8 and 1.2 msec.

The results obtained by Deutsch and Szabo within an ICSS site were replicated in various other experiments using different methodologies. Coons' group (Smith & Coons, 1970; Coons et al., in press) established a criterion response rate and manipulated current intensity. Between C-T intervals of .3 and .8 msec., current threshold was similar to that used for the C pulse alone. As C-T interval increased to 5 msec., current threshold needed to maintain criterion responding decreased. The same results (Kestenbaum, Deutsch, & Coons, 1970, 1973; Brauth & Coons, 1975) were also found in passive stimulation of pain pathways. Gallistel (1969,a,b) demonstrated the same results when he used pulse pairs as a priming stimulus in a

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running-speed situation; as C-T interval increased past .8 msec., running speed increased. Rose (1974) demonstrated the same temporal patterning for the elucidation of stimulation-produced analgesia, while several studies found the same relationship in stimulus-bound eating and drinking (Rolls, 1972; Halboth & Coons, 1973; Hu, 1975; Hawkins, 1974). Finally, studies demonstrated that brainstem single-unit activity is increased when pulse pair stimulation in medial forebrain bundle electrodes was presented at C-T intervals greater than 0.8 msec. (Gallistel, Rolls, & Greene, 1969; Rolls, 1971,a; Matthews & Gallistel, 1975).

Two studies have attempted to investigate the nature of ICSS interactions between hypothalamic sites using the C-T pulse pair technique (Ungerleider & Coons, 1970; German & Holloway, 1973). Ungerleider and Coons implanted rats with electrodes in two contralateral hypothalamic placements. Using latency to-lever-press and inter-stimulation interval stimulation as a response measure, they found in all cases that when both C and T pulses were delivered to a single hypothalamic site, interstimulation intervals and latencies were significantly longer at short C-T intervals and with C pulses alone, than at longer C-T intervals. These data are in accordance with data showing an operant level of ICSS behavior elicited at short C-T intervals and for C pulses alone, but as C-T intervals increase, ICSS rates increase (Deutsch, 1964; Szabo et al., 1972,b; Gallistel, 1969,a,b). However, when the C and T pulses were split between the two contralateral hypothalamic placements, the latency to press and the inter-stimulation intervals were significantly shorter at short C-T intervals as compared to the within-site condi-

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tion. Ungerleider and Coons interpreted this data as an elimination of refractoriness. Since the two pulses were not delivered to the same site, there could be no chance for the second pulse ever to be refractory to the neuronal activity caused by the first pulse. However, even though the C and T pulses were delivered to different sites, an interaction occurred as evidenced by the shorter latencies to press. This interaction was hypothetically due to a common pool of neurons upon which contralateral hypothalamic placements synapse. The sooner that the second pulse arrives after the first pulse, the more their effects would summate. German and Holloway (1973) tested these interactions with assymetric contralateral hypothalamic placements, the lateral hypothalamus and the lateral preoptic area. Once again, when the C and T pulses were split between sites, an interaction occurred. In this study, response rate was the measure, and at short C-T intervals in the split-pulse condition, responding was similar to that attained at longer C-T intervals, in contrast to low responding when both C and T pulses were delivered to a single site at short C-T intervals. Moreover, at one C-T interval, responding was greater when the C pulse was delivered to the more anterior (preoptic) site and the T pulse to the more posterior site, than when the C pulse was delivered to the more posterior site and the T pulse to the more anterior site. From these results, German and Holloway (1973) concluded that the point of summation for these placements must be posterior to both, inferring that increased responding was a consequence of optimal summation of the C and T pulses, the high data point representing the transmission times from the two electrode sites. Since the point of summation was posterior, then the C

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pulse would have to be placed in the anterior site. German and Holloway (1973) provided behavioral evidence for directionality in neurophysiological interactions; thus, this technique offers possibilities of detecting the respective influences of each site on the interaction and on each other.

Thus, it is apparent that the pulse pair technique offers opportunities to investigate the directionality of interactions and enable one to judge the characteristic properties of individual ICSS sites within the system. Hence, one can ascertain the locus, neuronal properties, interactive properties, and integrity of single ICSS sites with relation to other ICSS sites.

The central purpose of this series of studies was to further delineate the role of brain areas in ICSS behavior by defining characteristics of the ICSS system. In Experiment I, a mapping study of various brain loci was undertaken using monopolar stimulation with bipolar electrodes. This provided an opportunity to selectively study the capability of very discrete neuroanatomical loci to sustain ICSS behavior. In Experiment II, within-site pulse pair functions were studied in order to elucidate the axonal/synaptic properties of ICSS from different sites. In Experiment III, which is the pivotal point of this dissertation, neurophysiological interactions were done between various ICSS sites in order to determine the summation characteristics of the system. Furthermore, if ICSS does correlate with catecholaminergic systems, then subtle differences in type of interaction should occur. Specifically, ascending excitatory influences should appear in certain combinations when the two sites belong to the same system, but not appear if they belong

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to different systems. If two ICSS sites belong to the same system, differences in responding should occur in interactions, dependent only upon which site of the combination is stimulated first. If the excitatory influence is ascending, then the condition in which the C pulse of the pulse pair is delivered to the posterior site should yield greater responding that the condition in which the C pulse of the pulse pair is delivered to the anterior site. If the excitatory influence is descending, then the condition in which the C pulse of the pulse pair is delivered to the anterior site should yield greater responding than the condition in which the C pulse of the pulse pair is delivered to the posterior site. If the excitatory influence is neither predominantly ascending nor predominantly descending, then responding under both conditions should be similar. Obviously, this model utilizes only excitatory mechanisms. Experiment IV investigates the feasibility of an alternate hypothesis for Experiment III. Rather than excitatory influences explaining differences in interactions, one could explain the same results by suggesting inhibition of one site upon another. In order to investigate this possibility, the neurophysiological interactions of specific animals in Experiment III were tested after administration of strychnine or picrotoxin. These drugs are potent inhibitors of glycine and GABA respectively, both of which have been implicated in inhibitory mechanisms. Experiment V combined this interaction technique with lesions in order to determine if predictions independent of histological verification could be made about the effect of lesions of the hindbrain site on the forebrain sites on the basis of the interactions. Cumu-

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latively, these studies should provide answers towards the underlying mechanisms of the ICSS system.

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Experiment I. Specificity of ICSS Within an Electrode Site

Rationale

In the introduction section, a catecholaminergic hypothesis accounting for ICSS was proposed, based on the following facts: a) all electrodes supporting ICSS have catecholaminergic cell bodies or projections either around the electrode or within the stimulating field of the electrode; b) agents that block catecholaminergic activity facilitate ICSS; and c) significant ICSS reductions caused by lesions or knife cuts occur only when catecholamine systems are disrupted. It is possible, however, that disruptions of other systems (e.g., cholinergic) (Shute & Lewis, 1967), which parallel catecholamine systems, could also mediate ICSS. It is even possible that heretofore undiscovered transmitters could mediate ICSS. This latter point is given credence when one considers that the putative transmitter for about 90% of central nervous system neurons are still unknown (Eccles, 1964).

Several authors (Routtenberg & Malsbury, 1969; Clavier & Routtenberg, 1974; Cooper & Rolls, 1974, Yeomans, 1975) have advocated alternatives to the catecholaminergic hypothesis. For example, Routtenberg (1968) suggested a motor hypothesis in which ICSS produces a reinforcing, and redundant, motor pattern. Thus, Routtenberg and Malsbury (1969) found that the substantia nigra, the brachium conjunctivum, and the peduncle of the fifth cranial nerve supported ICSS. However, the red nucleus, a pivotal structure in the extra-pyramidal system, did not support ICSS and was indeed

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aversive. Also, the other components of Routtenberg's motor ICSS system either have catecholamines present within the site (e.g., substantia nigra), or have proximal catecholaminergic cell bodies (e.g., locus coeruleus proximal to the mesencephalic trigeminal nucleus) or fiber tracts (e.g., dorsal noradrenergic bundle traverses the brachium conjunctivum). Routtenberg (Amaral & Routtenberg, 1975; Clavier & Routtenberg, 1974; Simon, Lemoal, & Cardo, 1975) claims, however, in contradiction to other studies (Farber et al., 1972; Crow et al., 1972; Ritter & Stein, 1973; Steiner et al., 1973; Ellman et al., 1974, 1975; Phillips et al., 1975) that the locus coeruleus and its ascending fiber tracts do not support ICSS.

Because the locus coeruleus is primarily noradrenergic, several catecholaminergic theories assign a pivotal role to it. Ellman (1975) proposes that ICSS, as an artificially motivated behavior, taps certain specific and non-specific systems. The specific systems are involved in primary reinforcement, and most are located in the hypothalamus. He further suggests that rapid eye movement (REM) sleep activates these specific systems during the sleep cycle and lowers their thresholds for that behavior. Jouvet (1969, 1973) demonstrated in cats that REM sleep can be disrupted by lesions of the locus coeruleus and the adjacent dorso-lateral tegmentum. Thus, in Ellman's (1975) hypothesis, the locus coeruleus, in addition to triggering the REM sleep system, must also be part of the ICSS system in order to modulate specific-behavior systems. In support of this hypothesis, a) Steiner and Ellman (1972) demonstrated that a reciprocal relationship exists between REM sleep and hypothalamic ICSS, where REM deprivation lowered ICSS thresholds and ICSS lowered REM

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rebound; b) Ellman et al. (1974, 1975) found that the locus coeruleus is an ICSS site; and c) Ellman et al. (1975) demonstrated that physiological interactions occur between locus coeruleus and hypothalamus. These studies implied a functional relationship between the REM sleep system and the ICSS system. Therefore, an important theoretical consideration is the source of the rewarding aspects of anterior mesencephalic brain stimulation, be it trigeminal nerve or locus coeruleus.

Major methodological differences exist between Routtenberg's technique and those of other laboratories. First, Routtenberg employs one intensity, 25 uA, an intensity which often fails to support even hypothalamic ICSS, while the other laboratories test the animals over a range of intensities (5-200 uA). Routtenberg claims that his method controls for current spread, a cautionary argument initiated by Valenstein (1964). Though current spread should be accounted for, the fact that an animal does not self-stimulate for 25 uA delivered to a site does not negate the possibility that it would self-stimulate for some other intensity. Ellman et al. (1975) demonstrated that intensities needed to support locus coeruleus ICSS do not differ significantly from intensities needed to support hypothalamic ICSS. Most importantly, Routtenberg does not shape his animals to lever-press, on the assumption that all sites eliciting ICSS react in the same way as hypothalamic ICSS. Many studies (see review, German & Bowden, 1974) report that hypothalamic ICSS, particularly medial forebrain bundle ICSS, is characterized by behavioral exhibitions of affect, such as furious grooming, biting, penile erections, urination, defecation, hyper-excitability, hyper-activity, and con-

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vulsions. On the other hand, behavioral reports of locus ceoruleus ICSS (Ritter & Stein, 1973; Crow et al., 1972; Ellman et al.,1975) indicate methodical, "clock-work" lever-pressing often accompanied by either head or body movements, jaw or teeth movements, and relative quiescence, with no convulsions. Since locus coeruleus ICSS is qualitatively different from hypothalamic ICSS (Steiner et al., 1973) and is often accompanied by motor artifacts, if animals are not shaped, and if one stimulus intensity is offered, ICSS behavior may fail to appear even though the animal would self-stimulate if it were shaped at different current intensities.

A similar difficulty is encountered with midbrain periaqueductal gray electrode sites. In several studies (Margules, 1969; Liebman et al., 1973) in which a single intensity was employed and in which animals were not shaped, ICSS was elicited only from the mid-ventral portion of the gray; the remainder of the gray was described as aversive. Conversely, other investigators, employing a variety of intensities (Cooper & Taylor, 1967; Bodnar et al., 1974; Ellman et al., 1974; Ackermann, in preparation,b) have demonstrated ICSS could be elicited from the lateral portions of the gray as well as the mid-ventral portion.

In addition to the above-described methodological difficulties, electrode size can also present difficulties in interpreting ICSS data. In order to elicit ICSS, a large number of reward-relevant neurons must be activated; electrodes with small transverse surface areas do not elicit ICSS (Valenstein & Beer, 1971). Therefore, the bipolar electrodes often used in ICSS studies have a transverse diameter approaching that of the neuroanatomical area in question.

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Therefore, it is difficult to make precise histological determinations of the area stimulated, especially when pertinent sites for particular theories are close to one another (e.g., locus coeruleus and mesencephalic trigeminal nucleus). Since electrode size is a crucial factor, any technique which could reduce the area of stimulation would resolve some of the questions concerning the locus of effective stimulation. In virtually all studies, bipolar electrodes and either a sinusoidal or biphasic rectangular pulse-pair stimulation technique are employed. In these types of stimulation, half of the time one pole of the bipolar electrode is the cathode and the anode the other half of the time. By contrast, in the C-T technique, monophasic stimulation is utilized in which one pole of the bipolar electrode is always the cathode, and either the other pole or some other referent serves as anode. This type of stimulation, delivered over long periods of time, produces anodal lesions because of ionic hyper-polarization. However, the C-T technique delivers stimulation briefly (.1 mesc.), with long intervals between pulses to allow for ionic repolarization. This technique was used (Szabo et al., 1972, b; Szabo & Milner, 1973) to demonstrate the existence of anodal hyper-polarization and to demonstrate that ICSS excitation travels in a caudal-to-rostral (ascending) direction.

In the present experiment, the cathode source of stimulation was manipulated for each pole of a bipolar electrode aimed at one of five areas: Locus coeruleus, periaqueductal midbrain central gray, substantia nigra, hypothalamus, or septum. Each bipolar electrode was aligned in a medio-lateral axis perpendicular to the mid-

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sagittal plane because: a) this avoided anodal hyperpolarization in the direction of ascending stimulation(Szabo et al., 1972(b); Szabo & Milner, 1973), and b) the pathways of the alternate hypotheses proceed in an posterio-anterior direction, but are situated medial and lateral with respect to each other. If response rate of one pole acting as cathode was less than the response rate of the other pole .3 mm away, acting as cathode, it could be assumed that the pole which elicited the higher response rate was closer to the source of ICSS reward at that level. Hence, through this technique and subsequent histological localization, a precise mapping study of ICSS sites was possible. Both bipolar conditions (stimulating between two poles of the bipolar electrode) and monopolar conditions (stimulating between one pole of the bipolar electrode and a cortical screw) were tested.

Method

Subjects. Fifty (50) male, albino Holtzman Sprague-Dawley rats, weighing between 350 and 500 grams, were anesthesized with 2 ml. of sodium pentobarbitol solution (Chloropent) per kilogram of body weight. After sedation, incisions were made exposing the skull and each animal was stereotaxically (Kopf) implanted with two bipolar electrodes aimed at one of the following combinations of sites: locus coeruleus/hypothalamus, midbrain periaqueductal gray/hypothalamus, substantia nigra/hypothalamus, and locus coeruleus/septum. The bipolar electrodes (Plastic Products, MS 303-.018-.312-SS-.010) were insulated except at the tips, and were aligned in a medio-lateral direction, perpendicular to the mid-sagittal plane.

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Two stainless steel cortical screws were attached to the skull and connected to a third electrode by uninsulated wires; these screws served as anchors to hold a cap of dental acrylic to the skull, and also as anodal indifferents. The scalp was then sutured around the cap and the animal returned to its home cage where he had ad libitum access to food and water.

Locus coeruleus electrode coordinates were: a) 1.5-2.0 mm. posterior to the lambda suture, b) 1.0 mm. lateral to a line extrapolated from the saggital suture, and c) 7.0 mm. from the top of the skull. Periaqueductal midbrain central gray coordinates were: a) 0.6 mm. anterior to the lambda suture, b) 1.5 mm. lateral to the saggital suture, c) 7.5 mm. from the top of the skull, and d) inserted at a 12^o angle to the mid-sagittal plane. Substantia nigra coordinates were: a) 2.0 mm. anterior to the lambda suture, b) 2.0 mm. lateral to the saggital suture, and c) 8.2 mm. from the top of the skull. Hypothalamic coordinates were: a) 4.2-4.4 mm. posterior to the bregma suture, b) 1.5 mm. lateral to the sagittal suture, and c) 8.7 mm. from the top of the skull. Septal coordinates were: a) even with the bregma suture, b) 0.3 mm. lateral to the sagittal suture, and c) 6.4 mm. from the top of th skull. All electrode combinations were implanted ipsilateral with respect to one another, except for the periaqueductal midbrain central gray placement, where contra-lateral implanation and the 12^o angle placed the tip along the midline. In all cases, the incisor bar was set at 5 mm. below the interaural line.

Apparatus. After recovery (ten days), each animal was tested for ICSS behavior for each site. Each animal was placed in an isolated operant conditioning chamber which was constructed of Plexiglas

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and stainless steel, with measurements of 20 cm. by 20 cm. by 22 cm. A 4-cm. by 2-cm. retractable lever was located 4 cm. above the grid floor on one wall of the chamber; a force of 0.2 Newtons was sufficient to depress the lever and constituted a response. Electrical and solid-state switching circuitry, located in an adjacent room, monitored the animal's response rate, recorded minute-by-minute response rates on a data tape, controlled the amount of time the lever was available, and controlled contingencies of reinforcement.

Reinforcements were pulses of electrical stimulation delivered to the animal's electrode site from a stimulator constructed from Digi-Bit solid-state logic circuitry, through an insulated and shielded cable, and commutator equipment which allowed the animal unrestricted movement. Each depression of a lever activated this circuit. The Digi-Bit solid-state logic circuitry provided a continuous stream of pulses, the contingencies of which could be manipulated by the experimenter. One multivibrator (MV262; BRS/LVE), three one-shot timers (OS204; BRS/LVE), one or-gate (OG205; BRS/LVE) and one inverter (IN202; BRS/LVE) received positively-going pulses generated by a -12 VDC power supply (PS007; BRS/LVE). These components allowed the experimenter to independently manipulate the C-C interval, the C-T interval, the C pulse duration, the T pulse duration, and whether the T pulse was placed into the circuit or not. Independent of this circuitry was logic which determined whether or not the animal would be placed into the current stream. When the animal pressed the lever, a make-before-break relay (RY205; BRS/LVE) was activiated, and the animal began to receive stimulation. Simul-

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taneously, a one-shot timer which determined the length of the stimulation train was triggered. When the train duration ended, the make-before-break relay was deactivated and the animal was removed from the stimulation circuit until it depressed the lever again. Any responses made once a stimulation train had been initiated, but prior to its termination, did not affect contingencies of reinforcement. The amplitude of the monophasic pulses was controlled by a variable 50,000-ohm potentiometer (Clarostat) in series with a one-microfarad capacitor and a 100-ohm precision resistor to regulate the current intensity between a range of 0 to 1200 microamperes. Stimulation entered the cathodal pole and left the brain via the anodal pole or an indifferent. A 68,000-ohm precision resistor allowed ions to leak back slowly during inter-pulse intervals to prevent hyper-polarization. Current travelled out of the brain through a 100-ohm return resistor. Wave form, C-C interval, C-T interval, pulse and train durations, and stimulus intensity were continuously monitored by observing the current drop across the 100-ohm precision resistor in series with the animal on a cathode ray oscilloscope (Hewlett-Packard 1200B). All parameters of stimulation were preset before the stimulation period by monitoring across the 100-ohm precision resistor on a cathode ray oscilloscope a 10,000-ohm precision resistor which substituted for the animal and then obtaining a ratio between the amplitude of that resistor and the actual resistance of the animal.

Procedure. After recovery from surgery (ten days), each animal was tested for ICSS in the apparatus described above. For

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each electrode site, each animal was shaped to lever-press on a continuous reinforcement schedule for a maximum of 15 daily sessions at a variety of current intensities, C-C intervals, C-T intervals, and train durations. Each pole was tested as a cathode, and both bipolar (the second pole acting as an anode) and monopolar (the cortical screw acting as the anode) stimulation was utilized. If, after 15 daily sessions, the rat did not self-stimulate, the second site was tested in the same manner. If the rat did not self-stimulate from either electrode site, it was eliminated from the study. If the animal self-stimulated from at least one electrode site, it entered the following paradigm.

For each electrode site, an animal self-stimulated for the following electrical stimulation parameters. The duration of the C and T pulses were set at 0.1 msec. and the C-T interval was set at 5 msec. The C-C interval was 10 msec., unless the animal exhibited motor artifacts which interferred with its lever-pressing, or if convulsions occurred. In such cases, the C-C interval was lengthened in order to eliminate these interferences. The train duration was set at 700 msec.; this value was selected because this was comparable to previous studies which used response rate as a measure (Deutsch, 1964; German & Holloway, 1973). The current intensity was varied in 50-microampere steps until the lowest intensity was found which would elicit peak responding from one of the poles of the bipolar electrode. When this intensity was set, the animal was tested for specificity of response rate in the following manner. This threshold intensity was monitored for any changes in animal impedence for

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each pole either within a testing session or between sessions; if this occurred, the entire paradigm was re-run. This was done in all experiments, except Experiment V where the lesion could reasonably account for impedence changes.

Over three days for each electrode site, each animal was tested for six 7-min. periods at the stimulus parameters described above. Data collected from the first two minutes of each 7-min. period was discarded to control for any carry-over effects from the previous period. Response rates over the last five minutes of the 7-min. period were averaged, recorded, and constituted the dependent variable. During three of the six 7-min. periods, the lateral pole of the bipolar electrode served as cathode; the medial pole of the bipolar electrode served as cathode during the other three periods. The order in which each pole served as cathode was alteranted in an a-b-b-a-a-b manner over the six periods each day and counterbalanced over the three-day testing session.

After determination of response rate from each pole of the bipolar electrode for the first site was made, the same procedure was repeated for the second electrode site to determine if it also supported ICSS. Data for each electrode site was analyzed separately; t-tests determined if there was a significant difference between the response rates elicited from the medial and lateral poles when each served as the cathode.

If an animal self-stimulated from both electrode sites, it continued into Experiment II. Selected animals which self-stimulated from only one electrode site continued into Experiment II; the others were sacrificed in the following histological procedure.

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Histology. After completion of the experiment, each rat was injected with an overdose of Chloropent and perfused with 0.9% normal saline solution, followed by 10% Formalin solution. After the perfusion, the brain was removed and blocked around the electrode tracks. The block was mounted on a stand and frozen; 40-micron slices of the track were collected by cutting the sections with a sliding microtome (Spencer 860). Sections were mounted on glass slides and stained with luxol fast blue for fibers, and cresyl violet for cell bodies, by the method described by Kluver and Barrera (1953). Microscopic examination of each electrode site with precise localization of each pole of the bipolar electrode was done by comparing the stained sections with available rat atlases (Konig & Klippel, 1963; Craigie, 1963).

Electrode localization was performed in the following manner. All electrode calls were made by two raters, independent of one another. One rater knew the data of each animal, while the second rater was blind with respect to the data. The independent rating had a .98 correlation with one another; in cases where the raters were in slight disagreement, the call was remade by the rater who was blind to the data.

Results

Eighty-two (82) electrode placements in 46 animals were tested for ICSS responding, with each electrode tip serving as cathode. Thirty-seven (37) placements were located in the hypothalamus; 23 placements were in the locus coeruleus area; 12 placements were in the periaqueductal midbrain central gray area; three were in the substantia incra area; and seven were septal placements. Table 1 provides precise localizations of each tip of each electrode placement along with response rates elicited from each tip when it served as cathode source.

Response rates elicited from the lateral and medial tips were compared with one another by a t-test and the results were broken into one of three categories: a) the lateral tip elicited response rates significantly ($p \leq .05$) greater than response rates elicited by the medial tip; b) the lateral and medial tips elicited response rates which did not significantly ($p > .05$) differ from one another; and c) the medial tip elicited response rates significantly ($p \leq .05$) greater than response rates elicited by the lateral trip. In 42 of the 82 instances, response rates between the two tips were significantly (t-test $p \leq .05$) different when the two tips were located in neuroanatomically distinct areas; in 16 placements, no difference was found neuroanatomical location between the two tips of the bipolar electrode and in no case was there a significant difference in response rates between these two tips. For the remaining 66 electrode placements, the number of significant differences in response rate (t-test, $p \leq .05$) between the lateral and medial tips of the bipolar electrode were as follows: a) hypothalamic area: 16 of 33 placements; b) locus coeruleus area: 13 of 18 placements; c) periaqueductal midbrain central gray area: 10 of 12 placements; d) substantia nigra area: 3 of 3 placements; e) septal area: 1 of 1 placement. Table 2 summarizes these results by dividing each area into sub-sections which are neuroanatomically distinct. Table 1 provides the alpha level achieved for each comparison between tips. It is apparent that the hypothalamic placements do not

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differentiate as much as the locus coeruleus, periaqueductal midbrain central gray, substantia nigra, and septal placements. The following is an electrode tip analysis for each ICSS area.

Hypothalamic placements were compared in three ways: a) medial forebrain bundle-perifornical area; b) medial forebrain bundle-lateral to medial forebrain bundle, and c) perifornical area-dorso-medial hypothalamic area. The first comparison included all cases where the lateral tip of the bipolar electrode was impinging upon the medial forebrain bundle and the medial tip of the bipolar electrode was impinging either in the perifornical area or any area dorso-medial or ventro-medial to the medial forebrain bundle. In Table 2, the A-1 section summarizes these results; in seven of 14 animals, the tip in the medial forebrain bundle exhibits response rates which are significantly greater than the tip in the perifornical area. In the remaining seven animals, electrode tips in the medial forebrain bundle elicited higher rates than tips in the perifornical area in five of the seven instances. Perifornical ICSS rates were never significantly greater than medial forebrain ICSS rates in any animal tested. The second comparison included all cases where the medial tip of the bipolar electrode was impinging upon the medial forebrain bundle and the lateral tip was impinging on an area dorso-lateral, lateral, or ventro-lateral to the medial forebrain bundle. In Table 2, the A-2 section summarizes these results; in six of 11 animals, the tip in the medial forebrain bundle exhibits response rates which are significantly greater than the tip

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lateral to it, whereas in no case is the reverse true. The third comparison included all cases where the lateral tip was impinging upon the perifornical area and the medial tip was impinging upon the medial hypothalamic area. In Table 2, the A-3 section summarizes these results; in three of the eight animals, the tip in the perifornical area exhibits response rates which are significantly greater than tips either dorsal to the perifornical area or medial to it. In the remaining animals, four of five animals produced higher response rates when stimulating from the perifornical area than when stimulating from an area dorsal or medial to it. In no case did dorsal or medial placements elicit significantly higher response rates than perifornical placements. Figure 1 provides a schematic representation of these findings where the medial forebrain bundle yields the highest responding, followed by areas bordering it, such as the perifornical area and crus cerebri, followed by areas bordering medially to the perifornical area.

Locus coeruleus placements were compared in two ways: locus coeruleus-medial to locus coeruleus area, and locus coeruleus-lateral to locus coeruleus area. The first comparison included all cases where the lateral tip of the bipolar electrode was impinging upon <u>or</u> was closer to the locus coeruleus/dorsal noradrenergic bundle, while the medial tip of the bipolar electrode was impinging upon an area medial to the locus coeruleus and dorsal noradrenergic bundle. Section B-1 of Table 2 summarizes these results; in nine of 13 animals, the tip either impinging upon or closer to the locus coeruleus elicits response rates which are significantly greater

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than the tip medial to it. Of the remaining four animals, three had higher response rates for the tip in the locus coeruleus than for the tip medial to the locus coeruleus. No animals in this study elicited significantly higher rates for stimulation delivered to areas medial to the locus coeruleus as compared to the locus coeruleus itself. In three cases, the tip impinging upon or closer to the locus coeruleus elicited ICSS responding, while the tip medial to it did not elicit any ICSS responding at any set of parameters. In two of these cases, the animal was tested in the monopolar condition, that is, the indifferent was a cortical screw. When each of these animals was tested for ICSS behavior under the bipolar condition, it failed to exhibit ICSS behavior from either tip. In both of these cases, the placement was not impinging upon the locus coeruleus but close to it. Therefore, either bipolar stimulation with its small stimulating field or monopolar stimulation at a tip more distant from the locus coeruleus could not elicit ICSS behavior, whereas monopolar stimulation with a proximal electrode tip to the locus coeruleus does elicit the behavior. The second comparison included all cases where the medial tip of the bipolar electrode was impinging upon or was closer to the locus coeruleus and dorsal noradrenergic bundle, while the lateral tip of the bipolar electrode was impinging upon an area lateral to the locus coeruleus and dorsal noradrenergic bundle. Section B-2 of Table 2 summarizes these results; in three of five animals, the tip either impinging upon or closer to the locus coeruleus elicits response rates which are significantly greater than the tip lateral to it. In one instance, the

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reverse was true; in this case, both tips impinged upon the locus coeruleus, with the lateral tip also impinging upon the brachium conjunctivum. In one case where ICSS rates were significantly greater for the medial tip closer to the locus coeruleus, the lateral tip did not sustain ICSS behavior. Figure 2 provides a schematic representation of these findings where tips either impinging upon or closer to the locus coeruleus elicit higher responding than tips in areas lateral or medial to locus coeruleus placements.

Periaqueductal midbrain central gray placements were compared as to whether one tip was impinging upon midline structures while the other tip was lateral to midline structures. Section C of Table 2 summarizes these results; in ten of 12 animals, the tip impinging upon midline central gray structures elicited response rates which were significantly greater that the tip lateral to it. In all instances, the electrode tip impinging upon midline central gray structures yielded higher response rates than the tip lateral to it. In three cases, the tip impinging upon midline central gray structures elicited ICSS responding while the tip lateral to it did not elicit ICSS responding for any set of parameters. Figure 3 provides a schematic representation of these findings where tips impinging upon midline central gray structures elicit higher responding than tips located in areas lateral to midline.

Substantia nigra placements were compared as to their impinging upon or proximity to the pars compacta of the substantia nigra. Section D of Table 2 summarizes these results; in all three cases, electrode tips located in or close to the pars compacta of

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the substantia nigra elicited response rates which were significantly greater than response rates elicited by electrode tips ventral or medial to the pars compacta. Figure 3 provides a schematic representation of these findings where tips impinging upon or closer to the pars compacta elicit higher responding than tips ventral or medial to it.

Septal placements were compared as to whether the tips were located in the lateral versus the medial septum. In the only case, the medial septum elicited response rates significantly greater than those elicited by the lateral septum. In the remaining animals, no further distinction could be made about site specificity.

Discussion

The results in Experiment I correlate in large part with existing literature in the field. However, it should be stressed at the outset that this was the first systematic attempt to map ICSS sites using two poles of a bipolar electrode to test two specific populations of neurons for ICSS. With this technique, four criticisms can be overcome. First, current spread cannot be used as a criticism. Since the electrode tips and hence cathodal sources are .3 mm. apart, any difference between the two tips should necessarily be attributed to activity beneath the tip since each tip has an equipotent chance of spreading current to equivalent-sized areas. Under a current-spread hypothesis, electrode tips in such proximity should exhibit similar response rates, whereas in fact, 75% of the placements tested elicited significantly different response rates

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from their electrode tips.

A second criticism levelled at previous mapping studies was the use of response rate as a measure, where ceiling effects in some animals might confound the results. Most mapping studies (Olds & Olds, 1962; German & Bowden, 1974 - reviews) in the past have used a separate-groups design, that is, only one electrode site was tested in each animal. Therefore, if particular animals had response limitations endemic to themselves, then response rate would not be affected by locus of stimulation, but rather by some peculiarity of the animal. In this study, comparisons are made within a particular electrode locus within a particular animal. Thus, any between animal differences are conrolled.

A third possible criticism is differences in tissue conductivity under each electrode tip, as measured by differences in resistance level. As described in the method, the amplitude of the current intensity delivered to the animal is pre-set by comparing the animal's resistance level to a 10,000-ohm resistor. This is done for each electrode tip. Therefore, for each tip there is a ratio established between the electrode tip and the resistor. Large differences in resistance between electrode tips would be mirrored in large differences in the ratios established across the resistor. In virtually every case, there was no difference between the resistance levels of the two tips, even though there may have been a significant difference in response rate. In one case (rat 30H, locus coeruleus(Table 1), the respective resistances between the two tips were drastically different; this animal was not included in the ana-

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lysis. Thus, possible differences in tissue conductivity under each electrode tip was not a factor which affected the outcome of the experiment.

The fourth possible criticism is that differences in response rate are caused by tissue damage. Again, since this is a within-animal design and in particular, a within-locus design, the possibilities of large amounts of tissue damage under only one tip of the bipolar electrode is at chance level. Furthermore, histological verification of electrode tips did not reveal any noticeable pattern in tissue damage which could explain the results. In most animals, very little tissue damage was noted, and when it occurred, the damage was under both tips of the bipolar electrode. It is apparent that the differentiation in response rate was a result of electrode locus placement and not because of some uncontrolled non-specific effect.

In 59 of 80 placements tested, response rates elicited under one tip serving as cathode was significantly greater than response rates elicited by the other tip serving as cathode located .3 mm. away. This fact suggests very strongly that the areas supporting the ICSS phenomena are very discrete, and small changes in electrode placement produce significant changes in ICSS behavior.

Hypothalamic ICSS, particularly medial forebrain bundle ICSS, has been most widely studied by researchers (see review, German & Bowden, 1974). In most studies, hypothalamic ICSS has been thought of as uniform. However, recent lesion (Farber et al., in preparation) and pharmacological (Stephens & Herberg, 1975) studies have

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demonstrated different influences on various aspects of hypothalamic ICSS. This study provides definite proof that hypothalamic ICSS can be differentiated by response rate. As noted previously in other studies (see review, German & Bowden, 1974), the medial forebrain bundle elicits the highest level of responding. This area is followed by areas immediately bordering it, such as the perifornical area and crus cerebri/internal capsule. The medial aspects of the hypothalamus, including the dorso-medial, ventro-medial, periventricular, and anterior nuclei of the hypothalamus, elicit the lowest levels of responding when directly compared to other hypothalamic areas. Since the hypothalamus has been extensively mapped, comparisons of the present and previous studies' results are a means of validating this particular procedure. German and Bowden (1974) reviewed over 500 hypothalamic placements described in over 30 separate studies and reported the same response differentiations as noted in the present study. It is apparent that the results of this procedure are in accordance with the large amount of literature reported on hypothalamic ICSS. Therefore, this procedure should be an accurate indicator of differentiations in responding for brain loci where controversy exists as to the source of ICSS from that area. This is apparent for dorsal pontine and periaqueductal midbrain placements.

This study provides direct evidence for involvement of the locus coeruleus and dorsal noradrenergic bundle in ICSS behavior. Several laboratories (Farber et al., 1972; Crow et al., 1972; Ritter & Stein, 1973; Ellman et al., 1974, 1975; Phillips et al., 1975)

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report that ICSS behavior can be elicited from locus coeruleus and dorsal noradrenergic bundle electrode sites. On the other hand, four studies (Routtenberg & Malsbury, 1969; Clavier & Routtenberg, 1974; Amaral & Routtenberg, 1975; Simon et al., 1975) report that no ICSS behavior could be elicited from locus coeruleus cell bodies. In this study, 21 pontine placements supported ICSS behavior; in all cases, one or both of the electrode tips was located in the region of the locus coeruleus. In 12 of 18 cases, electrode tips impinging upon or closer to the locus coeruleus elicited significantly greater response rates than electrode tips either medial or lateral to the locus coeruleus. In four cases, the tips located too far medial or too far lateral from the locus coeruleus did not elicit any ICSS behavior at all. It is apparent from this study that ICSS is elicited from only a discrete dorsal pontine area, and that this area is exclusively the locus coeruleus.

Periaqueductal placements exhibited the same degree of response specificty with respect to electrode tip location. In ten of 12 placements, electrode tips situated in the midline central gray elicited response rates which were significantly greater than rates elicited by electrode tips situated lateral to the midline. This data correlates well with reports of both ICSS and non-ICSS behavior reported previously (Crow, 1972; Liebman et al., 1973; Ellman et al., 1974). Midline placements supporting ICSS behavior were the oculomotor/Edinger-Westphal nuclei, the medial longitudinal fasciculus, the nucleus linearis, and the dorsal and ventral tegmental decussations. Most of these sites correlate with mesencephalic area A-10 (Dahlstrom & Fuxe, 1964), and are considered to be

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dopaminergic (Ungerstedt, 1971). In three cases, areas lateral to midline failed to support ICSS, while the midline placement did. In these three cases and in a fourth case for both tips, the electrode tip was located in the red nucleus.

Substantia nigra and septal placements also demonstrate differentiation between electrode tips in close correlation with previous studies (Crow, 1972). Substantia nigra, pars compacta placements elicit higher rates than surrounding placements; this agrees with other mapping studies (Routtenberg & Malsbury, 1969; Crow, 1972). Medial septal placements both differ within the placement and elicit greater responding than lateral placements.

The results of the present study provides data by which one can analyze competing hypotheses. Routtenberg (1968; Routtenberg & Malsbury, 1969) suggest that ICSS in the hindbrain is mediated by the extrapyramidal system. Therefore, he postulated that the locus of ICSS in the dorsal pons and posterior midbrain was the mesencephalic trigeminal nerve and the brachium conjunctivum, while mesencephalic ventral tegmental ICSS is mediated by the substantia nigra and red nucleus. Routtenberg (Clavier & Routtenberg, 1974) specifically discounted the role of the locus coeruleus and its ascending projections in ICSS. Contrary to this hypothesis, several investigators (Ellman et al., 1974, 1975; Ritter & Stein, 1973; Crow et al., 1972) assigned a pivotal role for the locus coeruleus and its ascending projections in ICSS behavior. The results of this study strongly suggest that Routtenberg's extrapyramidal hypothesis cannot account for hindbrain ICSS. The locus coeruleus and dorsal noradre-

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nergic bundle consistently supported higher rates of ICSS through one tip of a bipolar electrode when compared with areas adjacent to, but outside of it. Perhaps, the most convincing point is the consistent absence of ICSS behavior in the red nucleus; indeed, the area when stimulated elicited clear aversive properties. The red nucleus, according to Routtenberg's hypothesis, should occupy the nodal point of an extrapyramidal ICSS system; however, it clearly does not. The results of these experiments clearly support a pivotal role for the locus coeruleus and its projections, as well as mesencephalic ventral tegmental areas which highly correlate with the dopaminergic cell bodies of Dahlstrom and Fuxe's area A-10.

This technique clearly differentiates locus of responding, and should be used in future mapping studies.

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Experiment II. Determination of Within-Site C-T Interval/Response Rate Functions

Rationale

As described in the background section, Deutsch (1964) and others (Gallistel, 1969, a,b; Smith & Coons, 1970; German & Holloway, 1972, 1973; Ungerleider & Coons, 1970; Szabo et al., 1974), using a variety of response measures, found that responding at short C-T intervals (0.3-1.2 msec.) was similar to responding in situations when C pulses were delivered alone. As C-T interval was increased (0.8-1.5 msec.), the response measure for those intervals approached response levels similar to when the C-T interval was 5 msec. or greater. Deutsch (1964) attributed this increase in responding to a recovery from refractoriness: at short C-T intervals, responding was similar to that of C pulses alone because the T pulse arrived within the refractory period caused by the C pulse. As C-T interval increased, the T pulse was no longer within the refractory period caused by the C pulse and the neurons were fired twice as often, causing increased summation post-synaptically, and hence increased behavior. Szabo et al. (1974) attributed this change in responding to synaptic events occurring on the peripheral field of stimulation. Szabo described hypothalamic ICSS as a result of summating potentials upon both fibers of passage and upon path neurons (Millhouse, 1969) intrinsic to the hypothalamic area. In Szabo's hypothesis, the C pulse fired neurons in the center of the stimulating field, but did not fire neurons in the periphery. Rather, the C

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pulse activated neurons which caused excitatory post-synaptic potentials (EPSP) on the peripheral neurons. The delivery of the T pulse enabled more EPSP's to occur on the periphery and these in turn were fired, causing an increase in behavior. The closer the C-T interval, the more optimal the summation EPSP's will be. Szabo's hypothesis, however, fails to explain why increased behaviors do not occur at very short C-T intervals.

The thrust of C-T studies done within a site has been to manipulate other behaviors (Gallistel, 1969(a),(b); Gallistel et al., 1969; Rolls, 1971(a),(b). Only two studies have attempted to use the within-site C-T function in order to determine if differences exist between ICSS loci. Halboth (1973) and German and Holloway (1972) have reported differences in the C-T interval/response rate function across sites. Halboth found that, at very long C-T intervals, medial forebrain bundle electrode sites capable of eliciting stimulus-bound eating behavior do not show a decay in responding. On the other hand, hypothalamic sites incapable of sustaining stimulus-bound eating behavior do show a decay in responding at long C-T intervals. German and Holloway (1972) found that C-T interval/response rate functions for preoptic hypothalamic sites had lower thresholds than lateral hypothalamic sites. Thus, C-T interval and response rate is a way of discerning differences among ICSS sites.

In Experiment II, C-T interval/response rate functions for the locus coeruleus, periaqueductal midbrain central gray, substantia nigra, hypothalamus, and septum were obtained. The data for the periaqueductal midbrain central gray area and for the substantia

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nigra were combined for the following reasons: a) pharmacological studies have indicated that both areas respond similarly under a variety of drug conditions (Phillips & Fibiger, 1973; Ellman et al.,1976; Liebman & Butcher, 1973; Lippa et al., 1973) and have both been classified as dopaminergic (Ungerstedt, 1971); and b) on the basis of Experiment I, ICSS behavior was limited to these areas and no others tested in the ventral mesencephalon. On the other hand, hypothalamic areas will be divided into two areas: medial forebrain bundle/perifornical area, and those hypothalamic sites outside the medial forebrain bundle and perifornical area. This was done because the results of Experiment I and the results of previous studies, both mapping (see review, German & Bowden, 1974) and pharmacological (Stephens & Herberg, 1975), indicate differences between these two categories.

The functions of these five areas were analyzed to determine if there were differences in the shape of the function between and within neuroanatomical loci.

Method

Subjects and apparatus. Forty-six (46) selected subjects described in Experiment I were used in Experiment II. The apparatus used in Experiment II was identical to Experiment I.

Procedure. After completion of Experiment I, electrode sites in each animal which elicited ICSS behavior were tested under the following stimulus parameters. Stimulation consisted of .1-msec. pulses of monophasic square wave current delivered during a 700-msec.

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train. The pole of the bipolar electrode which elicited the higher response rate in Experiment I served as cathode and either the other pole or the same bipolar electrode (bipolar condition) or the cortical screw (monopolar condition) served as the anode. The C-C interval was lengthened from 10 msec. to 20, 30, or 50 mesc. in order to prevent summation between succeeding C pulses, yet still elicit ICSS behavior. An intensity was chosen which yielded optimal response rates at a C-T interval of 5 msec., yet yielded rates below ten responses per minute when the T pulse was omitted. When these parameters were determined, the animal entered the following paradigm.

Nine 7-min. periods of stimulation were run daily. As in Experiment I, the data from the first two minutes of the 7-min. period was discarded and the average response rate over the last five minutes of the 7-min. period constituted the dependent variable. Eight C-T intervals, 0.5, 0.8, 1.0, 1.2, 1.5, 2.0, 3.0, and 5.0 msec. and a condition where the T pulse was omitted were randomly presented in each of nine days in a Latin Square design. The mean response rate elicited under each C-T interval was determined. With animals with two ICSS sites, the second site was tested in the same manner.

For each function in each animal, the response rate data was transformed into a Z-score distribution and the response rate which corresponded to a Z-score of 0.0 was determined. The corresponding C-T interval was determined by plotting the response rate/C-T interval function on a log-log plot and estimating the C-T interval from the 0.0 Z-score response rate. This estimated C-T interval was de-

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signated as the point of inflection and measured in milliseconds. This point of inflection served as a single-value estimate of the area of the function in which response rates began to increase as a function of C-T interval. A second single-value estimate of response rate/C-T interval functions was the product-moment r-score between response rate and C-T interval. In this estimate, both the response rate data and the C-T intervals were respectively transformed into Z-score distributions. A Pearson's product-moment correlation was performed between the two Z-score distributions and a single value, the r-score was obtained, this r-score was a valid estimate of the slope of the function because when the two variables of a function are transformed into Z-score distributions, the point of intercept automatically equals zero. Thus, any correlative measure between the two variables of the function indicates the slope and singly represents the acceleration of the curve. For each site, point of inflection, r-score and microamperage was determined. To compare differences between different sites, an analysis of variance was done using the microamperage, point of inflection, and slope as scores.

After completion of Experiment II, all animals with two ICSS electrode sites were continued into Experiment III; selected animals with only one ICSS electrode site were also continued into Experiment III. All others were sacrificed and histologically prepared, as described in Experiment I.

Results

Forty-six (46) animals completed Experiment II; 109 within-

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site functions were collected over 83 electrode sites. In 17 electrode sites, the same within-site function was tested under both the bipolar and monopolar conditions. In nine electrode sites, the same within-site function was tested with either the lateral and medial tips of the same bipolar electrode. Fifty-six (56) within-site functions were collected over 36 hypothalamic electrode sites; 27 within-site functions were collected over 23 locus coeruleus electrode sites; 19 within-site functions were collected over 14 periaqueductal midbrain central gray/substantia nigra electrode sites; and seven within-site functions were collected over seven septum electrode sites. Table 1 lists the precise electrode localization of each site, while Table 3 lists the mean of the microamperages, points of inflection, and r-scores for each site grouping.

Electrode sites were divided into five groups: a) hypothalamic medial forebrain bundle/perifornical area; b) hypothalamic areas outside the medial forebrain bundle/perifornical area; c) periaqueductal midbrain central gray/substantia nigra; d) locus coeruleus; and e) septum. There was no significant difference (F test $p > .05$) over sites for the minimum level of microamperage used to produce a function, that is, to elicit near optimal response rates for delivery of C and T pulses five milliseconds apart while eliciting threshold (less than ten responses per minute) response rates for delivery of C pulses with T pulses omitted. There was a significant difference across sites for the point of inflection of a given within-site function (F test, $p \leq .05$).

The septum C-T function yielded a significantly later (Duncan,

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$p \leq .05$) point of inflection (1.30 msec.) than the other four sites combined (Table 3). The locus coeruleus C-T function yielded a significantly later (Duncan, $p \leq .05$) point of inflection (1.15 msec.) than the remaining three sites combined. The septal point of inflection (1.30 msec.) and locus coeruleus point of inflection (1.15 msec.) were not significantly (Duncan, $p > .05$) different from one another; however, there was a definite trend in the direction of difference between the two sites. There were no significant differences (Duncan, $p > .05$) in point of inflection between the periaqueductal midbrain central gray/substantia nigra group (1.02 msec.), or the medial forebrain bundle/perifornical area group (1.13 msec.) and the hypothalamic areas outside of the medial forebrain bundle/perifornical area (1.03 msec.).

Corresponding with point of inflection, a significant difference was found (F test, $p \leq .05$) across sites for r-scores which serve as an indicator of slope. However, in using post-hoc comparisons, no significant comparisons (Duncan, $p > .1$) was found across sites. Complete correspondence between point of inflection and r-scores were found across sites. The periaqueductal midbrain central gary electrode sites had the lowest r-score of .7380. This indicates that among all of the sites with C-T intervals held constant, the periaqueductal midbrain central gray/substantia nigra electrode sites had the lowest correlation between increases in response rate corresponding with increases in C-T interval. Since this site also had the shortest response activation as measured by point of inflection (1.02 msec.), both measures give an indication of typical periaqueductal midbrain central gray/substantia nigra ICSS, which involves a short C-T interval (.5-.8

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msec.) response activation for ICSS, followed by attainment of peak rates, and then as asymptote in responding. Figure 4 shows four representative within-site functions for animals stimulating from periaqueductal midbrain central gray/substantia nigra electrode sites. As can be seen, response rates are at low levels for stimulation with C pulses alone. In all functions, response rates a) rise quickly as the C-T interval is increased, b) achieve near-peak response rates usually around a C-T interval of 1.5 msec. (t-test, $p \leq .05$) asymptote after approaching peak responding (t-test, $p > .05$). A shorter point of inflection score is attained in these sites because of the rapid rise in response rates over short C-T intervals; response rates at .5 msec. were significantly (t-test, $p \leq .01$) greater than response rates elicited by the C pulse alone. The lower r-scores are attained because of the asymptotic response rate function after reaching peak.

The medial forebrain bundle/periofrnical area group and the non-medial forebrain bundle/perifornical area group had the next lowest r-scores (.7943 and .7235 respectively) when compared with other loci (Table 3). Correspondingly, the points of inflection for the two groups (1.13 msec. and 1.03 msec. respectively) also occupied the same rank position as r-scores. No significant (Duncan, $p > .1$) difference was found between the two hypothalamic groups in comparisons of point of inflection and r-score. Figure 5 represents four within-site functions for animals stimulating from hypothalamic electrode sites; Figures 5B and 5D are functions produced by medial forebrain bundle/perifornical area placements, while Figures 5A and 5C are functions produced by hypothalamic placements outside of those areas. Hypothalamic placements showed more variability in the shape of the C-T function

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than did periaqueductal midbrain central gray/substantia nigra sites. Figure 5A, a non-medial forebrain bundle placement, resembles the periaqueductal midbrain central gray/substantia nigra functions where a) the response rates rise quickly at short C-T intervals, b) reach peak responsing at a C-T interval around 1.5 msec. (t-test, $p \leq .05$), and then c) response rate is asymptotic out to C-T intervals of 5 msec. (t-test, $p > .05$). Figures 5B and 5C, a medial forebrain bundle and non-medial forebrain bundle placement respectively, represent hypothalamic within-site functions where response rates rise quickly at short C-T intervals, but do not reach peak responding until longer C-T intervals. In Figure 5D, a medial forebrain bundle placement, the representative function neither rises quickly at short C-T intervals nor reaches peak with asymptotic response rates before C-T intervals of 5 msec. Thus, in contrast to periaqueductal midbrain central gray/substantia nigra electrode sites, hypothalamic placements are more variable in point of inflection and r-score.

Overall locus coeruleus placements had a significantly (Duncan, $p \leq .05$) later point of inflection (1.15 msec.) and higher r-score (.8150) than hypothalamic and periaqueductal midbrain central gary/substantia nigra placements (Table 3). Figure 6 represents four within-site functions for animals stimulating from locus coeruleus electrode sites. As in hypothalamic within-site functions, locus coeruleus functions vary in shape and acceleration of the function. Figures 6A and 6B show rises at short C- intervals, peak responding before the 5-msec. C-T interval, and asymptotic responding at longer intervals, while Figures 6C and 6D demonstrate a high correlation between increases in response rate con-

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commitant with increases in C-T interval.

The seven septal placements had the significantly (Duncan, $p \leq .05$) latest point of inflection (1.30 msec.) and the highest r-score (.8590) of all sites (Table 3). Figure 7 represents four within-site functions for animals stimulating from septal electrode sites. Septal animals have the highest correspondence between changes in C-T interval and concommitant changes in response rate. Small changes at the shorter C-T intervals elicit small changes in response rate (Figures 7A, 7B, 7C), while larger changes in C-T interval at longer C-T intervals elicit large changes in response rate. This is particularly striking at the C-T interval of 5 msec., the longest interval tested. In all animals, responding at 5 msec. was higher than at any other and as seen particularly in Figures 7C and 7D, responding increased markedly at this interval. This surge in responding corresponded with the C-T intervals tested (i.e., the 5-msec. interval was much higher than the others) and also influenced the point of inflection. The septal placements apparently generated functions most closely resembling linearity, indicating a high correlation between C-T interval tested and resultant response rate.

In addition to attaining comparisons of within-site functions across sites, the relative influence of anodal source of stimulation was measured. In 17 animals, 34 within-site functions were collected. Seventeen (17) functions in 17 animals were tested under the bipolar condition, that is, one pole of the bipolar electrode served as cathodal source, while the other pole of the bipolar

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electrode served as anodal source. Subsequently, 17 more functions in the same animals at the same C-C intervals were tested under the monoplar condition, that is, one pole of the bipolar electrode served as cathodal source while a cortical screw served as the indifferent anodal source. For each pair of within-site functions, differences between the bipolar condition and the monopolar condition were determined for a) the minimum microamperage to produce the function, b) the point of inflection, and c) the product-moment r-score. Table 4 indicates the results of each of these measures by site location.

There was a significant difference (t-test, $p \leq .05$) in minimum microamperage needed to produce a within-site function which would differentiate between C pulses alone which elicited low ICSS rates, and a C-T interval of 5 msec. which elicited peak ICSS rates. Moreover, there was no significant (t-test, $p > .1$) difference between the bipolar and monopolar conditions with respect to response rate. The monoplar condition, regardless of electrode site, had a significantly lower microamperage to produce a within-site function than the bipolar condition (Table 4). No significant differences (t-tests, $p > .05$) were found in point of inflection and r-scores between the monoplar and bipolar conditions when all sites were analyzed collectively.

However, as can be seen in Table 4, differences in reaction to the bipolar and monopolar condition for point of inflection and r-score were noted across sites. Twelve animals of the 17 tested had hypothalamic placements, while the other five placements were

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either in the locus coeruleus or periaqueductal midbrain central gray. Hypothalamic placements when tested in the monopolar condition had significantly (t-test $p \leq .05$) shorter points of inflection than when those same placements were tested under the bipolar condition. Correspondingly, hypothalamic placements when tested in the monopolar condition had significantly (t-test, $p \leq .05$) lower r-scores than when those same placements were tested under the bipolar condition. Thus for hypothalamic placements, the monopolar condition a) elicits the mean response rate over the entire within-site function at an earlier C-T interval than the bipolar condition, b) reaches peak responding at an earlier C-T interval than the bipolar condition, and c) is not as closely correlated with changes in C-T interval as the bipolar condition.

Both hindbrain placements (locus coeruleus and periaqueductal midbrain central gray) yielded opposite results to the hypothalamic placements for the bipolar and monopolar conditions as measured by point of inflection and r-score. In contrast to hypothalamic functions, hindbrain placements when tested in the monopolar condition has later points of inflection than when those same placements were tested under the bipolar condition. Similarly, hindbrain placements when tested in the monopolar condition had higher r-scores than when those same placements were tested under the bipolar condition. In both cases, although the difference was not significant (t-test, $p > .05$), the trend was definitely in the opposite direction to the hypothalamic placements and significance level was highly affected by the small number of subjects. Thus, for hindbrain placements,

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the monopolar conditon a) elicits the mean response rate over the entire within-site function at a later C-T interval than the bipolar condition, b) reaches peak responding at a later C-T interval than the bipolar condition, and c) is more closely correlated with changes in C-T interval as compared to the bipolar condition.

Finally, all 109 functions collected demonstrated differentiation in responding. In all cases, animals yielded low response rates for the C pulse alone condition as compared to the rates elicited by the pulse pair C-T interval of 5 msec. Furthermore, in most cases, animals yielded low response rates for short C-T intervals (.5 and .8 msec.), and did not reach peak responding usually until 1.2 to 1.5 msec. This data is in accordance with the literature (Deutsch, 1964; Gallistel, 1969a; Smith & Coons, 1970; Ungerleider & Coons, 1970; German & Holloway, 1972; Szabo, 1973).

Discussion

When stimulation pulse pairs are delivered to one electrode site at short C-T intervals, responding resembles the C pulse alone condition rather than the stimulation pulse pair condition at longer C-T intervals. Deutsch (1964) and others (Gallistel, 1969a; German & Holloway, 1972; Yeomans, 1975) attribute this response level at short C-T intervals to refractoriness properties of the neurons stimulated, while Sazbo (1974) attributes this response level mainly to a lack of synaptic activation. It is apparent that this data is in accordance with previous studies which show low responding at short C-T intervals, which increases as the C-T interval increases.

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From this data, it seems that there are subtle differences in the type of function elicited which is dependent upon electrode locus. German and Holloway (1972) found that preoptic hypothalamic ICSS electrode sites had a longer recovery from refractoriness than did medial forebrain bundle hypothalamic electrode sites. The present study demonstrates that septal ICSS electrode sites had the highest point of inflection and the highest correlation of response rate to changes in the C-T interval. Small changes in short C-T intervals elicited small increases in response rate while as the C-T interval increased, the response rate increased accordingly. The C-T intervals tested in this study are in the range of absolute refactory period, relative refractory period, supra-normal period, and normal resting state (Yeomans, 1975). Septal electrode placements seem to correlate to a high degree with these delineations (Figure 7). As one can see, septal response rates at short C-T intervals (.5-.8) were similar to response rates elicited by the C pulse alone; seemingly, this part of the function corresponds with the absolute refractory period where a second pulse causes no change in behavior because it is refractory to the first pulse. As the C-T interval increases (.8-2 msec.), the septal response rate increases in small steps. This corresponds with the relative refractory period where the second pulse increases behaviors some of the time, specifically when enough excitatory post-synaptic potentials overcome the increase in summation threshold. It is obvious from the septal data that this increased threshold in summation does not dissipate quickly. Of all of the groups tested, only the

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septal animals failed to reach peak responding before 5.0 msec. In fact, the septal response rate in several animals at a C-T interval of 5 msec. demonstrated an abrupt rise as compared to shorter C-T intervals. It seems that by 5 msec., septal placements have recovered fully from the relative refractoriness caused by C pulses. The locus coeruleus group seems to produce functions similar to the septum.

In contrast, functions elicited from periaqueductal midbrain central gray/substantia nigra ICSS electrode sites had the lowest point of inflection and lowest correlation of response rate to changes in the C-T interval. A quick rise in responding was noted in most of these functions (Figure 4) with response rates approaching peak at C-T intervals of .5 to 1.0 msec. and then asymptoting for longer C-T intervals. It seems, therefore, that these electrode sites experience very fast recovery from refractoriness, or else, the behavior isn't measured at all. It is possible that the C pulse is stimulating one group of neurons in the central area, but not effectively stimulating the peripheral neurons. When the T pulse arrives, the central area is refractory to the C pulse, but the peripheral area is receptive to this pulse and fires. An implicit assumption is that this area is rich in reward-relevant neurons. The two hypothalamic groups seem to produce functions similar to the periaqueductal midbrain central gray/substantia nigra group.

Thus, differences in within-site functions across sites may be suggestive of the relative number of reward-relevent elements in particular ICSS loci. In ICSS loci largely dependent upon increments in

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response rates, the relative number of reward-relevant elements would be small (e.g., locus coeruleus and septum). This interpretation is supported by data presented in Experiment I in which small deviations in the placement from the locus coeruleus area resulted in either sharply attenuated or an absence of ICSS behavior. In ICSS loci relatively independent of C-T interval for response rate increments, the relative number of reward-relevant elements would be larger and more diverse. Hence, at shorter C-T intervals, response rates may increase because some elements recover from refractoriness occurs before others. Periaqueductal and hypothalamic sites possess diverse reward-relevant elements, if catecholaminergic hypotheses are assumed.

Differences between bipolar and monopolar stimulation were also found. Stimulation under the monopolar condition required significantly less microamperage than stimulation under the bipolar condition to produce a differentiated function. This is in accordance with the results of Valenstein and Beer (1961) and is attributed to two factors. The first factor is that monopolar stimulation produces a larger stimulating field, since when cathodal and anodal sources are distant from each other, the possible paths which the current could take becomes greater. The size of the field correlates with the distance between cathodal and anodal source; hence, bipolar stimulation reduces the number of possible paths which the current could take. The second factor is anodal hyperpolarization which increases the firing thresholds for the influenced neurons. Anodal hyperpolarization affects the bipolar condition more than the monopolar condition because the hyperpolarized neurons which do not fire under the bipolar condition could fire and increase ICSS behavior in the monopolar condition. In the bipolar con-

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dition, anodal hyperpolarization is a factor because the anode is the other tip of the same bipolar electrode as the cathodal source. Hence, the anode is .3 mm. away from the cathode. The cathodal stimulation may stimulate the tissue under the tip in any number of directions. However, since the anode is in close proximity to the cathodal source, some of the tissue which could be fired by the cathode could also be hyperpolarized by the anode. Hyperpolarization increases the threshold for summation; hence, tissue normally accessible to firing by the cathode without hyperpolarization is no longer accessible because of hyperpolarization by the other tip in the bipolar condition. Hyperpolarization is removed when monopolar stimulation is used because the cortical screw acts as anode and the cortical screw is usually at least one millimeter or more away from the cathode. Thus, in the monopolar condition, the cathodal and anodal fields do not overlap the same tissue.

For hypothalamic sites, stimulation under the monopolar condition elicited significantly lower points of inflection and lower correlations between response rate and C-T interval than stimulation under the bipolar condition. Conversely, for hindbrain sites, stimulation under the monopolar condition elicited higher points of inflection and higher correlations between response rate and C-T interval than stimulation under the bipolar condition. As reviewed in the introduction, hindbrain ICSS is limited to highly discrete areas, while ICSS can be elicited from virtually any part of the hypothalamic area. Monopolar stimulation produces a larger stimulating field than bipolar stimulation. Hence, hypothalamic stimulation under the monopolar condition should activate more ICSS fibers

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in the area than the bipolar condition. As discussed earlier, if more reward elements are activated as monopolar stimulation does, the response rate will rise more quickly at short C-T intervals, reach peak responding at an earlier C-T interval, and then asymptote for the longer C-T intervals. From this model, it would be predicted that hypothalamic functions should activate quicker under monopolar stimulation.

The same model explains the opposite results found in the hindbrain. Monopolar stimulation elicited higher points of inflection and higher correlates between response rate and C-T interval than bipolar stimulation in hindbrain sites because these sites have concentrated areas of ICSS-sustaining neurons. In the bipolar condition, stimulation of these areas triggered a large number of these neurons, while in the monopolar condition, these neurons plus adjacent structures were stimulated. In these cases, the adjacent structures, such as the red nucleus or the mesencephalic V nuclei could elicit aversive responses. Hence, at the shorter C-T intervals where optimal triggering of the system has not taken place, that is, where the pulse pair did not fire the stimulated area twice, the aversive component could exert influence over response rate. This influence diminishes with intervals where the pulse pair fires the stimulated area twice. Since the monopolar condition stimulated these areas, the response rate is lower at these points. Thus, under the monopolar condition, the function rises slower and is more sensitive to changes in C-T interval than under the bipolar condition.

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Thus, within-site functions yield reliable differences across sites and yields differential effects as to the type of stimulation (monopolar vs. bipolar) condition used. Therefore, the ICSS system does not react uniformly to stimulation; rather, important differences occur depending upon where in the system one stimulates. This data provides further evidence that the stimulation delivered is discrete and confined to a small area as compared to a hypothesis which would suggest that no matter where the stimulation is delivered, the same result would occur because the stimulation locus is only an indirect source of reward.

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Experiment III. Directionality of Interactions Determined by C-T Interval/Response Rate Functions

Rationale

Neurophysiological interactions have been demonstrated for locus coeruleus/hypothalamus, substantia nigra/hypothalamus, periaqueductal midbrain central gray/hypothalamus, hypothalamus/contralateral hypothalamus, amygdala/hypothalamus, and substantia nigra/septum (Ackermann et al., in preparation,a; Ellman et al., 1975; Szabo et al., 1972a; Albino & Lucas, 1962; Ungerleider & Coons, 1970; German & Holloway, 1973). In interactions involving either the locus coeruleus, substantia nigra, or periaqueductal midbrain central gray with the hypothalamus, there is ample evidence to suggest that these cell bodies possess fibers which ascend through the hypothalamus through the dorso-medial nucleus, crus cerebri, Fields of Forel, periventricular area, and zona incerta (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971; Lindvall & Bjorklund, 1974). Data from Farber's (in preparation) lesion studies also suggest ascending influences from locus coreuleus to crus cerebri, but not to the medial forebrain bundle.

The C-T technique proved to be a useful tool in measuring interactions. Ungerleider and Coons (1970) obtained within-site C-T interval/response rate functions for two contralateral hypothalamic placements, then split the pulses between the two sites. This produced an interaction, as measured by increased responding during the interaction for C-T intervals which support minimal responding when delivered within a site. Moreover, if the hypothalamic sites are

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assymetrical, the C-T technique can differentiate directionality of the interaction by varying which site receives the C pulse (German & Holloway, 1973).

For the site combinations which have as its hypothalamic electrode placement the crus cerebri or Fields of Forel, and which have either the locus coeruleus, substantia nigra, or periaqueductal central gray as its caudal placement, an ascending directionality of interaction would be predicted assuming that the catecholaminergic hypothesis is indeed valid. If the C pulse was delivered to the locus coeruleus and the T pulse was delivered to the crus cerebri, a catecholaminergic hypothesis would predict that this condition should elicit a higher response rate than if the C pulse was delivered to the crus cerebri and the T pulse to the locus coeruleus. This prediction is made because the source of the interaction would probably lie rostral to both ICSS sites, so hence the locus coeruleus would be more distant from this pool. In order to achieve optimal summation (both pulses arriving at the same point at about the same time), the initial pulse would necessarily have to be delivered to the more distal site. From this reasoning, response rates should be higher when the C pulse is placed in the caudal site of a combination which has as its hypothalamic placement either the crus cerebri or Fields of Forel.

Due to the lack of strong ascending influences upon its ICSS response rates (Farber et al., in preparation), the medial forebrain bundle should not react in the same way as the crus cerebri or Fields of Forel. Therefore, response rates elicited when the C pulse is delivered to the locus coeruleus should not differ from the condition

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when the C pulse is delivered to the medial forebrain bundle. Both conditions of the locus coeruleus/medial forebrain bundle combination should produce an interaction, with little dependence upon which site is stimulated first.

Seven combinations of sites were tested in Experiment III for three findings: a) whether a neurophysiological interaction took place such that response rates in the interaction condition were significantly higher than the sum of response rates elicited by delivery of C pulses to each site singly; b) given that a neurophysiological interaction occurred, would elimination of within-site pulse pair function refractoriness occur in the interaction condition; and c) would there be differences in response rate dependent upon which site received the C pulse first. The seven combinations of electrode sites tested were: a) locus coeruleus-crus cerebri/Fields of Forel; b) locus coeruleus-medial forebrain bundle/perifornical area; c) substantia nigra-medial forebrain bundle/perifornical area; d) substantia nigra-_non_-medial forebrain bundle; e) periaqueductal midbrain central gray-medial forebrain bundle/perifornical area; f) periaqueductal midbrain central gray-_non_-medial forebrain bundle; and g) locus coeruleus-septum.

Method

Subjects and apparatus. Thirty-six (36) selected subjects from Experiment II were used. The apparatus used in Experiment III is as described in Experiment I.

Procedure. After completion of Experiment II, each animal

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was tested under the following parameters. The C-T interval/response rate function parameters for each electrode site was held constant with the C-C interval set at 30 msec. The C and T pulses were then split between the two electrode sites with the C-C interval set at 30 msec. and delivered at the sites' respective intensities. In one condition, in an animal with a locus coeruleus/hypothalamus combination, the C pulse was delivered to the locus coeruleus at its within-site intensity, and the T pulse was delivered to the hypothalamus at its within-site intensity. In the reverse condition, the C pulse was delivered to the hypothalamus at its within-site intensity and the T pulse was delivered to the locus coeruleus at its within-site intensity. Nine days of C-locus coeruleus, T-hypothalamus and nine days of C-hypothalamus, T-locus coeruleus, alternating in an a-b-b-a manner were randomly tested over the nine C-T intervals each day. To compare differences between the two conditions over different C-T intervals, a two-way analysis of variance was done. Furthermore, the sum of the response rates elicited by the sites stimulated by C pulses alone was compared with the split-site response rate to determine whether significant differences are found, indicating the presence of an interaction.

The same procedure was used for substantia nigra/hypothalamus, periaqueductal midbrain central gray/hypothalamus, and locus coeruleus/septum combinations. After completion of Experiment III, selected animals were tested separately in Experiments IV or V; the remainder were sacrificed as described in Experiment I.

Results

Thirty-six (36) animals with 36 combinations of ICSS sites

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completed Experiment III. Fourteen (14) animals were tested for interactions for the locus coeruleus/hypothalamus combination; 13 animals were tested for interactions for the periaqueductal midbrain central gray area/hypothalamus combination; four animals were tested for interactions for the substantia nigra/hypothalamus combination; and five animals were tested for interactions for the septum/locus coeruleus combination. Table 5 lists the electrode localization for each site of each combination.

In 31 of the 36 combinations, the animal sustained reliable ICSS behavior from both electrode sites. In the remaining five combinations (88G, 7H, 8H, 10H, 23H), the animal self-stimulated from only one electrode site. In four of these cases, the animal demonstrated qualitative interest in the stimulation, but would not sustain ICSS behavior. By qualitative interest, the animal would lever-press either when primed with stimulation or when actively shaped to the lever by the experimenter. The intensity at which the animal demonstrated maximal interest was used in the interaction. In one case (8H), one electrode site supported ICSS while the second electrode site elicited aversive behaviors (red nucleus); this animal was tested over a variety of intensities in the interaction.

An interaction was determined by comparing the response rates elicited when both electrode sites were stimulated by simultaneous delivery of C-pulse trains, with the sum of response rates elicited when each site alone was stimulated by delivery of C-pulse trains. In 35 of 36 combinations, simultaneous stimulation of both sites with C-pulse trains elicited significantly (t-tests, $p \leq .05$)

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higher response rates than the sum of the response rates elicited when each site alone was stimulated by delivery of C-pulse trains. The remaining case, 8H, had one site which elicited aversive behaviors. In this case, simultaneous stimulation of both sites with C-pulse trains elicited significantly (t-test, $p \leq .05$) lower response rates than when its ICSS site alone was stimulated by delivery of C-pulse trains. Thus, in cases where either an animal supported ICSS behavior for both electrode sites or sustained interest in a non-ICSS electrode site, an interaction took place such that simultaneous stimulation of both electrode sites yielded higher response rates than the sum of the response rates for each site stimulated singly.

As determined in Experiment II, all electrode sites when delivered trains of pulse pairs will demonstrate decrements in response rate when C and T pulses are presented at short C-T interval (.5-1.5 msec.) as compared to response rates elicited when longer C-T intervals (2-5 msec.) are presented. Moreover, the response rates elicited short C-T intervals are comparable to response rates elicited for the C-pulses alone. The term refractoriness will be used to describe this within-site function condition and the term elimination from refractoriness will be used to describe when the decrement in response rate at short C-T intervals is eliminated. In the elimination from refractoriness condition, therefore, response rates for any pulse pair regardless of C-T interval will be similar. Figure 8 shows the hypothalamic within-site function of Rat 67G; mean response rate per 5-min. period is on the ordinate, while C-T interval

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in milliseconds is on the abscissa. As can be seen, response rates at short C-T intervals are similar to the C-pulse alone condition and significantly (ANOVA $p \leq .05$) less than response rates at longer C-T intervals. Figure 9 shows the locus coeruleus within-site function of Rat 67G; again, response rates at short C-T intervals are similar to the C-pulse alone condition and significantly (ANOVA, $p \leq .05$) less than response rates at longer C-T intervals. Figure 10 shows the interaction function of Rat 67G when the C-pulse was delivered to the hypothalamus and T-pulse was delivered to the locus coeruleus. Unlike the within-site functions, the interaction function response rates do not differ from one another significantly (ANOVA, $p > .05$); response rates elicited at short C-T intervals are similar to response rates at longer C-T intervals.

In 35 of 36 cases, an elimination of refractoriness took place such that response rates at short C-T intervals did not differ significantly (ANOVA, $p > .05$) from response rates at longer C-T intervals when the C and T pulses were split across sites. Moreover, in all of these cases, the response rate elicited at all C-T intervals when the pulse pair was split between sites was significantly greater (ANOVA, $p \leq .05$) than the sum of response rates elicited when each site alone was stimulated by the C-pulse trains. Thus, in both the simultaneous condition (pulses split between sites and delivered at the same time) and the staggered condition (pulses split between sites and delivered at different times with respect to one another), both interactions and elimination from refractoriness took place. The remaining case involved again the animal with one elec-

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trode placement which, when stimulated, elicited aversive behavior. Both under the simultaneous and staggered conditions, there was no significant difference (ANOVA, $p > .05$) between response rates elicited by delivery of the pulse pair split across sites and response rates elicited by the ICSS site stimulated by C-pulse trains. Therefore, in order to maintain an interaction in both the simultaneous and staggered conditions, either both sites must sustain ICSS or one site must at least elicit interest in the stimulation and paired with an ICSS site.

Fourteen (14) animals were tested under the staggered pulse pair condition split between the locus coeruleus and hypothalamus. In five animals, response rates were significantly (ANOVA, $p \leq .05$) higher when the C pulse of the pulse pair was delivered to the locus coeruleus and the T pulse of the pulse pair was delivered to the hypothalamus, than when the C pulse of the pulse pair was delivered to the hypothalamus and the T pulse of the pulse pair was delivered to the locus coeruleus. Figure 11 demonstrates this realtionship in four representative animals. As can be seen, there was no overlap between response rates over any C-T interval. All five animals had their brainstem electrodes localized in and around the locus coeruleus. Figure 12 displays these placements with closed triangles. Three of the five animals which demonstrated differential responding between the C-locus coeruleus, T-hypothalamus condition and the C-hypothalamus, T-locus coeruleus condition had their hypothalamic placements in the area of the internal capsule and crus cerebri. The remaining two animals had their hypothalamic electrodes located

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in the anterior H1 Fields of Forel. Figure 13 displays these placements with closed triangles. Table 5 lists precise histological electrode localizations for each animal. These five animals will subsequently be referred to as the locus coeruleus-crus cerebri/ Fields of Forel group.

In seven of nine animals, response rates did not differ signigicantly (ANOVA, $p > .05$) between the C-locus coeruleus T-hypothalamus condition and the C-hypothalamus, T-locus coeruleus condition. In two cases, C-hypothalamus, T-locus coeruleus was significantly ($p \leq .05$) greater than C-locus coeruleus, T-hypothalamus. Figure 14 demonstrates this relationship in four representative animals, two with similar rates and two with C-hypothalamus, T-locus coeruleus higher rates. Like the previous group, all nine of these animals had their brainstem electrodes localized in and around the locus coeruleus; Figure 12 displays these placements with closed circles. All nine of these animals had their hypothalamic electrodes located in either the medial forebrain bundle or the perifornical area; Figure 13 displays these placements with closed circles. Table 5 lists precise histological electrode localizations for each animal. These nine animals will subsequently be referred to as the locus coeruleus-medial forebrain bundle/perifornical group.

In summary, the locus coeruleus-crus cerebri/Fields of Forel group elicit differential response rates across the two staggered split pulse-pair conditions, that is, response rates elicited by C-locus coeruleus, T-crus cerebri/Fields of Forel ICSS is greater than response rates elicited by C-crus cerebri/Fields of Forel, T-locus coeruleus ICSS (Figure 11). On the other hand, the locus coeruleus-

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medial forebrain bundle/perifornical group elicits similar response rates across both staggered split-pulse-pair conditions (Figure 14). As Figure 12 shows, locus coeruleus placements for both the C-locus coeruleus-crus cerebri/Fields of Forel condition (closed triangles) and for the locus coeruleus-medial forebrain bundle/perifornical area condition (closed circles) overlap each other. Conversely, in Figure 13 there is a clear difference with no overlap between conditions for the hypothalamic electrode placements. Hypothalamic placements in the crus cerebri or Fields of Forel (closed triangles) exhibit differential responding while placements in the medial forebrain bundle and perifornical area (closed circles) do not exhibit differences in responding across the two conditions. Therefore, it seems apparent that the placement of the hypothalamic electrode determines the type of differentiation attained between the two conditions.

Four animals were tested under the staggered pulse pair condition split between the substantia nigra and hypothalamus. In two animals, response rates were significantly (ANOVA, $p \leq .05$) higher when the C pulse of the pulse pair was delivered to the substantia nigra and the T pulse was delivered to the hypothalamus, than when the C pulse was delivered to the hypothalamus and the T pulse was delivered to the substantia nigra.

In the top two parts of Figure 15 (A and B), this relationship is demonstrated in the two animals; again, there was no overlap in response rates across C-T interval. Brainstem electrodes were localized in the pars reticulata and pars compacta of the substantia nigra; Figure 16 displays these placements with closed triangles.

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The hypothalamic placements were the periventricular nucleus of the hypothalamus and the area ventro-lateral to the medial forebrain bundle. Figure 17 displays these placements with closed triangles. Table 5 lists precise histological electrode localizations for each animal. These two animals will subsequently be referred to as the substantia nigra-_non_-medial forebrain bundle group.

In the remaining two animals, response rates did not differ significantly (ANOVAs, $p > .05$) between the C-substantia nigra, T-hypothalamus condition and the C-hypothalamus, T-substantia nigra condition. In the bottom two parts of Figure 15 (C and D), this relationship is demonstrated in the two animals. Like the previous group, brainstem electrodes were localized in the pars reticulata and pars compacta of the substantia nigra; Figure 16 displays these placements with closed circles. The hypothalamic placements were localized in the medial aspects of the medial forebrain bundle for both animals; Figure 17 displays these placements with closed circles. Table 5 lists precise histological electrode localizations for each animal. These two animals will be subsequently referred to as the substantia nigra-medial forebrain bundle group.

In summary, the locus coeruleus-crus cerebri/Fields of Forel group and the substantia nigra-_non_-medial forebrain bundle groups exhibit differential response rates between the two staggered split-pulse conditions where response rates elicited when the C pulse is in either the locus coeruleus or substantia nigra and the T pulse is in a hypothalamic area outside the medial forebrain bundle and perifornical area, is greater than response rates elicited when the C

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pulse is delivered to the hypothalamic area outside the medial forebrain bundle and the T pulse is delivered to either the substantia nigra or the locus coeruleus (Figures 11 and 15 (A and B); Table 5). Conversely, the locus coeruleus-medial forebrain bundle/perifornical area and the substantia nigra-medial forebrain bundle groups exhibit similar response rates between the two staggered split-pulse conditions where response rates elicited by either C-locus coeruleus or C-substantia nigra, and T-medial forebrain bundle/perifornical area are similar to response rates elicited by C-medial forebrain bundle/perifornical area and either T-locus coeruleus or T-substantia nigra (Figures 14 and 15 (C and D); Table 5). Hence, despite overlap in locus coeruleus and substantia nigra placements (Figures 12 and 16), hypothalamic placements in the medial forebrain bundle and perifornical area exhibit a single type of interaction in both staggered conditions (Figures 13 and 17, closed circles), while hypothalamic placements in the crus cerebri, Fields of Forel and periventricular area exhibit two types of interactions dependent upon whether the hindbrain placement receives the C or the T pulse (Figures 13 and 17, closed triangles).

Thirteen (13) animals were tested under the staggered pulse pair condition split between the periaqueductal midbrain central gray area and the hypothalamus. In four of the 13 animals, response rates were significantly (ANOVAs, $p \leq .05$) higher when the C pulse was delivered to the periaqueductal midbrain central gray area and the T pulse was delivered to the hypothalamus, than when the C pulse was delivered to the hypothalamus and the T pulse was delivered to

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the periaqueductal midbrain central gray area. Figure 18 displays the relationship for the four animals involved. Brainstem electrodes were localized in the mesencephalic dorsal tegmental decussation, the medial longitudinal fasciculus, the Edinger-Westphal/oculomotor nuclei and the mesencephalic nucleus linearis; Figure 19 displays these placements with closed triangles. No overlap occurred between response rates across C-T intervals. The hypothalamic placements were located in either the medial forebrain bundle or the perifornical area; Figure 20 displays these placements with closed triangles. Table 5 lists precise histological electrode localizations for each animal. These four animals will subsequently be referred to as the periaqueductal midbrain central gray area-medial forebrain bundle/perifornical area group.

In eight of the 13 animals tested for interactions in the periaqueductal midbrain central gray area and hypothalamus, response rates did not differ significantly (ANOVAs, $p > .05$) between the C-periaqueductal midbrain central gray area, T-hypothalamus condition, and the C-hypothalamus, T-periaqueductal midbrain central gray area condition. In two of these animals (Rat 75H), response rates under the C-hypothalamus, T-periaqueductal midbrain central gray was significantly (ANOVA, $p \leq .05$) greater than the reverse. Figure 21 displays this relationship for four representative animals. Like the previous group, brainstem electrodes were localized in the mesencephalic dorsal tegmental decussation, oculomotor nuclei, and surrounding area ventro-laterally, and the mesencephalic nucleus linearis. In addition, brainstem electrodes were localized in the mesencephalic

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ventral tegmental decussation; also, one animal (Rat 75H) had an anterior mesencephalic placement dorsal to the third ventricle and medial to the fasciculus retroflexus. Figure 19 displays all of these placements with closed circles. The hypothalamic placements in this group were clustered in two areas; four animals were located in the far lateral part of the medial forebrain bundle, while the remaining four were located around the anterior H_1 Fields of Forel and dorso-medial nucleus of the hypothalamus. Figure 20 displays all of these placements with closed circles. Table 5 lists precise histological electrode localizations for each animal. These eight animals will subsequently be referred to as the periaqueductal midbrain central gray area-non-medial forebrain bundle group.

In the remaining animal (8H) tested for interactions in the periaqueductal midbrain central gray area and hypothalamus, response rates for the C-hypothalamus, T-periaqueductal midbrain central gray area condition were significantly (ANOVA, $p \leq .05$) lower than response rates for either the C-periaqueductal midbrain central gray area, T-hypothalamus condition, or the condition where C pulses were delivered to the hypothalamus alone. The latter two conditions did not differ significantly (ANOVA, $p > .05$) from one another in terms of response rate. This was the same animal which: a) demonstrated an aversive reaction to periaqueductal midbrain central gray area stimulation, b) did not show an interaction when comparing response rates elicited when the C-pulses were delivered to the hypothalamus alone condition, and when C-pulses were delivered simultaneously to the periaqueductal midbrain central gray area and the hypothalamus,

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and c) did not demonstrate elimination from refractoriness when the pulse pair was split in a staggered fashion across the two sites. Figure 22 displays this interaction relationship for Rat 8H; the periaqueductal midbrain central gray area electrode placement was localized in the red nucleus, while the hypothalamic electrode placement was localized in the medial forebrain bundle. Figure 19 displays this placement with a closed square.

In summary, the periaqueductal midbrain central gray area-medial forebrain bundle/perifornical area group exhibited differential response rates between the two staggered split-pulse conditions where response rates elicited by the C-periaqueductal midbrain central gray area, T-medial forebrain bundle/perinfornical area condition are greater than response rates elicited by the C-medial forebrain bundle/perifornical area, T-periaqueductal central gray area condition (Figure 18). On the other hand, for the periaqueductal midbrain central gray area-non-medial forebrain bundle group, response rates did not differ between the two staggered conditions (Figure 21). Hence, despite overlap in periaqueductal midbrain central gray area placements (Figure 19), hypothalamic placements not in the medial forebrain bundle and perifornical area exhibit a single type of interaction in both staggered conditions (Figure 20, closed circles), while hypothalamic placements located in the medial forebrain bundle and perifornical area exhibit two types of interactions dependent upon whether the hindbrain placement receives the C or the T pulse (Figure 20, closed triangles). In one case, a) no interaction took place, b) no elimination from refractoriness took place, c) the

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periaqueductal midbrain central gray area electrode placement was localized in the red nucleus (Figure 19, closed square) which was behaviorally aversive, and d) exhibited differential response rates in which one staggered condition was significantly lower than either the other staggered condition or C pulses delivered to the ICSS electrode alone.

Five animals were tested under the staggered pulse pair condition split between the locus coeruleus and septum. In one case, response rates were significantly (ANOVA, $p \leq .05$) higher when C pulse was delivered to the locus coeruleus and the T pulse was delivered to the septum, than when the C pulse was delivered to the septum and the T pulse was delivered to the locus coeruleus. The left panel of Figure 23 (A) displays this relationship for Rat 88G. Histological localization of the electrodes revealed the placements to be in the dorsal noradrenergic bundle and the lateral septum respectively (Figures 24 and 25, closed triangles). In the remaining four cases, response rates did not differ significantly (ANOVA, $p > .05$) between the C-locus coreuleus, T-septum condition and the C-septum, T-locus coeruleus condition. The right panel of Figure 23 (B) displays this relationship for a representative animal of this group. The placements for these animals were localized in the dorsal noradrenergic bundle and the medial septum respectively (Figures 24 and 25, closed circles). Hence, despite overlap in locus coeruleus placements (Figure 24), medial septal placements exhibit a single type of interaction in both staggered conditions, while the lateral septal placement exhibits two types of interactions dependent

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upon whether the hindbrain placement receives the C or T pulse (Figure 25).

These results are encapsulated in Table 6, and can be summarized by the following points:

1) When both sites tested support ICSS behavior, an interaction takes place such that the response rates elicited by simultaneous delivery of C pulses to both sites are significantly greater than the sum of the response rates elicited when each site is stimulated by C pulses alone.

2) The same type of interaction can occur if one site can support ICSS, while the other site sustains interest. This interaction does not occur if one site supports ICSS, while the other site demonstrates behavioral aversion.

3) The sites which demonstrate interactions under simultaneous conditions will also demonstrate interactions when the pulses delivered to each site are staggered. This has been described as an elimination of refractoriness where response rates under the split pulse condition are similar across all C-T intervals. Normally, when both pulses are delivered within a given site, response rates are significantly lower at short C-T intervals as compared to longer C-T intervals. The sites which did not demonstrate an interaction under the simultaneous condition did not eliminate refractoriness under the staggered condition.

4) In all combinations of sites tested, differential results were obtained, a) dependent upon hypothalamic placement within each combination, and b) dependent upon which brainstem area was tested

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across combinations.

5) For the locus coeruleus-crus cerebri/Fields of Forel group, response rates were significantly greater when the C pulse was placed in the locus coeruleus and the T pulse was placed in the crus cerebri/Fields of Forel, than when the reverse took place. For the substantia nigra-non-medial forebrain bundle group, response rates were significantly greater when the C pulse was placed in the substantia nigra and the T pulse was placed in a non-medial forebrain bundle hypothalamic placement, than when the reverse took place.

6) For the locus coeruleus-medial forebrain bundle/perifornical area group, response rates were similar, regardless of which site received the C pulse. In two cases, response rates were significantly greater for C-medial forebrain bundle, T-locus coeruleus rather than the reverse. For the substantia nigra-medial forebrain bundle group, response rates were similar regardless of which site received the C pulse.

7) Therefore, in locus coeruleus/hypothalamus combinations and substantia nigra/hypothalamus combinations, different rates of responding will occur under the two staggered conditions only when the hypothalamic placement is outside of the medial forebrain bundle/perifornical area.

8) For the periaqueductal midbrain central gray area-medial forebrain bundle/perifornical area group, response rates were significantly greater when the C pulse was placed in the periaqueductal midbrain central gray area and the T pulse was placed in the medial forebrain bundle/perifornical area group, than when the reverse took

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place.

9) For the periaqueductal midbrain central gray area-non-medial forebrain bundle group, response rates were similar, regardless of which site received the C pulse. In two cases, response rates were significantly greater when the C pulse was placed in a hypothalamic area outside the medial forebrain bundle and the C pulse was placed in the periaqueductal midbrain central gray area, than when the reverse took place.

10) Therefore, in periaqueductal midbrain central gray area/hypothalamus combinations, differential rates of responding will occur under the two staggered conditions only when the hypothalamic placement is inside the medial forebrain bundle/perifornical area.

11) For locus coeruleus/septum combinations, the location of the septal placement elicits differential results. If the septal placement placement is lateral, then response rates elicited under C-locus coeruleus, T-lateral septum is greater than the reverse. If the septal placement is medial, then response rates are similar regardless of which site, locus coeruleus or medial septum, received the C pulse.

Discussion

When two sites are capable of supporting ICSS, an interaction took place such that simultaneous delivery of C pulses to both ICSS sites elicited response rates which were significantly greater than the sum of the response rates elicited by stimulating each site with C pulses alone at the same intensities used in the simultaneous

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condition. This data is in accordance with virtually all interaction studies which measured this variable (Ungerleider & Coons, 1970; Szabo, 1972; Ellman et al., 1975; Coons et al., in press; Ackermann et al., in preparation,a). In addition, an interaction took place even when one of the sites does not sustain reliable ICSS responding, but only qualitative behavioral interest. In four cases, while the animal would respond reliably from one electrode site, the second placement would support intermittent responding operantly and approach-behavior towards the lever when shaped. Administration of one and two mg/kg doses of d-amphetamine, a catehcolamine releaser and an agent which enhances ICSS (Stein, 1964; Steiner & Stokely, 1973) would not increase responding above this level. Yet, when tested for interactions with an ICSS site, response rates elicited by simultaneous delivery to both sites were significantly greater than the sum of response rates elicited by stimulating each site with C pulses alone. In all four cases, the non-ICSS electrode placement was in a site which supported ICSS in the present or other studies. Therefore, one must qualify this statement by reporting that ICSS could not be maintained within the site by this stimulation technique and these stimulus parameters. As previously noted in Experiment I, it is not feasible to report that an electrode placement is incapable of sustaining ICSS based on a study which utilizes restrictive parameters (Routtenberg & Malsbury, 1969). Still, interactions occurring between an ICSS site and a non-ICSS site are unreported in the literature. In fact, four studies (Ellman et al., 1975; Ungerleider & Coons, 1970; Ackermann et al., in preparation,a;Coons et al., in press)

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have demonstrated that interactions will not take place if one or both of the two sites does not support ICSS. However, procedural and histological differences can account for the results. Two studies (Ellman et al., 1975; Ackermann et al., in preparation, a) tested the non-ICSS over a wide range of intensities with sinusoidal stimulation which delivers current continuously during the train. Therefore, their report of a non-ICSS site actually not sustaining ICSS has more validity because of a more comprehensive screening procedure. In addition, these studies and the two others (Ungerleider & Coons, 1970; Coons et al., in press) localized their non-ICSS electrode placements in sites where either no ICSS reports in the literature existed or where behavioral aversion took place. As was pointed out previously, the non-ICSS sites which elicited an interaction when paired with an ICSS site, sustained qualitative behavioral interest and were located in areas where ICSS placements were reported previously.

In one case, an interaction did not take place between an ICSS site and non-ICSS site; response rates did not differ when stimulation was delivered to the ICSS site as compared to simultaneous stimulation of both sites. In contrast to the four animals described above, the non-ICSS placement was localized in the red nucleus in which previous studies (Routtenberg & Malsbury, 1969; Crow, 1972) have reported no ICSS behavior. In this animal, stimulation of the red nucleus elicited behavioral aversion, and when stimulated simultaneously with a hypothalamic ICSS site, did not produce a behavioral facilitation. This is in accordance with studies (Ellman et al.,

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1975; Ungerleider & Coons, 1970; Coons et al., in press; Ackermann et al., in preparation,a) where interactions will not take place if one or both sites does not support ICSS.

A further finding was that if a site combination was capable of supporting an interaction under simultaneous conditions, then it was able to support the interaction under staggered conditions, that is, the C pulse and T pulse are delivered to different sites with a varied interval in milliseconds between C and T pulses. This data for the seven site combination tested constitutes a so-called elimination from refractioriness since pulses delivered at short C-T intervals within a site do not support ICSS behavior at the level gained at longer C-T intervals. However, when pulses are split between sites, staggered response rates are similar over all C-T intervals tested when one particular site receives the C pulse and a second particular site receives the T pulse. This elimination from refractoriness has been demonstrated previously (Ungerleider & Coons, 1970; German & Holloway, 1973), although at very long C-T intervals (100 msec. or more), this elimination from refractoriness dissipates. Hence, it seems that the interaction under simultaneous and staggered conditions is triggered by synaptic and summative, rather than axonal, events. As delay between succeeding pulses increases, chances of optimal summation decreases.

Further evidence of summation is apparent (Figure 10) when one considers that responding at all C-T intervals in the split-pulse staggered condition is not uniform. Note that in Figure 10, response rates at 1.2 msec. are non-significantly lower than at the other C-T intervals, while response rates at 1.0 msec. are non-significantly higher. This small deviation could be due to the optimal summative ability of the common point of influence is at 1.0 msec. If the hypothalamic site is stimulated 1.0 msec. before the locus coeruleus site, optimal summative capacity for that particular ordered combination is reached and many cells are fired. However, if the hypothalamic pulse is delivered 1.2 msec. after the locus coeruleus pulse, then either: a) limited refractoriness to the effects of the locus coeruleus pulse is indicated by decline in response rate, or b) stimulation by the locus coeruleus pulse triggered concommitant transmitter which creates IPSP's at the summation point. This latter possibility will be explored in Experiment IV.

The split-pulse staggered condition was tested in two ways for each combination. In one condition,the C pulse was delivered to the hindbrain electrode site and the T pulse was delivered to the forebrain electrode site. In the second condition, the C pulse was delivered to the forebrain electrode site and the T pulse was delivered to the hindbrain electrode site. Seven site combinations were tested; the locus coeruleus-septum combination will be discussed later. Of the six remaining site combinations tested, three of them demonstrated significant differentiation in response rate which was dependent upon which electrode site of the particular combination received the C pulse. In the locus coeruleus-crus cerebri/Fields of Forel group, the substantia nigra-non-medial forebrain bundle group, and the periaqueductal midbrain central gray-medial forebrain bundle/perifornical area group, response rates were significantly

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higher when the C pulse was delivered to the hindbrain electrode site and the T pulse was delivered to the hypothalamic electrode site, than when the reverse condition was tested. In Table 5, sections A, C, and E summarize these results. These groups will be collectively called the differential interaction group. In the other three combinations, most of the animals demonstrated similar response rates under both conditions of the interaction. In the locus coeruleus-medial forebrain bundle/perifornical area group. the substantia nigra-medial forebrain bundle group, and the periaqueductal midbrain central gray-non-medial forebrain bundle group, response rates were usually similar regardless of whether the C pulse was delivered to the hindbrain electrode site and the T pulse was delivered to the hypothalamic electrode site or whether the reverse condition was tested. In a minority of animals (N = 4), response rates were significantly higher when the C pulse was delivered to the hypothalamic electrode site and the T pulse was delivered to the hindbrain electrode site, than when the reverse condition was tested. In Table 5, sections B, D, and F summarize these results. These groups will be collectively called the similar interaction group.

There are several explanations for the differences in responding under the two conditions for the differentiated interaction group and the similar interaction group. First, four possible explanations of the data produced by the differential interaction group will be discussed, followed by four possible explanations of the data produced by the similar interaction group.

The four explanations for the differentiated interaction group are: a) the differential interaction is caused by a direct ascending excitatory influence by the hindbrain electrode site upon the hypothalamic electrode site; b) the differential interaction is caused by ascending excitatory influences by both electrode sites upon a common summation point anterior to both sites; c) the differential interaction is caused by excitement of an ascending system and re-excitement of that same system by path neurons; and d) the differential interaction is caused by a direct inhibitory influence by the hypothalamic electrode site upon the hindbrain electrode site.

The first explanation postulates that the differential interaction is caused by a direct ascending excitation by the hindbrain electrode site upon the hypothalamic electrode site. Evidence for this explanation are found is histofluorescence, assay, and drug studies. In the locus coeruleus-crus cerebri/Fields of Forel group, histofluorescence studies indicate that the locus coeruleus sends fibers of passage through the crus cerebri and Fields of Forel via the ventral noradrenergic bundle and dorsal noradrenergic bundle respectively (Ungerstedt, 1971; Lindvall & Bjorklund, 1974), although there is negative evidence of locus coeruleus terminals in the hypothalamus (Ungerstedt, 1971; Lindvall & Bjorklund, 1974). This relationship is still unclear, however, since studies (Ross & Reis, 1974; Ross, Joh, & Reis, 1975) have demonstrated that locus coeruleus lesions reduce hypothalamic dopamine-beta-hydroxylase activity while hypothalamic lesions reduce the retrograde reaction of tyrosine hydroxylase and dopamine-beta-hydroxylase in locus coeruleus cell bodies. This evidence has been interpreted as sup-

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port for locus coeruleus terminals in hypothalamic areas. In the substantia nigra-non-medial forebrain bundle group, one animal's hypothalamic placement was ventro-lateral to the medial forebrain bundle, an area where histofluorescence studies have located the nigro-striatal pathway (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971; Lindvall & Bjorklund, 1974). In the second animal, however, the hypothalamic placement was localized in the periventricular hypothalamic nuclei, certainly an area where ascending nigro-striatal fibers do not pass. In the periaqueductal midbrain central gray-medial forebrain bundle group, the evidence for exclusively ascending fiber systems is small, although the meso-limbic system passing from mesencephalic cell group A-10 to forebrain nuclei in the accumbens, amygdala, septum and striatum passes through the medial forebrain bundle (Lindvall & Bjorklund, 1974). It is more medial to the nigro-striatal or ventral noradrenergic bundles and thus be more affected by medial forebrain bundle and perifornical ICSS. Hence, it seems that this first explanation, although tenable, does not apparently have overwhelming evidence in support of it.

The second explanation for differential interactions suggests the existence of a common summation point for the two electrode sites of each combination where each electrode site exerts an ascending excitatory influence. Since the data for the differential interaction group demonstrates that response rates are greater in the condition when the C pulse is delivered to the hindbrain site, than in the condition when the C pulse is delivered to the hypothalamic site, this suggests that the summation point must

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be anterior to both electrode sites so as to allow the excitatory influence from the hindbrain site adequate lead time to optimally summate with the excitatory influence from the hypothalamic site. Evidence for this notion for the locus coeruleus-crus cerebri/Fields of Forel group indicates that the nigro-striatal tract clearly traverses the crus cerebri area (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971; Lindvall & Bjorklund, 1974) and innervates the striatum, anterior cingulate cortex and the amygdala. The Fields of Forel does not have as strong histochemical evidence for ascending fibers, although lateral aspects of the dorsal noradrenergic bundle and medial aspects of the incerto-hypothalamic bundle could be involved (Lindvall & Bjorklund, 1974; Bjorklund, Lindvall, & Nobin, 1975). Stimulation of the locus coeruleus and crus cerebri or Fields of Forel could result in summation of limbic nuclei in the forebrain. Delivery of the C pulse to the locus coeruleus provides the adequate lead time so that transmitters are released by both locus coeruleus and crus cerebri/Fields of Forel terminal areas which in turn create summative EPSPs upon forebrain nuclei. If the C pulse is delivered to the crus cerebri or Fields of Forel and the T pulse is delivered to the locus coeruleus, then the crus cerebri or Fields of Forel terminal areas will release transmitter well in advance of locus coeruleus terminals. Furthermore, transmitter released by locus coeruleus terminals will create forebrain nuclei EPSPs after the EPSPs of crus cerebri/Fields of Forel, transmitter has been largely dissipated, causing less than optimal summation. It appears then, that while both conditions cause an enhancement in responding over delivery of

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C-pulses from either site alone, the greater enhancement occurs when the C pulse is in the locus coeruleus because of more optimal summative capacities in forebrain nuclei. In the substantia nigra-non-medial forebrain bundle group, one animal had its hypothalamic electrode ventro-lateral to the medial forebrain bundle in a location near the nigro-striatal pathway. Thus, it is possible that hypothalamic stimulation excites the same terminal areas as the nigro-striatal pathway. The second animal's hypothalamic placement was the periventricular nucleus where ICSS rates are low, corresponding with response rates elicited by midbrain periaqueductal bundle ICSS (Ackermann et al., in preparation, a). The periaqueductal bundle (Lindvall & Bjorklund, 1974) arises from the locus coeruleus and ascends through the lateral periaqueductal and periventricular gray, and then traverses ventro-laterally into the preoptic area and amygdala, areas innervated by the nigro-striatal tract. Another possibility is that ICSS is triggering A-12, A-13, or A-14 hypothalamic cell groups (Lindvall & Bjorklund, 1974) which activate the incerto-hypothalamic system (Bjorklund et al., 1975). The nigro-striatal and incerto-hypothalamic systems are both ascending and may have common forebrain terminal areas. As in the case of the locus coeruleus-crus cerebri/Fields of Forel group, the most optimal summation in forebrain areas would involve initial C-pulse activation of the poserior site (substantia nigra) to provide adequate lead time so that the transmitters are released by both substantia nigra and non-medial forebrain bundle terminal areas in close temporal succession. In the periaqueductal midbrain central gray-

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medial forebrain bundle/perifonrical area group, if one assumes that the hypothalamic placement is directly stimulating parts of the mesolimbic pathway, then the common summation point hypothesis is valid. Again, evidence for this second explanation is available, though not overwhelming.

The third explanation for directional interactions contends that the differentiation is caused by excitement of an ascending system and re-excitement of that same system by path neurons. Response rates are higher if the ascending system component is excited initially and the path neurons are excited at some point after the ascending component passes. In the locus coeruleus-crus cerebri/Fields of Forel group, path neurons in the Fields of Forel region or the crus cerebri region are activated by stimulation to these areas and trigger ascending fibers of passage. If the C pulse is delivered to the locus coreuleus, activation of these fibers occur before the path neurons are activated by the T-pulse; hence, both pulses are propagated along the same axons and maximal summation occurs. However, if the C pulse is delivered to the path neurons in the area of the crus cerebri or Fields of Forel, then they in turn propogate pulses along the same axons where T pulses from the locus coeruleus are travelling. Some of the path neuron propogations travel orthodromically and trigger EPSPs; some of the path neuron propogations travel antidromically and cancel out the ascending T pulse propogations. Thus, the C-crus cerebri/Fields of Forel T-locus coeruleus condition allows less activation of terminal areas with less than maximal summation with correspondingly lower response

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rates. In the substantia nigra-non-medial forebrain bundle group, the path neurons described above will act in the same way. In the periaqueductal midbrain central gray-medial forebrain bundle/perifornical area group, path neurons in the medial forebrain bundle are involved. Delivery of the C pulse to the periaqueductal midbrain central gray activates an ascending excitatory influence on forebrain nuclei. Delivery of the T pulse in the medial forebrain bundle or perifornical area activates path neurons which propagate impulses onto the same axons which the C pulse has activated. However, since the C pulse activation has already passed, this system is then stimulated twice in short temporal succession insuring maximal transmitter release and optimal EPSP summation on forebrain nuclei. In the reverse condition, C pulse activation of medial forebrain bundle or perifornical path neurons create orthodromic excitability but also antidromic cancelling of advancing T-pulses ascending from periaqueductal midbrain central gray cell bodies. This cancelling reduces amount of transmitter release and less than optimal EPSP summation on forebrin nuclei.

Therefore, it seems that behavioral evidence exists for three ascending excitatory systems: the dorsal-periaqueductal-ventral noradrenergic bundle system, the nigro-striatal system, and the mesolimbic system. If the posterior aspect of a system is stimulated initially by the C pulse and the anterior aspect of a system is stimulated by the T pulse, elicited response rates are greater than when the reverse condition is tested. For an ascending system to work at maximal capacity, the posterior part must be stimulated

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initially to allow lead time for maximal forebrain summation. In the cases of the locus coeruleus-crus cerebri/Fields of Forel group, the substantia nigra-non-medial forebrain bundle group and the periaqueductal midbrain central gray area-medial forebrain bundle/perifornical area group, both behavioral and histochemical evidence suggest an ascending excitatory influence.

The fourth and final explanation for the results produced by the differential interaction group postulates that the differentiation is caused by direct inhibitory influences by the hypothalamic electrode site upon the hindbrain electrode site. In this model, when the C pulse is delivered to the hypothalamic electrode site, it interacts in an inhibitory fashion with the T pulse arriving later from the hindbrain site, causing a decrease in behavior. If the C pulse is delivered to the hindbrain electrode site, however, the resulting excitation will produce a behavior before the T pulse in the hypothalamus produces its inhibitory effects. Hence, response rates in the C-hindbrain, T-hypothalamus condition are higher because of less inhibition than rates in the C-hypothalamus, T-hindbrain condition which produces more inhibition. Experiment IV provides a direct pharmacological test of this hypothesis.

The four explanations for the similar interaction group are: a) the similar interaction is caused by an ascending excitatory influence from the hindbrain electrode site and an equally strong descending excitatory influence from the hypothalamic electrode; b) the similar interaction is caused by two ascending excitatory influences with indirectly connected summation points; c) the similar interaction is caused by excitement of an ascending system and in-

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direct re-excitement of the same system by path neurons; d) the similar interaction would be differential except there exists inhibitory influences.

The first explanation suggests equally strong ascending and descending influences. Hence, the C-hindbrain, T-hypothalamus condition more effectively excites the ascending influence while the C-hypothalamus, T-hindbrain condition more effectively excites the descending influence resulting in similar response rate behavior. The evidence for descending systems is small, involving a cholinergic network (Shute & Lewis, 1967) heretofore not involved in ICSS behavior as evidenced by the lack of effect of pharmacological cholinergic manipulations upon ICSS (Newman, 1972; Domino, Olds, & Wilson, 1975). A second descending system not involved in ICSS behavior is the pallido-nigral pathway which contains GABA (Groves et al., 1975). The last descending pathway is the medial forebrain bundle itself (Nauta, 1958), but it does not heavily innervate the hindbrain areas of the interaction. Thus, this hypothesis does not have much evidence to support it.

The second explanation postulates that the similar interactions are caused by two ascending excitatory influences with indirectly connected summation points. In the locus coeruleus-medial forebrain bundle/perifornical area group, the path of interaction can be ascending in both cases, but with slightly overlapping summation points rather than heavily overlapping summation points. The data indicating that response rates are greater under the C-medial forebrain bundle/perifornical area, T-locus coeruleus condition

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than the reverse could still be explained in terms of ascending influences where the locus coeruleus stimulation has less synaptic connections to cross to reach the common summation point than the medial forebrain bundle or perifornical area placement. In the substantia nigra-medial forebrain bundle group and in the periaqueductal midbrain central gray-non-medial forebrain bundle group, forebrain placements from each electrode placement should be in close proximity to one another, but not directly synapsing on a common post-synaptic receptor site. Therefore, the polysynaptic connections will dissipate the temporality of the summation. Again, little direct evidence can support this position.

The third explanation implicates path neuron connections which are polysynaptic between the source of the ascending influence and the source of the hypothalamic stimulation. The path neuron system (Millhouse, 1969) is certainly capable of activating neurons several synapses away, but again direct evidence for this hypothesis is not readily available.

The fourth explanation for similar interactions by way of inhibition will be tested in Experiment IV along with the differential interaction group.

Experiments which can effectively isolate suspected summation points from the interaction sites should be done in order to provide further insight into the mechanisms of the interactions themselves.

The seventh combination of sites involved the locus coeruleus and septum, where an interaction took place such that response rates

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elicited under either the simultaneous or staggered delivery of pulses split between the two sites were significantly greater than the sum of response rates elicited by delivery of C pulses to each site alone. This is the first report of interactions between these two sites and thus further corroborates the premise (Ungerleider & Coons, 1970; Ellman et al., 1975; Ackermann et al., in preparation,a) that any two ICSS sites should produce a neurophysiological interaction. In four of the septal-locus coeruleus combinations, response rates were similar under both the C-septum, T-locus coeruleus and C-locus coeruleus, T-septum conditions. The septal placements in these cases were located medially, where a confluence of noradrenergic and meso-limbic dopaminergic terminals are located (Lindvall, 1975; Brownstein et al., 1974). The fact that these placements exhibit a bidirectional influence when interacting with the locus coeruleus correlates with the bidirectional interactions between locus and medial forebrain bundle-perifornical area where meso-limbic influences were also apparent. In one case, the septal placement was lateral and response rates elicited by the C-locus coeruleus, T-septum condition were significantly greater than the response rates elicited by C-septum, T-locus coeruleus condition. Catecholaminergic terminals in this area have been found to be primarily noradrenergic (Ungerstedt, 1971); this correlates with locus-coeruleus-crus cerebri/Fields of Forel interactions.

It seems from this data that there are two distinct ICSS systems which interact but do not overlap to any great degree. The first system is comprised of: a) the ascending noradrenergic fiber

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system arising principally from the locus coeruleus, and b) the nigro-striatal fiber system arising from the substantia nigra. Interactions between aspects of this system will be greater if the posterior part is stimulated initially, causing a greater enhancement of the ascending excitatory influence as demonstrated by locus coeruleus-crus cerebri/Fields of Forel interactions and substantia-nigra-non-medial forebrain bundle interactions. Further support for this system is that locus coeruleus lesions selectively abolish crus cerebri/Fields of Forel ICSS (Farber et al., in preparation) and reduce substantia nigra ICSS (Farber et al., in preparation; Belluzzi et al., 1975). The second system is comprised of the mesolimbic fiber system arising from the mesencephalic ventral tegmental aream notably dopaminergic cell group A-10. Interactions between aspects of this system will be greater if the posterior part is stimulated initially causing a greater enhancement of the ascending excitatory influence as demonstrated by periaqueductal midbrain gray-medial forebrain bundle/perifornical area interactions. When the two systems are tested, an interaction without exclusive directionality occurs as demonstrated by locus coeruleus medial forebrain bundle/perifornical area interactions, substantia nigra-medial forebrain bundle/perifornical area interaction and periaqueductal midbrain central gray-non-medial forebrain bundle/perifornical area interactions.

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Experiment IV. Effect of Gamma-butyric Acid and Glycine Inhibitors upon C-T Function Interations

Rationale

The results of Experiment III demonstrated that differential interactions could occur depending upon the site combinations. In the differential interaction group, response rates were higher in the C-hindbrain, T-hypothalamus condition than in the reverse condition. This data was analyzed in terms of three separate excitatory hypotheses and one inhibitory hypothesis which suggested that the interaction is differential because of hypothalamic inhibition upon hindbrain excitation. Hence, response rates are higher in the C-hindbrain, T-hypothalamus condition because the hypothalamic pulse cannot inhibit the hindbrain pulse because it is occurring after the hindbrain pulse. In the C-hypothalamus, T-hindbrain condition, the C-pulse in the hypothalamus has a more ideal chance of inhibiting the T pulse in the hindbrain, producing less responding and thus the differentiation. In the similar interaction group, response rates were similar under both conditions. An inhibitory hypothesis here would suggest that there really are differences in response depending upon placement of the C pulse, but they are masked by inhibition. This experiment will attempt to reconcile the inhibitory hypothesis by pharmacological manipulation.

Specifically, the inhibitory mechanism upon excitatory activity is usually explained in terms of neurohumoral transmitter substances producing inhibitory post-synaptic potentials (IPSPs) and thus increasing the threshold for firing. Two transmitter substances

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have been implicated in largely inhibitory roles; they are gamma-butyric acid (GABA) and glycine (Groves et al., 1975). Thus direct pharmacological manipulation of these two transmitters may allow for direct testing of inhibitory hypotheses particularly by inhibiting the inhibitor, or disinhibiton. If disinhibition occurs, response rates which are differential should become similar, and, correspondingly, response rates which are similar should become differential.

A potent inhibitor of GABA is picrotoxin, which fills GABA receptors without activating the post-synaptic membrane with IPSPs, while a potent inhibitor of glycine is strychnine which also occupies glycine receptor sites. Porrino (1975) demonstrated that stimulation of the medial hypothalamus inhibits lateral hypothalamic ICSS at different C-T intervals. Decreases in response rate were found at 1.0 and 3.0 msec. respectively. Intraperitoneal injections of strychnine, a glycine inhibitor, eliminated inhibition at 1.0 msec., while intraperitoneal injections of picrotoxin, a GABA inhibitor, eliminate inhibition at 3.0 mesc. From this data, Porrino inferred that glycine inhibition occurs at short C-T intervals, while GABA inhibition occurs at longer C-T intervals.

The effects of strychnine and picrotoxin on the C-T function interactions of selected animals in different brainstem combinations with the hypothalamus were tested in this experiment.

Method

Subjects and apparatus. Ten selected subjects from Experiment

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III were used. The apparatus used in Experiment IV is described in Experiment I.

Procedure. After completion of Experiment III, each animal was tested under the following procedure. Holding all of the parameters described in Experiment III constant, an animal was tested: a) under picrotoxin for the C-brainstem, T-hypothalamus condition; b) under picrotoxin for the C-hypothalamus, T-brainstem condition; c) under strychnine for the C-brainstem, T-hypothalamus condition, and d) under picrotoxin for the C-hypothalamus, T-brainstem condition. Condition order and drug order was counterbalanced across animals.

Each condition described above consisted of a minimum four-day sequence. On days one, two, and four, each animal received an intraperitoneal injection of normal saline solution (1 ml. of saline solution/kg. of body weight) 20 minutes before the ICSS session. On day three, each animal received an intraperitoneal injection of either strychnine sulfate (.5 mg. of strychnine sulfate/ml. of saline solution/kg. of body weight; Sigma Laboratories) or picrotoxin (.75 mg. of picrotoxin/ml. of saline solution/kg. of body weight; Sigma Laboratories) 20 minutes before the ICSS session. If response rates of days one and two were similar to those of day four, the animal entered the next condition. However, if day four response rates differed from those of days one and two, the animal was tested under saline on subsequent days until rates returned to those of days one and two. The doses chosen for this study were those found effective in Porrino's study, yet were non-toxic and free of behavioral convulsions.

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After completion of all drug sequences, all animals in this experiment were sacrificed as described in Experiment I.

Results

Of the 35 combinations of sites tested in Experiment III, ten combinations were selected for Experiment IV. Table 7 gives the combination group of each animal,which is the particular group which a site combination in an animal was assigned, based on the interaction data in Experiment III. The number of animals tested in the drug screen for each particular group were: locus coeruleus-crus cerebri/ Fields of Forel: 2 animals; locus coeruleus-medial forebrain bundle/perifornical area: 2 animals; substantia nigra-medial forebrain bundle: 1 animal; substantia nigra-non-medial forebrain bundle: 1 animal; periaqueductal midbrain central gray-medial forebrain bundle/ perifornical area: 2 animals; and periaqueductal midbrain central gray-non-medial forebrain bundle: 2 animals. Four conditions were tested for each animal involving combination condition and drug: a) C-hindbrain, T-hypothalamus: strychnine; b) C-hindbrain, T-hypothalamus: picrotoxin; c) C-hypothalamus, T-hindbrain: strychnine; d) C-hypothalamus, T-hindbrain: pcirotoxin.

There were no overall excitatory effects for either of the drugs under either condition. Table 8 lists the mean multiplicative effect of strychnine and picrotoxin for each C-T interval in each condition for all animals as compared to saline baseline. Multiplicative effect is measured by dividing an animal's drug response rate by the animal's saline response rate for the same C-T interval

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and condition. Thus, across two conditions, there was neither a discernable rise in one condition nor a discernable decrease in responding in another condition.

However, if one just looks at the effects of strychnine or picrotoxin within the nine C-T intervals tested in each condition, the results of the drugs is apparent. The major effect of strychnine and picrotoxin was a selective abolition of troughs in the interaction function. As reported in Experiment III, neurophysiological interactions between two ICSS sites took place such that elimination from refractoriness took place, that is, response rates elicited throughout the C-T function were similar and were significantly greater than response rates elicited by the C pulse alone. However, in one case (Rat 82G), response rates across the C-T function were significantly (ANOVA, $p \leq .05$) different from one another; responding at the 1.2 msec. C-T interval was significantly lower than the other C-T intervals. However, this rate was still significantly (ANOVA, $p \leq .05$) higher than the sum of response rates elicited by delivery of C pulses to each site alone. In many other cases, the elimination of refractoriness phenomenon did not result in equal response rates across all C-T intervals. Rather, as seen in Figure 26A (circles-saline), some C-T intervals elicited response rates higher than the rest (peaks), but not significantly so (Duncan, $p > .05$). On the other hand, some C-T intervals elicited response rates lower than the rest (troughs), but not significantly so (Duncan, $p > .05$). In nine cases, administration of strychnine or picrotoxin selectively abolished troughs in one condition of the interaction function

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(Table 7). Figure 26 displays a representative animal showing abolition of troughs: Rat 11H: C-locus coeruleus, T-medial forebrain bundle - strychnine; b) Rat 7H; C-periaqueductal midbrain central gray, T-fornix - picrotoxin; c) Rat 75G: C-periventricular hypothalamus, T-substantia nigra - strychnine; d) Rat 69: C-Fields of Forel, T-locus coeruleus - picrotoxin. Table 7 summarizes the effects of picrotoxin and strychnine upon eliminating troughs in one condition of an interaction function. Strychnine abolished troughs in four cases, three of which were in the C-hypothalamus, T-hindbrain conditon of the interaction. Picrotoxin abolished troughs in five cases, three of which were in the C-hindbrain, T-hypothalamus condition of the interaction. In combinations where response rates elicited by the C-hindbrain, T-hypothalamus condition were significantly greater than response rates elicited by the C-hypothalamus, T-hindbrain condition, picrotoxin eliminated troughs in three animals while strychnine eliminated it in one. On the other hand, in combinations where response rates elicited by the C-hypothalamus, T-hindbrain condition were either significantly greater than or similar to response rates elicited by the C-hindbrain, T-hypothalamus condition, strychnine eliminated troughs in three animals while picrotoxin eliminated it in two. Strychnine eliminated troughs in two animals with medial forebrain bundle/perifornical area electrodes, and in two animals with electrodes outside that hypothalamic area. Picrotoxin eliminated troughs in two animals with medial forebrain bundle/perifornical area electrodes in three animals with electrodes outside that hypothalamic area.

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In no case did either strychnine or picrotoxin abolish the overall significant differences in response rates for those animals which demonstrated significantly greater response rates for the C-hindbrain, T-hypothalamus condition over the reverse. In addition, in no case did either strychnine or picrotoxin establish a significant difference in response rates for those animals which demonstrated similar responding for both conditions.

Discussion

In Experiment III, two different types of interactions occurred across combinations. In the differential interaction group, response rates were significantly higher in the C-hindbrain, T-hypothalamus condition when compared to the reverse, while in the similar interaction group, response rates were similar regardless of whether the hindbrain or the hypothalamus received the C pulse. Two general types of interactions were suggested for these results; one type was generally excitatory while the other was generally inhibitory.

An ascending excitatory system was proposed as the main reason for differential effects of magnitude of interaction in Experiment III; the effect was determined within a particular hindbrain-hypothalamic combination (e.g., locus coeruleus-hypothalamus) by the placement of the hypothalamic electrode (e.g., ascending - crus cerebri; bidirectional - medial forebrain bundle). It was suggested that differential interactions occur in the locus coeruleus-crus cerebri combination because the crus cerebri hypothalamic placement is in the direct ascending line of the locus coeruleus/

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substantia nigra system; similar interactions occur in the locus coeruleus-medial forebrain bundle combination because the medial forebrain bundle placement does not belong to the same ascending system, but rather another system which indirectly connects with the locus coeruleus/substantia nigra system.

The alternate hypotheses which explained the same data was inhibition. In this explanation, differential effects occur in the locus coeruleus-crus cerebri combination because of hypothalamic inhibition, that is, the C-locus coeruleus, T-crus cerebri condition is greater than the C-crus cerebri T-locus coeruleus condition because the C pulse in the crus cerebri is acting as an inhibitor upon the pulse in the locus coeruleus. This inhibiton does not occur when the C pulse is placed in the locus coeruleus. This inhibitory process does not work in the locus coeruleus-medial forebrain bundle combination because the medial forebrain bundle inhibits the periaqueductal midbrain central gray, and not the locus coeruleus; therefore, similar rates occur under both conditions of the locus coeruleus-medial forebrain bundle combination. This inhibitory hypothesis would explain each of the combinations in the same way. A prime candidate for the mechanism which would underlie the inhibitory mechanism would be GABA-containing and/or glycine-containing neurons in the hypothalamus and forebrain. They have been shown to be selectively inhibitory on catecholaminergic excitatory activity in eating (Kimura, 1975), motor modulation (Groves et al., 1975) and ventro-medial/lateral hypothalamic ICSS interactions (Porrino, 1975).

However, in this study, the inhibitory activity of GABA and

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glycine did not account for the differentiation in interactions when it occurred in particular combinations. In the five animals selected from the differential interaction group, the combination was such that response rates were higher in the C-hindbrain, T-hypothalamus condition than the C-hypothalamus, T-hindbrain condition. The disinhibitory effects of strychnine and picrotoxin could have equalized this differentiation in three ways: a) depressing C-hindbrain T-hypothalamus rates to C-hypothalamus, T-hindbrain levels; b) raising C-hypothalamus, T-hindbrain rates to C-hindbrain, T-hypothalamus levels; or c) both depressing C-hindbrain, T-hypothalamus rates slightly and raising C-hypothalamus, T-hindbrain rates slightly, thus equalizing these rates. An inhibitory hypothesis with GABA and glycine as prime mechanisms would predict one of these results. In no animal with differentiation in interaction combination did this occur. Hence, it is apparent that the differentiation in interaction combinations reported in Experiment III cannot be accounted for in terms of a GABA or glycine inhibitory mechansim acting upon one part of particular combinations.

This is not to say that GABA and glycine do not exert any effect upon the interaction functions. In nine of ten animals, either picrotoxin or strychnine selectively eliminated one or more troughs (decrements in interaction response rate at a particular C-T interval) in one condition of the C-T function. This disinhibitory effect of strychnine and picrotoxin suggests an inhibitory role for GABA and glycine upon catecholaminergic interactions which occurs postsynaptically at the summation point or one synapse beyond. The

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peaks and troughs in the interaction C-T function are probably determined by the phase in the neural excitability cycle of the post-synaptic summation neuron. In all cases, both of the stimulation outcomes produced by the C and T pulses are excitatory upon the post-snyaptic receptor sites. At some point in the interaction function, many post-synaptic neurons are in one of the two stages of neuronal refractoriness; some of these neurons will be in the absolute refractory period and hence be incapable of firing in response to any stimulus, while many other neurons will be in the relative refractory period. During the relative refractory period, the neuron will not propagate another action potential unless a higher threshold of summative excitability is reached (Erlanger & Gasser, 1937). The summative capacity is governed by the relative amounts of excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs). At this particular point in the interaction function, despite the amount of potentially excitable transmitter released by the interaction stimulation, too many post-synaptic neurons are in relative refractoriness where IPSPs in part determine the ability to fire. These IPSPs are produced by the normal activation of GABA and glycine-containing neurons which are acting as modulators. Depending upon which type neuron is impinging upon this particular set of post-synaptic receptors, the disinhibitory effects of strychnine or picrotoxin should exert an influence such that the IPSPs produced by GABA or glycine neurons will be cancelled. With the influence of IPSPs negated, the relative refractoriness threshold is reduced and the EPSPs caused by the transmitter release

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of interaction neurons should fire the post-synaptic receptors at a higher rate, thus eliminating the trough in the function.

This model can also account for the findings which show that both strychnine and picrotoxin do not exert overall excitatory effects upon the C-T function, that is, response rates elicited at C-T intervals where no trough exists are not facilitated by either drug. Reasons for the lack of facilitation by strychnine or picrotoxin can be explained. The baseline function upon which the drugs are administered is an interaction function where maximal EPSP summation is occurring at post-synaptic receptor sites. Therefore, theoretically, more than enough EPSPs are occurring to produce summation post-synaptically. Thus, administration of strychnine and picrotoxin which act to lower IPSPs will not affect response rates simply because their action is redundant. If summation occurs even with a normal level of IPSPs, then it will still occur at the same rate with a lower level of IPSPs. Thus, this same level in summation still produces the same level of response rate behavior.

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Experiment V. Effects of Locus Coeruleus Lesions on Hypothalamic C-T Functions and on Locus Coeruleus/ Hypothalamus C-T Interactions

Rationale

Unilateral locus coeruleus lesions selectively abolished or reduced crus cerebri/Fields of Forel ICSS, while sparing medial forebrain bundle ICSS (Farber et al., in preparation). The results of Experiment III have demonstrated that locus coeruleus/crus cerebri-Fields of Forel combinations should show an ascending interaction; that is, response rates were greater when the C pulse is delivered to the locus coeruleus and the T to the crus cerebri-Fields of Forel than response rates elicited in the reverse condition. However, locus coeruleus/medial forebrain bundle-perifornical area combinations did not show corresponding differentiations in interaction.

Farber (in preparation) used sinusoidal stimulation which does not allow the study of the summative characteristics of the effect. The C-T technique holds current constant and could provide answers as to why the reductions take place, such as less synaptic activation as proposed by Szabo (1973). This change is measured by changes in response rate for particular C-T intervals.

Based on the results of Experiment III which suggested an ascending excitatory hypothesis for locus coeruleus-crus cerebri/ Fields of Forel animals and an indirect excitatory hypothesis for locus coeruleus-medial forebrain bundle/periofrnical area animals,

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certain predictions as to the effects of locus coeruleus lesions upon hypothalamic ICSS can be made. If an animal demonstrates response rates which are significantly higher in the C-locus coeruleus, T-hypothalamus condition as compared to the reverse, it is predicted that: a) the hypothalamic electrode will be located in the crus cerebri or Fields of Forel, and b) a locus coeruleus lesion will abolish or reduce hypothalamic ICSS over the entire C-T function. However, if an animal demonstrates similar response rates under both interaction conditions for the locus coeruleus-hypothalamus combination, it is predicted that: a) the hypothalamic electrode will be located in the medial forebrain bundle or perifornical area, and b) a locus coeruleus lesion will not affect hypothalamic ICSS over the entire C-T function.

Subjects. Eight selected subjects with locus coeruleus/ hypothalamus combinations from Experiment III were used.

Apparatus. The C-T apparatus described in Experiment I was used. Lesions were made with a Grass (LM-4) radio-frequency lesion maker, which controlled for the possibility of iron deposits and blockade of stimulation (Aranow, 1960; DiCara, Weaver, & Wolf, 1974; Reynolds, 1963).

Procedure. Pre-lesion baseline consisted of ten days in which ten days of hypothalamic C-T interval/response rate functions, five days of C-locus coeruleus, T-hypothalamus functions, and five days of C-hypothalamus, T-locus coeruleus functions were collected. The two interaction conditions were alternated over the ten days in an a-b-b-a manner and were randomly presented each day in a Latin

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Square design; the hypothalamic function was randomly presented daily in a Latin Square design. The order of the hypothalamic function and the interaction function were alternated in an a-b-b-a manner over days.

After baseline, each animal was lightly anesthetized (.70 cc Equithesin) and underwent a 25-volt radio-frequency lesion for 30 seconds, to the locus coeruleus. Both poles of the bipolar electrode served as cathode; a plate situated under the animal served as ground. All overt behavior and the milliamperage delivered was noted through the lesion.

After the lesion, each animal was tested daily for 30 days, broken into three 10-day segments. Each segment of post-lesion testing was identical to the pre-lesion baseline. Every tenth day, each animal was retested through its locus coeruleus electrode to determine if it recovered the capability to elicit ICSS.

After the post-lesion testing, all animals were sacrificed as described in Experiment I.

Results

Eight rats completed Experiment V; in the pre-lesion condition, seven animals produced interaction response rates which were similar (ANOVA, $p > .05$) under both interaction conditions. Histological verification revealed that the placements were in the locus coeruleus and medial forebrain bundle/perifornical area; these animals will collectively be referred to as the locus coeruleus-medial forebrain bundle group (see annotations, Table 5, section A). In the pre-lesion condition, one animal produced interaction response

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rates in which the C-locus coeruleus, T-hypothalamus condition yielded significantly higher (ANOVA, $p \leq .05$) response rates than the reverse condition. Electrode placements were localized in the locus coreuleus and internal capsule; this animal will be referred to as the locus coeruleus internal capsule group (see annotation, Table 5, section B).

Results of the locus coeruleus lesion will be analyzed in the following manner. First, the locus coeruleus-medial forebrain bundle group will be analyzed for: a) extent of locus coeruleus lesion, b) effects of the lesion upon the within-site hypothalamic C-T function, and c) effects of the lesion upon the two interaction conditions between the locus coeruleus and medial forebrain bundle. The locus coeruleus-internal capsule group will be analyzed later.

A. Locus Coeruleus-Medial Forebrain Bundle Group: Extent of Lesion

In the locus coeruleus-medial forebrain bundle group, three of the locus coeruleus lesions (Rats 33H, 38H, 77H) effectively eliminated 100% of the anterior one-third of the cell group located in the posterior mesencephalon and anterior pontine area. The anterior part of the dorsal tegmental nucleus was also destroyed by the lesion; in all three cases, the posterior two-thirds of the pontine cell bodies of the locus coeruleus and dorsal tegmental nucleus were spared. In two of these cases (Rat 33H and 77H), the lesion spread to the contra-lateral side and also eliminated the contra-lateral locus coeruleus and dorsal tegmental nucleus. In four animals *Rats 31H, 80H, 6I, 17I), the lesion destroyed the posterior

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two-thirds of the locus coeruleus while sparing the anterior one-third of the locus coeruleus cell bodies.

B. Locus Coeruleus-Medial Forebrain Bundle Group: Effect of Lesions upon Within-Site Hypothalamic C-T Function

In three animals with anterior locus coeruleus lesions, the lesion significantly (ANOVA, $p \leq .01$) increased response rates over the entire medial forebrain bundle/perifornical area pulse-pair function. However, the time course, magnitude, and specific intervals affected by the lesion varied from animal to animal. In rat 33H, after one day post-lesion, hypothalamic response rates returned to baseline ICSS rates, and after three days post-lesion, response rates were significantly (ANOVA, $p \leq .01$) elevated at all C-T intervals (Figure 27). Peak responding for this site doubled in rate; responding remained at significantly (ANOVA, $p \leq .05$) elevated levels through the 30-day post-lesion test period. In contrast to Rat 33H, Rat 38H (Figure 28) demonstrates a very gradual change in responding. During the first five days post-lesion, response rates were depressed across all C-T intervals of the hypothalamus. However, as can be seen in Figure 28, response rates elicited at short (0, .5, .8 msec.) C-T intervals during the next five days post-lesion became significantly (ANOVA, $p \leq .05$) greater than pre-lesion baseline. By 20 to 30 days post-lesion (panel C of Figure 28), response rates at C-T intervals from 0 to 1.2 msec. were significantly (ANOVA, $p \leq .05$) greater than baseline. By 40 days post-lesion (panel D of Figure 28), response rates were greater at all C-T intervals except 5 msec. Rat 77H, the third animal with an anterior locus coeruleus

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lesion, had medial forebrain bundle response rates returned to baseline levels within two days post-lesion (Figure 29). Between five days post-lesion and 15 days post-lesion, response rates were significantly (ANOVA, $p \leq .05$) greater than pre-lesion levels over all C-T intervals tested (Figure 29). Bewteen 15 days post-lesion and 25 days post-lesion, response rates returned (ANOVA, $p > .05$) to baseline levels (Figure 29). The facilitations demonstrated by anterior locus coeruleus lesions upon medial forebrain bundle were not as pronounced with the animal (Rat 31H) with a posterior locus coeruleus lesion (Figure 30). After two days of sub-baseline responding, post-lesion response rates elicited by medial forebrain bundle ICSS recovered to baseline levels at short (0 to 1.5 msec.) C-T intervals and were significantly (ANOVA, $p \leq .05$) greater than pre-lesion response rates at long C-T intervals (Figure 30). This increase lasted until 15 days post-lesion, after which post-lesion response rates were similar (ANOVA, $p > .05$) to pre-lesion response rates. In the remaining three animals (rats 80H, 6I, and 17I), initial post-lesion response rates were significantly (ANOVA, $p \leq .05$) depressed over all C-T intervals. After five to ten days post-lesion, response rates at all C-T intervals recovered (ANOVA, $p > .05$) to baseline levels and demonstrated no facilitation.

C. Locus Coeruleus-Medial Forebrain Bundle Group: Effect of Lesions upon Interactions

During the same period as post-lesion hypothalamic functions were tested, both interaction functions were tested. In the seven animals tested in the locus coeruleus-medial forebrain bundle group, interactions still persisted such that post-lesion response rates

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elicited under either interaction condition were significantly greater (ANOVA, $p \leq .05$) than the sum of the pre-lesion response rates elicited by delivery of C pulses to each site alone. Therefore, despite the absence of post-lesion locus coeruleus ICSS at any parameters, an interaction between the locus coeruleus and medial forebrain bundle still took place. Differences were also noted within animals when comparing pre-lesion interaction response rates with post-lesion interaction response rates. In Rat 33H, a significant (ANOVA, $p \leq .05$) difference between pre-lesion interactions and post-lesion interaction response rates (Figure 31). At short (.3-1.5 msec.) intervals, post-lesion response rates for both conditions of the interaction were lower than pre-lesion response rates through all phases of post-lesion testing. On the other hand, at longer (2-5 msec.) C-T intervals, post-lesion interaction response rates were either similar to or significantly greater (ANOVA, $p \leq .05$) than pre-lesion response rates (Figure 31). In Rat 38H, a gradual recovery in interaction response rates occurred. As with the hypothalamic function, the first ten days of post-lesion interaction responding yielded significantly lower (ANOVA, $p \leq .05$) rates as compared to baseline under both conditions (Figure 32). During the next 30 days post-lesion, response rates elicited at short C-T intervals (.3, .5, .8 msec.) were significantly (ANOVA, $p \leq .05$) lower than baseline, under both conditions, while response rates elicited at longer C-T intervals (2, 3, 5 msec.) were significantly (ANOVA, $p \leq .05$) higher than baseline under both conditions (Figure 32). In contrast to the other two animals with anterior locus coeruleus lesions, post-lesion

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interaction response rates under both conditions for the remaining five animals were significantly (ANOVA, $p \leq .05$) lower than pre-lesion interaction response rates at all tested C-T intervals. Figure 33 displays this relationship for rat 77H, a representative animal.

In summary, the locus coeruleus-medial forebrain bundle group: a) had locus coeruleus lesions which destroyed either the anterior or posterior extent, but never all of the cell bodies in any one animal; b) demonstrated facilitations in response rate elicited by within-site hypothalamic functions; c) maintained an interaction despite the locus coeruleus lesion; and d) in two animals, the interaction elicited higher responding post-lesion over pre-lesion baseline.

In the locus coeruleus-internal capsule group, only the hypothalamic function was tested after the lesion destroyed the anterior one-third of thelocus coeruleus cell bodies. In this animal, post-lesion response rates remained significantly (ANOVA, $p \leq .05$) lower than pre-lesion response rates throughout the 15-day post-lesion test period (Figure 34). Response rates elicited in the post-lesion condition only reached 50% of the level elicited pre-lesion. However, in the C pulse alone condition and at the shortest C-T interval tested (.5 msec.), post-lesion response rates were significantly (ANOVA, $p \leq .05$) higher than pre-lesion levels (Figure 34) from the fifth to fifteenth day post-lesion. As the C-T interval increased and pre-lesion response rates increased, concommitantly, post-lesion response rates asymptoted throughout the entire C-T function. No post-lesion locus coeruleus ICSS could be elicited under any parameters.

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Discussion

It is apparent that ipsilateral unilateral locus coeruleus lesions exert different effects on ICSS from different hypothalamic areas. Farber et al. (in preparation) found that crus cerebri or Fields of Forel ICSS is selectively reduced or abolished by locus coeruleus lesions, while medial forebrain bundle or perifornical area ICSS remains unaffected by the same size and location of lesion. The results of Experiment III demonstrated that a strong ascending excitatory influence exists between a locus coeruleus-crus cerebri/ Fields of Forel interaction combination while indirect excitatory influences not strongly directional in nature exists for a locus coeruleus-medial forebrain bundle/perifornical area interaction combination.

The results of Experiment V joins these two findings and, in effect, validates the capacity of interaction data to predict the outcome of a lesion. In seven animals with locus coeruleus-medial forebrain bundle/perifornical area combination, response rates under both conditions were similar. Lesions of the locus coeruleus produced significant facilitations in medial forebrain bundle/perifornical ICSS in four of the seven animals, and baseline recovery in the remaining three animals. In one animal with a locus coeruleus-internal capsule combination, response rates elicited by the C-locus coeruleus, T-internal capsule condition were significantly higher than response rates elicited by the C-internal capsule, T-locus coeruleus condition. Lesion of the locus coeruleus produced a significant reduction to 50% of baseline response rates in internal

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capsule ICSS.

Preliminary indications suggest that the area of the locus coeruleus destroyed influences the size of the effect upon hypothalamic ICSS. In this study, the strongest facilitations in medial forebrain bundle/perifornical area ICSS occurred when the anterior one-third of the locus coeruleus cell bodies situated in the posterior midbrain and anterior pons were destroyed. Correspondingly, the decrement in internal capsule ICSS occurred when the anterior one-third of the locus coeruleus cell bodies situated in the posterior midbrain and anterior pons were destroyed. The smallest facilitation in medial forebrain bundle ICSS occurred when the posterior two-thirds of the locus coeruleus cell bodies were destroyed while the anterior one-third were spared. This data is in accordance with that of Farber et al. (in preparation).

Data presented in Experiment III may explain why ICSS elicited from the internal capsule area of the hypothalamus is reduced by locus coeruleus lesions while ICSS elicited from the medial forebrain bundle and perifornical area is facilitated. Both the locus coeruleus-crus cerebri/Fields of Forel group and the substantia nigra-non-medial forebrain bundle group demonstrated direct ascending excitatory influences. Projections of the locus coreuleus through the ascending ventral noradrenergic bundle pass the crus cerebri and internal capsule while projections of the substantia nigra also pass through this region; both locus coeruleus terminals and substantia nigra terminals are found in the amygdala and cingulate cortex (Lindvall & Bjorklund, 1974). Farber et al. (in preparation) reports

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that locus coeruleus lesions cause reductions in substantia nigra ICSS in addition to crus cerebri/Fields of Forel ICSS. All of this data suggests that the locus coeruleus and substantia nigra cell bodies are the nodal points of one ascending excitatory system and where the integrity of the locus coeruleus seems necessary for full maintenance of ICSS behavior in the substantia nigra and its ascending fiber tract. A reciprocal relationship has not been tested investigating whether locus coeruleus ICSS needs an integral substantia nigra. Therefore, lesions of the locus coeruleus remove a vital link in the maintenancee of internal capsule ICSS, assuming that this region's capability to support ICSS is dependent upon locus coeruleus and/or substantia nigra fibers of passage.

Data from Experiment III suggests explanations for the facilitations in medial forebrain bundle ICSS as a result of locus coeruleus lesions. Interactions between either the locus coeruleus-medial forebrain bundle/perifornical area combination or the substantia nigra-medial forebrain bundle combination were such that response rates elicited under both conditions of each combination were similar. In contrast, interactions between the periaqueductal midbrain central gray-medial forebrain bundle/perifornical area combination elicited response rates which were significantly higher in the C-periaqueductal midbrain central gray, T-medial forebrain bundle condition than in the reverse condition. This data suggested that within the medial forebrain bundle/perifornical area, an ascending excitatory influence exists, probably the meso-limbic system which emanates from the mesencephalic catecholaminergic cell group

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A-10 in the midline ventral tegmentum. It was postulated that this system overlapped with the locus coeruleus/substantia nigra system in such forebrain areas as the accumbens, olfactory tubercles, cingulate cortex, and septum (Lindvall & Bjorklund, 1974; Lindvall, 1975). Thus, two excitatory systems, independent yet overlapping, possess terminal areas in those forebrain areas, particulary the septum where ICSS is elicited at low rates and often produces convulsions (Olds & Milner, 1954; Reid,Gibson,Gledhill,& Porter, 1964). Mattiace et al. (1976) has demonstrated that locus coeruleus lesions produce facilitations in septal and accumbens ICSS. The present study demonstrated that lesions of the locus coeruleus produce facilitations in medial forebrain bundle ICSS. It is apparent that removal of one excitatory influence (the locus coeruleus system) potentiates the response activity of a second excitatory system (the meso-limbic system), perhaps through some gating mechanism. Excitatory locus coeruleus influences, acting in a modulatory role, may be potentiating inhibitory systems (e.g., cholinergic, GABA, glycine interneurons) which in turn dissipate the excitatory meso-limbic system. Lesions of the locus coreuleus would remove inhibitory potentiation and thus indirectly produce lower thresholds (EPSPs) for the excitatory meso-limbic system. In this way, the only manifestation of the indirect disinhibition would be by stimulating the meso-limbic system itself. Therefore, locus coeruleus lesions would facilitate A-10, medial forebrain bundle, and septal/accumbens ICSS, while A-10 ICSS should have no effect on locus coeruleus ICSS because the locus coeruleus indirectly affects meso-

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limbic ICSS and not vice versa.

Evidence for GABA and glycine-containing neurons, acting in an inhibitory role on post-synaptic receptor sites excited by catecholamines, is found in ventro-medial hypothalamic mediation of lateral hypothalamic activity (Kimura, 1975) and in a negative feedback loop between dopaminergic cell bodies in the mesencephalon and GABA-containing neurons in the striatum (Groves et al., 1975). Ventro-medial hypothalamic excitatory influences on satiety arise from the periaqueductal and ventral noradrenergic bundle (Ahlskog & Hoebel, 1973; Gold, 1967), while lateral hypothalamic excitatory mechanisms on feeding arise from dopaminergic mesencephalic cell bodies (Zigmond & Stricker, 1973; Marshall, Richardson, & Teitelbaum, 1975). Thus, in the eating literature, evidence for this dichotomous influence of two excitatory systems is apparent where one (the noradrenergic locus coeruleus) system thorugh excitatory activity indirectly inhibits the second (the dopaminergic meso-limbic) system by means of activating an inhibitory system (GABA and glycine interneurons). Further evidence for inhibitory roles for GABA and glycine was demonstrated in Experiment IV where the effects of these inhibitors were seen in interactions. GABA and glycine seemed to exert maximal effects at times when many stimulated neurons were either in their absolute or relative refractory periods. The disinhibiting effects of picrotoxin and strychnine reduced the effectiveness of GABA and glycine to produce IPSPs. Therefore, it is tenable to suggest that GABA or glycine-containing neurons can exert inhibitory influences on the excitatory systems

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in question.

Another explanation of the effects of locus coeruleus lesions upon hypothalamic ICSS is that the dorsal and ventral noradrenergic bundles exert paradoxical influences upon one another, that is, the dorsal bundle excites some systems and inhibits others, while the ventral noradrenergic bundle does the reverse. Thus, if the locus coeruleus lesion disrupts dorsal bundle input and the hypothalamic placement is in a system of dorsal bundle inhibition, a facilitation should take place. However, if the hypothalamic placement is in a system of dorsal bundle excitation, a reduction in behavior should take place. Lesions of the locus coeruleus input to the ventral bundle should have opposite effects in this model.

The second significant result in Experiment V was the maintenance of an interaction between the locus coeruleus and hypothalamus despite the absence of post-lesion locus coeruleus ICSS where response rates elicited by delivery of staggered pulse pairs to the locus coeruleus and hypothalamus in the post-lesion locus coeruleus condition were significantly greater than the sum of the response rates elicited by delivery of C pulses to each site alone. This occurred under both conditions of the interaction for all animals. In two of the four interaction combinations, an interaction still occurred, yet response rates elicited under both conditions of the post-lesion testing were significantly less than the pre-lesion condition (Figure 33). As can be seen, the respective relationships among C-T intervals within a condition was still maintained, that is,

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increments or decrements in responding elicited by particular C-T intervals in the pre-lesion condition still occur in the post-lesion condition at overall lower response levels. In two animals, response rates elicited at short (.3, .5, .8, 1.0 msec.) C-T intervals in the post-lesion condition were significantly lower than response rates elicited at the same intervals in the pre-lesion condition. In contrast, response rates elicited at longer (2, 3, 5 msec.) C-T intervals in the post-lesion condition were significantly higher than response rates elicited at the same intervals in the pre-lesion condition. As Figures 31 and 32 demonstrate, pre-lesion interaction response rates increase slightly, though non-significantly, as C-T intervals increase from short to long. The effects of the locus coeruleus lesion seem to have changed the slope of the increase in the interaction. It should be noted that in both animals, the change occurred under both the C-locus coeruleus, T-medial forebrain bundle condition and the C-medial forebrain bundle, T-locus coeruleus condition.

A possible explanation for any maintenance of the interaction despite the absence of locus coeruleus ICSS is recruitment and stimulation of other cells or fibers under the field of the locus coeruleus stimulating electrode. A typical occurrence after a locus coeruleus lesion was an increase in resistance under the locus coeruleus electrode. This increase usually persisted over a few days, but would then steadily decrease. In the three animals with interactions post-lesion, but lower rates than baseline, the resistance remained at higher levels than pre-lesion, indicating that the current was passing through tissue with lower conductance capacitance

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(i.e., neuroglia rather than neurons). On the other hand, in the two animals with interactions which changed slope in the post-lesion condition after the initial rise, the resistance was comparable to baseline. In the first case, it seems that some neurons from the original group stimulated are still intact, and that the stimulation is relegated to this area. In this case, these remaining neurons are insufficient in number to support ICSS behavior themselves, but are enough to support an interaction with another ICSS site. In the second case, some original neurons are intact, but in addition, neurons outside the original field are now being stimulated; this may be due either to current spread or dendritic sprouting. These newly-stimulated neurons are also insufficient in number to support ICSS themselves, but capable of supporting an interaction with another ICSS site. In addition, the relationship between the newly-stimulated neurons and the second ICSS site is different than the relationship between the original neurons and the second ICSS site. Hence, one would expect a difference between responses elicited under the pre- and post-lesion conditions. This interaction data strongly suggests that an integral locus coeruleus is essential to maintain ICSS behavior from the dorsal pontine area. Part of the locus coeruleus can support neurophysiological interactions with other brain loci, but cannot support self-stimulation behavior itself. In the post-lesion interaction, the remaining cell bodies of the locus coeruleus are triggered by the C or T pulse of the interaction condition and their projections interact with the intact hypothalamic placement. A test of this hypothesis would be to insure a complete locus

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coeruleus lesion either electrolytically or chemically. With a complete destruction of locus coeruleus cell bodies, the interaction should cease.

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General Discussion

A catecholamine hypothesis for ICSS had been suggested by many investigators (see review, German & Bowden, 1974). This series of studies has demonstrated further credence for this hypothesis. The mapping study in Experiment I strongly suggests that it is catecholamine-containing cell bodies and fibers of passage which discretely support ICSS while areas closely adjacent to catecholamines play either an irrelevent or aversive role in ICSS behavior. Experiment II demonstrated subtle differences between within-site C-T functions across ICSS loci. The data correlated with the premise that in some cases, cell bodies were the source of ICSS; in some cases, fibers were the source of ICSS, and in some cases terminal areas. The cell body case correlated with within-site functions for the locus coeruleus, a pontine noradrenergic nucleus (Dahlstrom & Fuxe, 1964). The fiber case correlated with within-site functions for the hypothalamus and periaqueductal midbrain central gray, through which the major ascending noradrenergic and dopaminergic fiber groups traverse towards forebrain cell bodies (Dahlstrom & Fuxe, 1964). Finally, the terminal area case correlated with within-site functions for the septum, a telencephalic nuclear group with noradrenergic and dopaminergic terminal areas (Brownstein et al., 1974; Lindvall, 1975). In Experiment III, neurophysiological interaction enhancements were obtained either when two ICSS sites were tested or when one ICSS site was tested with a site demonstrating behavioral interest and histologically located in a

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site previously capable of supporting ICSS. On the other hand, a combination involving one ICSS site and a second site eliciting aversion did not elicit enhancements in the interaction. Thus, in keeping with previous studies (Ungerleider & Coons, 1970; German & Holloway, 1973; Ackermann, in preparation,a), neurophysiological interaction enhancements only occur between two areas capable of supporting ICSS; all of these placements contain catecholamines (Dahlstrom & Fuxe, 1964; Ungerstedt, 1971; Lindvall & Bjorklund, 1974).

Directionality of interactions also correlated with existing catecholaminergic pathways. In the locus coeruleus-crus cerebri/ Fields of Forel interaction, response rates were higher in the C-locus coeruleus, T-crus cerebri condtion than in the C-crus cerebri, T-locus coeruleus condition. These areas of the hypothalamus are adjacent to or traversed by the fibers of passage of the dorsal and ventral noradrenergic bundles and the nigro-striatal tract (Lindvall & Bjorklund, 1974). The fibers which traverse this hypothalamic area possess common terminal areas with fibers emanating from the locus coeruleus. Therefore, activation of two fiber bundles with common forebrain terminal areas can explain this result. In contrast, activation of one fiber bundle, one directly at the locus coeruleus, the other through hypothalamic path neurons in the vicinity of the bundle can explain the same result. Both explanations would predict that initial stimulation of the locus coeruleus by the C pulse would produce greater summative capabilities than initial stimulation of the crus cerebri by the C pulse. The common forebrain

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terminal area hypothesis necessitates initial locus coeruleus stimulation to provide adequate lead time so the two terminal points would release transmitter simultaneously to insure maximal summation. The path neuron hypothesis necessitates initial locus coeruleus stimulation to insure that the propagation from the pulse passes the hypothalamic area before the second pulse enters this area. This is necessary because path neuron stimulation would propagate pulses along the fiber of passage both orthodromically and antidromically, the latter cancelling any oncoming pulse. Both hypotheses suggest direct ascending connections for the locus coeruleus-crus cerebri group, and also serve to explain directionality of interaction for the substantia nigra-non-medial forebrain bundle group and the periaqueductal midbrain central gray-medial forebrain bundle group. In both of these cases, there is either a direct ascending path neuron connection in the hypothalamus with the nigro-striatal tract and meso-limbic system or hypothalamic areas share common terminal points with these tracts in forebrain areas, probably through the incerto-hypothalamic system (Bjorklund & Lindvall, 1975).

In three cases, ascending directionality of interactions did not occur: locus coeruleus-medial forebrain bundle/perifornical area; substantia nigra-medial forebrain bundle, and periaqueductal midbrain central gray-non-medial forebrain bundle. In these cases, a direct ascending connection through path neurons is less likely and that the terminal areas for each of these combinations may slightly overlap, but are not comparable to those discussed in the previous section. In two animals, response rates were significantly higher

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when the C pulse was delivered to the hypothalamic placement and the T pulse was delivered to the hindbrain placement than in the reverse condition; moreover, other animals with these combinations elicited response rate differentiations in this direction, but did not significantly so. It could be postulated on the basis of this data that strong descending influences exist and this could indeed be the case, although no predominantly descending catecholaminergic system has been identified (Lindvall & Bjorklund, 1974). One cannot preclude the possibility that this descending system is not catecholaminergic (Rolls & Cooper, 1974; Clavier & Routtenberg, 1974).

However, this differentiation can occur and be explained within the existing ascending catecholaminergic system. In the combinations of locus coeruleus-medial forebrain bundle/perifornical area, substantia nigra-medial forebrain bundle and periaqueductal midbrain central gray/non-medial forebrain bundle, there are not direct ascending connections between the two parts of any of these combinations. The locus coeruleus projections extend through the dorso-medial, medial, and ventro-lateral hypothalamus, while substantia nigra projections extend through the ventro-lateral hypothalamus. In both cases, medial forebrain bundle or perifornical area placements will neither be directly stimulating fibers of passage from the locus coeruleus or substantia nigra, nor directly stimulating path neurons which directly impinge upon these fibers of passage. Rather, stimulation of the medial forebrain bundle or perifornical area can have one or both of two possibilities: a) the path neurons can stimulate other path neurons which directly activate the fibers

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of passage from the locus coeruleus and substantia nigra, or b) the path neurons can directly stimulate the meso-limbic system which has slightly overlapping terminals with locus coeruleus or substantia nigra terminal areas. It seems that the first possibility can better explain the reverse condition differentiation in the locus coeruleus-medial forebrain bundle/perifornical area combination as compared to the locus coeruleus-crus cerebri/Fields of Forel combination. The reason why the C-medial forebrain bundle/perifornical area, T-locus coeruleus conditions elicits greater response rates than the C-locus coeruleus, T-medial forebrain bundle condition is because the medial forebrain bundle path neurons are indirectly and hence polysynaptically connected to the locus coeruleus fibers of passage. Thus, if the C pulse is delivered to the locus coeruleus, a long monosynaptic activation occurs which is quick and allows little summation time with the delayed T pulse which has to activate a number of connections before summation. However, if the C pulse is delivered to the medial forebrain bundle or perifornical area, this lead time allows the multi-synaptic connections to activate and summate with the delayed and longer-travelling monosynaptic activation from delivery of the T pulse in the locus coeruleus. The same explanations hold for the substantia nigra-medial forebrain bundle and periaqueductal midbrain central gray-non-medial forebrain bundle. Since the hypothalamic placements do not stimulate the ascending system by a direct route, polysnaptic connections are necessary to activate the ascending system indirectly with the result being a reversal in the relationship between the optimal delivery of pulse

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pairs.

In summary, locus coeruleus-crus cerebri/Fields of Forel combinations, substantia nigra-non-medial forebrain bundle combinations and periaqueductal midbrain central gray-medial forebrain bundle combinations elicit optimal response rates by delivery of the C pulse to the hindbrain site because these combinations possess direct ascending influences. In contrast, locus coeruleus-medial forebrain bundle combinations and periaqueductal midbrain central gray area-non-medial forebrain bundle combinations elicit similar response rates under both conditions because these combinations have only indirect ascending influences upon each other. Thus, a catehcolamine hypothesis can explain these results parsimoniously.

Differentiations in interaction conditions cannot be explained by inhibitory processes, that is, response rates which are differential under one condition as opposed to the second condition for particular combinations seem to be due to excitatory influences and not inhibitory influences. Strychnine, a potent inhibitor of glycine and picrotoxim, a potent inhibitor of GABA did not eliminate differentiations between conditions in an interaction combination which exhibited them during baseline, nor did these agents establish differentiations beteen conditions in an interaction combination which were similar during baseline. However, strychnine and picrotoxin selectively eliminated decrements in interaction responding at particular C-T intervals within a particular interaction condition. This disinhibitory effect of a particular drug was limited to one

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condition only, and seemed to affect the level of IPSPs produced at the post-synaptic part of the summative area. Neither strychnine nor picrotoxin demonstrated any generalized excitatory influence. The limitations of strychnine and picrotoxin to a disinhibitory mechanism provides further credence for catecholaminergic substrates of neurophysiological interactions because avialable literature suggests that roles for GABA and glycine act as inhibitors post-synaptically where catecholamines act as excitatory influences (Groves et al., 1975; Kimura, 1975; Porrino, 1975).

Lesions of the locus coeruleus selectively reduce ICSS within the internal capsule while facilitating ICSS in the medial forebrain bundle or perifornical area. The reductions and facilitations occur across the entire C-T function and last from fifteen to forty days post-lesion. Differences in placement of lesion affects the magnitude of the effect; lesions in the anterior one-third of the locus coeruleus are more effective in producing reductions in internal capsule ICSS and producing facilitations in medial forebrain bundle/perifornical area ICSS than lesions of the posterior two-thirds of the locus coeruleus. Predictions as to effect of lesion could be made on the basis of the type of interaction. Interactions demonstrating directionality elicited reductions in hypothalamic ICSS, while interactions demonstrating similarities between conditions elicited hypothalamic facilitations. An interaction still persisted after the lesion despite the absence of post-lesion locus coeruleus ICSS, suggesting that either some neurons remained which could sustain an interaction but were incapable of supporting ICSS, or neurons

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outside the original stimulating field were recruited to support the interaction, yet were insufficient to support ICSS.

The underlying mechanisms of the locus coeruleus lesions producing either reductions or facilitations can be explained in terms of the catecholamine system. In internal capsule ICSS, lesions of the locus coeruleus reduce this behavior through a direct depletion of fibers of passage. Activation of local path neurons under the hypothalamic electrode in the internal capsule do not in turn activate fibers of passage which are depleted by the lesion. Thus, a long-lasting reduction in ICSS would be expected.

On the other hand, lesions of the locus coeruleus could facilitate medial forebrain bundle ICSS through indirect disinhibition, that is, the activation of locus coeruleus neurons excite neurons of undetermined transmitter (e.g., GABA, glycine, acetylcholine, serotonin) which in turn inhibit medial forebrain bundle path neurons. However, at the same time, locus coeruleus neurons could also be exciting post-synaptic receptor sites which are also excited by meso-limbic neurons. Thus, the locus coeruleus excites forebrain areas excited by the meso-limbic system, but modulates meso-limbic activity by indirectly inhibiting medial forebrain bundle path neurons. Lesions of the locus coeruleus deactivate the inhibitory neurons, disinhibiting the medial forebrain bundle path neurons. Stimulation of the medial forebrain bundle under disinhibition produces more optimal summation for the same excitation and hence the meso-limbic system fires at higher rates. Since locus coeruleus and meso-limbic terminals share overlapping areas and since post-synaptic sites are

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affected by the lack of post-lesion locus coeruleus transmitter, the more highly activiated meso-limbic system triggers the supersensitive forebrain post-synaptic sites of the locus coeruleus together with forebrain areas normally activated by the meso-limbic system itself. This increase in activation elicits greater ICSS behavior.

All of these experiments lend credence to the hypothesis which implicates the catecholamines in motivated behavior. Moreover, the delineation of two types of directional interactions along two discrete ascending fiber systems suggests a dual influence on reinforced behavior which has been postulated by the laboratory of Ellman and Steiner (1971). Ellman and Steiner propose two reinforcement systems, one specific, the other non-specific. The non-specific reinforcement system can lower the threshold of any or all of the specific system and thus acts as a modulator of goal-directed activity. This non-specific system seems to be embodied in the locus coeruleus and substantia nigra cell bodies and their ascending fiber tracts which project to striatal motor areas, hippocampus, and cortex. Both the locus coeruleus and substantia nigra act as modulators of motor outflow and hence can be often viewed as a checking or gating influence. In contrast, the specific system seems to emanate from the ventral tegmental area and more closely follow classic limbic pathways including the medial forebrain bundle and perifornical area. Projections of the ventral tegmental area primarily innervate affective-coding nuclei such as the accumbens, amygdala, and septum. Thus, the ventral tegmental nuclei provide input to nuclei

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which affectively influence incoming sensory information. The balance of the two excitatory systems, one modulatory, one affective, provides the decision-making mechanisms for motivated and reinforced behavior.

This pulse pair technique is an invaluable means by which titration of stimulation can be controlled, and in so doing, contingencies of ICSS reinforcement. If animal models of psychopathological behaviors (Stein, 1973, 1974; Snyder et al., 1974) have any credence, this technique provides simple, yet well-controlled manipulation over ICSS behavior by which one can effectively test excitatory system models. Further work in this area may allow a braoder understanding of how basic coding of incoming stimuli is processed, along with identifying the various structures responsible for various components of the stimuli. Selective pharmacological, lesion, stimulation, and recording techniques applied with the pulse pair technique may allow us to separate the basic constituents of organized and reinforcing behavior.

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REFERENCES

Ackermann, R. F., Bodnar, R. J., Jackler, F., Steiner, S. S., & Ellman, S. J. Behavioral interactions occur in rat brainstem hypothalamic self-stimulation sites. In preparation. (a)

Ackermann, R. F., Bodnar, R. J., Jackler, F., Steiner, S. S., & Ellman, S. J. In preparation. (b)

Ahlskog, J. E., & Hoebel, F. G. Overeating and obesity from damage to a noradrenergic system in the brain. *Science*, 1975, *182*, 166-196.

Albino, R. C., & Lucas, J. W. Mutual facilitation of self-rewarding regions within the limbic system. *J. Comp. Physiol. Psychol.*, 1962, *55*, 182-185.

Amaral, D. G., & Foss, J. A. Locus coeruleus lesions and learning. *Science*, 1975, *188*, 377-388.

Anden, N. E., Dahlstrom, A., Fuxe, K., Larsson, K., Olson, L., & Ungerstedt, U. Ascending monoamine neurons to the telencephalon and diencephalon. *Acta physiol. scand.*, 1966, *67*, 313-326.

Anzelark, G. M., Walter, D. S., Arbuthnott, G. W., Crow, T. J., & Eccleston, D. The relationship between noradrenaline turnover in cerebral cortex and electrical self-stimulation through electrodes in the region of the locus coeruleus. *J. Neurochem.*, 1975, *24*, 677-681.

Aranow, S. The use of radio-frequency power in making lesions in the brain. *J. Neurosurgery*, 1960, *17*, 431-438.

Arbuthnott, G. W., Crow, T. J., Fuxe, K., Olson, L., & Ungerstedt, U. Depletion of catecholamines in vivo induced by electrical

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

stimulation of central monoamine pathways. Brain Research, 1970, 24, 471-483.

Belluzzi, J. D,. Ritter, S., Wise, C. D., & Stein, L. Substantia nigra self-stimulation: Dependence on noradrenergic reward pathways. Behav. Biol., 1975, 13, 103-111.

Bjorklund, A., & Lindvall, O. Dopamine in dendrites of substantia nigra neurons: Suggestions for a role in dendritic terminals. Brain Research, 1975, 83, 531-537.

Bjorklund, A., Lindvall, O., & Nobin, A. Evidence of an incerto-hypothalamic dopamine neurone system in the rat. Brain Research, 1975, 89, 29-42.

Black, W. C., & Cooper, B. R. Reduction of electrically rewarded behavior by interference with monoamine synthesis. Physiol. Behav., 1970, 5, 1405-1409.

Bodnar, R. J., Ellman, S. J., & Steiner, S. S. The effects of d- and l-amphetamine on electrical self-stimulation in the periaqueductal midbrain. Presented at Eastern Psychological Association, Phila., April 1974.

Bower, G. H., & Miller, N. E. Rewarding and punishing effects from stimulating the same place in the rat's brain. J. Comp. Physiol. Psychol., 1958, 51, 669-674.

Boyd, E. S., & Gardner, L. C. Effect of some brain lesions on intracranial self-stimulation in the rat. Am. J. Physiol., 1967, 213, 1044-1052.

Brauth, S. E., & Coons, E. E. Correlation of overt escape behavior, multiunit thalamic activity, and midbrain lemniscal stimulation in

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

rats. J. Comp. Physiol. Psychol., 1975, 89, 371-378.

Brownstein, M., Saavedra, J. M., & Palkovits, M. Norepinephrine and dopamine in the limbic system of the rat. Brain Research, 1974, 79, 431-436.

Caggiula, A. R. Analysis of the copulation-reward properties of posterior hypothalamic stimulation in male rats. J. Comp. Physiol. Psychol., 1969, 70, 399-412.

Caggiula, A. R., & Hoebel, B. G. Copulation-reward site in the posterior hypothalamus. Science, 1966, 153, 1284-1285.

Carter, D. A., & Phillips, A. G. Intracranial self-stimulation at sites in the dorsal medulla oblongata. Brain Research, 1975, 94, 155-160.

Carter, D. A., Phillips, A. G., & Fibiger, H. C. Neuropharamcological correlates of brain stimulation reward in the neostriatum. Presented at Society for Neuroscience, New York, November 1975.

Clavier, R. M., Phillips, A. G., & Fibiger, H. C. Effects of unilateral nigrostriatal bundle lesions with 6-hydroxydopamine on self-stimulation from the A-9 dopamine cell group. Presented at Society for Neuroscience, New York, November 1975.

Clavier, R. M., & Routtenberg, A. Ascending monoamine-containing fiber pathways related to intracranial self-stimulation: Histochemical fluorescence study. Brain Research, 1974, 72, 25-40.

Clavier, R. M., & Routtenberg, A. Brainstem self-stimulation attenuated by lesions of medial forebrain bundle but not by lesions of locus coeruleus or the caudal ventral norepinephrine bundle. Brain Research, 1976, 101, 251-271. (a)

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Clavier, R. M., & Routtenberg, A. Fibers associated with brainstem self-stimulation: Fink-Heimer study. Brain Research, 1976, 105, 325-332.

Coons, E. E. Motivational correlates of eating elicited by electrical stimulation in the hypothalamic feeding area. Doctoral Dissertation, Yale University, 1964 (Microfilm available from University Microfilms Inc., Ann Arbor, Mich.).

Coons, E. E., Smith, N., & Ungerleider, L. G. Uses of double-pulse stimulation behaviorally to infer refractoriness, summation, convergence and transmitter characteristics of hypothalamic reward systems. J. Comp. Physiol. Psychol., in press.

Cooper, B. Self-stimulation of the lateral hypothalamus, substantia-nigra and locus coeruleus: A role for dopamine. In symposium: The Role of Catecholamines in Central Mechanisms of Reward and Motivation. Presented at Eastern Psychological Association, New York, April 1975.

Cooper, B. R., & Breese, G. R. A role for dopamine in the psychopharmacology of electrical self-stimulation. In: B. K. Bernard, Ed., Aminergic Hypotheses of Behavior: Reality or Cliche? NIDA Monograph #3, Rockville, Md., 1975.

Cooper, B. E., Cott, J. M., & Breese, G. R. Effects of catecholamine-depleting drugs and amphetamine on self-stimulation of brain following various 6-hydroxydopamine treatments. Psychopharmacologia (Berl.), 1974, 37, 235-248.

Cooper, R. M., & Taylor, L. H. Thalamic reticular system and central gray: Self-stimulation. Science, 1967, 153, 102-103.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Coyle, J. T., & Snyder, S. H. Antiparkinsonian drugs: Inhibition of dopamine uptake in the corpus striatum as a possible mechanism of action. Science, 1969, 166, 899-901.

Coyle, J. T., & S. H. Catecholamine uptake by synaptosomes in homogenates of rat brain; stereospecificty in different areas. J. Pharmacol. Exp. Ther., 1970, 170, 221-231.

Craigie, E. H. Neuroanatomy of the Rat. New York: Academic Press, 1963.

Crow, T. J. Catecholamine-containing neurons and electrical self-stimulation. 1) A review of some data. Psychol. Med., 1972, 2, 414-421. (a)

Crow, T. J. A map of the rat mesencepahlon for electrical self-stimulation. Brain Research, 1972, 36, 265-273. (b)

Crow, T. J., Spear, P. J., & Arbuthnott, C. W. Intracranial self-stimulation with electrodes in the region of the locus coeruleus. Brain Research, 1972, 36, 275-287.

Dahlstrom, A., & Fuxe, K. Evidence for the existence of monoamines in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta physiol. scand., Suppl, 1964, 232, 1-55.

Deutsch, J. A. Behavioral measurement of the neural refractory period and its application to intracranial self-stimulation. J. Comp. Physiol. Psychol., 1964, 58, 1-9.

Deutsch, J. A., Howarth, C. J., Ball, G. G., & Deutsch, D. Threshold differentiation of drive and reward in the Olds effect. Nature, 1962, 196, 699-700.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Deutsch, J. A., Adams, D. W., & Metzner, R. J. Choice of intracranial stimulation as a function of delay between stimulations and strength of competing drives. J.Comp. Physiol. Psychol., 1964, 57, 271.

DiCara, L., Weaver, L., & Wolf, G. Comparison of DC and RF for lesioning white and gray matter. Physiol. Behav., 1974, 12, 1087-1090.

Domino, E. F., Wilson, A. E., & Olds, M. E. Relation of rat brain acetylcholine levels to duration of self-stimulation and escape behavior. Pharmacol. Biochem. Behav., 1975, 3, 103-107.

Dresse, A. Importance du systeme mesencephalo-telencephalique noradrenergique comme substratum anatomique du comportement d'autostimulation. Life Sci., 1966, 5, 1003-1014.

Eccles, J. C. The Physiology of Synapses. Berlin: Springer-Verlang, 1964.

Ellman, S. J. The relationship between ICSS and REM sleep: Implications for catecholamine systems. In symposium, The Role of Catecholamines in Central Mechanisms of Reward and Motivation. Presented at Eastern Psychological Association, New York, April 1975.

Ellman, S. J., Ackermann, R. F., Bodnar, R. J., Jackler, F., & Steiner, S. S. Comparison of behaviors elicited by electrical brain stimulation in dorsal brainstem and hypothalamus of rats. J. Comp. Physiol. Psychol., 1975, 88, 816-828.

Ellman, S. J., Ackermann, R. F., Bodnar, R. J., Jackler, R., & Steiner, S. S. D- and l-amphetamine differentially mediates self-stimulation in rat dorsal midbrain. Physiol. Behav., 1976, 16, 1-8.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Ellman, S. J., Ackermann, R. F., Farber, J., Mattiace, L. A., & Steiner, S. S. Relationship between dorsal brainstem sleep sites and intracranial self-stimulation. Physiol. Psychol., 1974, 2, 31-34.

Ellman, S. J., & Steiner, S. S. REM sleep: A CNS self-stimulation system. N.I.M.H. Grant, 1971-1974.

Erlanger, J., & Gasser, H. S. Electrical Signs of Nervous Activity. London: Oxford University Press, 1937.

Farber, J., Ellman, S. J., Mattiace, L. A., Ippolito, P. M., Halperin, R., Holtzman, A., & Steiner, S. S. In preparation.

Farber, J., Steiner, S. S., & Ellman, S. J. The pons as an electrical self-stimulation site. Psychophysiol., 1972, 9, 105.

Felton, D. L., Laties, A. M., & Carpenter, M. B. Monoamine-containing cell bodies in the squirrel monkey brain. Am. J. Anat., 1974, 139, 153-165.

Ferris, R. M., Tang, F. L. M., & Maxwell, R. A. A comparison of the capacities of the isomers of amphetamnine, deoxypipradol, and methylphenidate to inhibit the uptake of titrated catecholamines into rat cerebral cortex slices, synaptosomal preparations of rat cerebral cortex, hypothalamus and striatum and into adrenergic nerves of rabbit aorta. J. Pharmacol. Exp. Ther., 1972, 181, 407-416.

Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta physiol. scand., Suppl., 1965, 247, 37-85.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Gallistel, C. R. The incentive of brain stimulation reward. J. Comp. Physiol. Psychol., 1969, 69, 713-721. (a)

Gallistel, C. R. Self-stimulation: Failure of pretrial stimulation to affect rats' electrode preference. J. Comp. Physiol. Psychol., 1969, 69, 722-729. (b)

Gallistel, C. R. Self-stimulation: The neurophysiology of reward and motivation. In J. A. Deutsch (Ed.), The Physiological Basis of Memory. New York: Academic Press, 1973.

Gallistel, C. R., Rolls, E., & Greene, D. Neuron function inferred from behavioral and electrophysiological estimates of refractory period. Science, 1969, 166, 1028-1030.

German, D. C., & Bowden, D. M. Catecholamine systems as the neural substrate for intracranial self-stimulation. Brain Research, 1974, 73, 381-419.

German, D. C., & Holloway, F. A. Behaviorally determined neurophysiological properties of MFB self-stimulation fibers. Physiol. Behav., 1972, 9, 823-839.

German, D. C., & Holloway, F. A. Directionality of rewarding impulses within the medial forebrain bundle self-stimulation system of the rat. Science, 1973, 179, 1345-1347.

Gold, R. M. Aphagia and adipsia following unilateral and bilaterally assymetrical lesions in rats. Physiol. Behav., 1967, 2, 211-220.

Groves, P. M., Wilson, C. J., Young, S. J., & Rebec, G. V. Self-inhibition by dopaminergic neurons. Science, 1975, 190, 522-529.

Halboth, L. Unpublished doctoral dissertation, New York University, 1973 (microfilm obtainable from University Microfilsm, Inc.,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Ann Arbor, Mich.).

Halboth, P. H., & Coons, E. E. Behavioral measurements of the neural poststimulation excitability cycle in the lateral hypothalamic eating system of the rat. J. Comp. Physiol. Psychol., 1973, 83,

Harris, J. E., & Baldessarini, R. J. Uptake of 3-H catecholamines by homogenates of rat corpus striatum and cerebral cortex: Effects of amphetamine analogues. Neuropharmacol., 1973, 12, 669-678.

Hawkins, R., & Chang, J. Behavioral measurement of the neural refractory periods for stimulus-bound eating and self-stimulation in the rat. J. Comp. Physiol. Psychol.,1974, 86, 942-948.

Hoebel, B. G., & Teitelbaum, P. Hypothalamic control of feeding and self-stimulation. Science, 1962, 135, 375-377.

Hokfelt, T., Ljungdahl, A., Fuxe, K., & Johansson, O. Dopamine nerve terminals in the rat limbic cortex: Aspects of the dopamine hypothesis of schizophrenia. Science, 1974, 184, 177-179.

Howarth, C. J., & Deutsch, J. A. Drive decay: The cause of fast "extinction" of habits learned for brain stimulation. Science, 1962, 137, 35-36.

Hu, J. W. Refractory period of hypothalamic thirst pathway in the rat. J. Comp. Physiol. Psychol., 1973, 85, 463-468.

Jouvet, M. Biogenic amines and the states of sleep. Science, 1969, 163, 32-41.

Jouvet, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. In: Ergebnisse der Physiologie: Biologischen Chemie und Experimentallen Pharmacologia, Vol. 64. Berlin: Springer-Verlag, 1972.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Keesey, R. E. The relation between pulse frequency, intensity and duration and the rate of responding for intracranial stimulation. J. Comp. Physiol. Psychol., 1962, 55, 671-678.

Keesey, R. E. Duration of stimulation and the reward properties of hypothalamic stimulation. J. Comp. Physiol. Psychol., 1964, 58, 201-207.

Kestenbaum, R. D., Deutsch, J. A., & Coons, E. E. Behavioral measurement of neural post-stimulation excitability cycle: Pain cells in the brain of the rat. Science, 1970, 167, 393-394.

Kestenbaum, R. S., Deutsch, J. A., & Coons, E. E. Inference of refractory period, temporal summation and adaptation from behavior in chronic implants: Midbrain pain systems. J. Comp. Physiol. Psychol., 1973, 83, 412-428.

Kimura, H., & Kuriyama. Distribution of gamma-amino butyric acid (GABA) in the rat hypothalamus: Functional correlates of GABA with activities of appetite controlling mechanisms. J. Neurochem., 1975, 24, 903-907.

Kluver, H., & Barrera, E. A method for combined staining of cells and fibers in the nervous system. J. Neuropathol. Exp. Neurol., 1953, 12, 400-402.

Konig, J. F. R., & Klippel, R. A. The Rat Brain: A Stereotaxic Atlas. Williams & Wilkins: Baltimore, 1963.

Koob, G. F., Balcom, G. J., & Meyerhoff, J. L. Increases in intracranial self-stimulation in the posterior hypothalamus following unilateral lesions in the locus coeruleus. Brain Research, 1976, 101, 554-560.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Liebman, J. M., & Butcher, L. L. Effects of self-stimulation behavior of drugs influencing dopaminergic mechanisms. Nauvn-Schmeideberg's Arch. exp. Path. Pharmak., 1973, 277, 305-318.

Liebman, J. M., Mayer, D. J., & Liebeskind, J. C. Self-stimulation loci in the midbrain central gray matter of the rat. Behav. Biol., 1973, 9, 299-306.

Lindvall, O. Mesencephalic dopaminergic afferents to the lateral septal nucleus of the rat. Brain Research, 1975, 87, 89-95.

Lindvall, O., & Bjorklund, A. The organization of the ascending catecholamine neuron systems in the rat brain. Acta physiol. scand., Suppl., 1974, 412.

Lindvall, O., Bjorklund, A., Moore, R. Y., & Stenevi, U. Mesencephalic dopamine neurons projecting to neocortex. Brain Research, 1974, 31, 325-331.

Lindvall, O., Bjorklund, A., Nobin, A., & Stenevi, U. The adrenergic innervation of the rat thalamus as revealed by the glyoxylic fluorescence method. J. Comp. Neur., 1974, 154, 317-348.

Lippa, A. S., Antelman, S., Fisher, A., & Canfield, D. Neurochemical mediation of reward: A significant role for dopamine. Pharmacol. Biochem. Behav., 1973, 1, 23-28.

Lloyd, D. P. C. Facilitation and inhibition of spinal motoneurons. J. Neurophysiol.,1946, 9, 421-438.

Loizou, L. A. Projections of the nucleus locus coeruleus in the albino rat. Brain Research, 1969, 15, 563-566.

Lorens, S. A. Effect of lesions in the central nervous system on lateral hypothalamic self-stimulation in the rat. J. Comp. Physiol. Psychol., 1966, 62, 256-262.

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Lucas, K. Quantitative researches on the summation of inadequate stimuli in muscle and nerve. J. Physiol., 1910, 39, 461-475.

Maeda, T., & Shimizu, N. Projections ascendantes du locus coeruleus et d'autres neurons aminergiques au niveau du prosencephale du rat. Brain Research, 1972, 36, 19-35.

Margules, D. L. Noradrenergic rather than serotonergic basis of reward in the dorsal tegmentum. J. Comp. Physiol. Psychol., 1969, 67, 32-35.

Marshall, J. F., Richardson, J. S., & Teitelbaum, P. Nigrostrial bundle damage and the lateral hypothalamic syndrome. J. Comp. Physiol. Psychol., 1974, 87, 808-830.

Matthews, V., & Gallistel, C. R. Bilateral interactions in single units driven by MFB self-stimulation electrodes. Physiol. Behav., 1975, 15, 543-549.

Mattiace, L. A. In preparation.

Mattiace, L. A., Tempel A., Halperin, J., Mallon, V., Holtzman, A., Pramberger, Steiner, S. S., & Ellman, S. J. The effect of hindbrain lesions on septal self-stimulation in the rat. Presented at Eastern Psychological Association, New York, 1976.

Miller, N. E. Central stimulation and other new approaches to motivation and reward. Amer. Psychologist, 1958, 13, 100-108.

Millhouse, O. E. A Golgi study of the descending medial forebrain bundle. Brain Research, 1969, 15, 341-363.

Nauta, W. J. H. Hippocampal projections and related neural pathways to the midbrain in the cat. Brain, 1958, 81, 319-340.

Newman, L. M. Effects of cholinergic agonists and antagonists on self-stimulation behavior in the rat. J. Comp. Physiol. Psychol.,

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

1972, 79, 394-413.

Nobin, A., & Bjorklund, A. Topography of the monoamine neuron systems in the human brain as revealed in fetuses. Acta physiol. scan., Suppl. 388, 1973.

Olds, J. Self-stimulation of the brain. Science, 1958, 127, 315.

Olds, J., & Milner, P. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psychol., 1954, 47, 419.

Olds, J., & Peretz, B. A motivational analysis of the reticular activity system. Electroenceph. clin. Neurophysiol., 1960, 12, 445-454.

Olds, J., Travis, R. P., & Schwing, R. C. Topographic organization of hypothalamic self-stimulation functions. J. Comp. Phsyiol. Psychol., 1960, 53, 23-32.

Olds, M. E., & Olds, J. Approach-escape interactions in rat brain. Amer. J. Physiol., 1962, 203, 803-810.

Olds, M. E., & Olds, J. Effects on lesions in medial forebrain bundle on self-stimulation behavior. Amer. J. Physiol., 1969, 217, 1253-1264.

Olson, L., & Fuxe, K. Further mapping out of central noradrenergic neuron systems: Projections of the subcoeruleus area. Brain Research, 1972, 43, 289-295.

Palkovits, M., & Jacobowitz, D. M. Topographic atalas of catecholamine and acetylcholinestrerase-containing neurons in the rat brain. II. Hindbrain (Mesencephalon, Rhombencephalon). J. Comp. Neur., 1974, 157, 29-42.

Phillips, A. G. Dopaminergic pathways of reward: The role of the nigro-neostriatal system. In symposium: The Role of Catechola-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

amines in central mechanisms of reward and motivation. Presented at Eastern Psychological Association, New York, April, 1975.

Phillips, A. G., & Fibiger, H. C. Dopaminergic and noradrenergic substrates of positive reinforcement: Differential effects of d- and l-amphetamine. Science, 1973, 179, 575-577.

Phillips, A. G., Brooke, S. M., & Fibiger, H. C. Effects of amphetamine isomers and neuroleptics on self-stimulation from the nucleus accumbens and dorsal noradrenergic bundle. Brain Research, 1975, 35, 13-22.

Phillips, A. G., Carter, D. A., & Fibiger, H. C. Dopaminergic substrates of intracranial self-stimulation in the caudate-putamen. Brain Research, 1976, 104, 221-232.

Porrino, L. Time-dependent inhibition of lateral hypothalamic reward. Presented at Eastern Psychological Association, New York, April 1975.

Poschel, B., & Ninteman, F. Excitatory (antidepressant) effects of monoamine oxidase inhibitors on the reward system of the brain. Life Sci., 1964, 3, 909-910.

Reid, L. P., Gibson, W. E., Gledhill, S. M., & Porter, P. B. Anticonvulsant drugs and self-stimulation behavior. J. Comp. Physiol. Psychol.,1963, 204, 60-62.

Reynolds, R. W. Ventro-medial hypothalamic lesions without hyperphagia. Amer. J. Physiol., 1963, 204, 60-62.

Rinvik, E. Demonstration of nigothalamic connections in the cat by retrograde axonal transport of horseradish perioxidase. Brain Research, 1975, 90, 313-318.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Ritter, S., Belluzzi, J. D., Wise, C. D., & Stein, L. In preparation.

Ritter, S., & Stein, L. Self-stimulation of noradrenergic cell group (A-6) in locus coeruleus of rats. J. Comp. Physiol. Psychol., 1973, 85, 443-452.

Ritter, S., & Stein, L. Self-stimulation in the mesencephalic trajectory of the ventral noradrenergic bundle. Brain Research, 1974, 81, 145-157.

Roberts, W. W. Both rewarding and punishing effects from stimulation of posterior hypothalamus of cat with same electrode at same intensity. J. Comp. Physiol. Psychol., 1958, 51, 400-407.

Rolls, E. T. Contrasting effects of hypothalamic and nucleus accumbens septi self-stimulation on brain stem single unit activity and cortical arousal. Brain Research, 1971, 31, 275-285. (a)

Rolls, E. T. Involvement of brainstem units in medial forebrain bundle self-stimulation. Physiol. Behav., 1971, 7, 297-310. (b)

Rolls, E. T. Refractory periods of neurons directly excited in stimulus-bound eating and drinking in the rat. J. Comp. Physiol. Psychol., 1973, 82, 15-22.

Rolls, E. T., & Cooper, S. J. Connection between the prefrontal cortex and pontine brain-stimulation reward sites in the rat. Exp. Neurol., 1974, 42, 687-699.

Rose, M. D. Pain-reducing properties of rewarding electrical brain stimulation in the rat. J. Comp. Physiol. Psychol., 1974, 87, 607-617.

Ross, R. A., Joh, T. H., & Reis, D. J. Reversible changes in the accumulation and activities of tyrosine hydroxylase and dopamine-beta-hydroxylase in neurons of nucleus locus coeruleus during the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

retrograde reaction. Brain Research, 1975, 92, 57-72.

Ross, R. A., & Reis, D. J. Effects of lesions of locus coeruleus on regional distribution of dopamine-beta-hydroxylase activity in the rat brain. Brain Research, 1974, 73, 161-166.

Routtenberg, A. The two-arousal hypothesis: Reticular formation and limbic system. Psychol. Rev., 1968, 75, 51-80.

Routtenberg, A., & Malsbury, C. Brainstem pathways of reward. J. Comp. Physiol. Psychol., 1969, 68, 22-30.

Routtenberg, A., & Olds, J. Stimulation of dorsal midbrain during septal and hypothalamic self-stimulation. J. Comp. Physiol. Psychol., 1966, 62, 250-255.

Sherrington, C. The Integrative Action of the Nervous System. New Haven: Yale University Press, 1906.

Shute, C. C. D., & Lewis, P. R. The ascending cholinergic reticular system: Neocortical and subcortical projections. Brain, 1967, 90, 497-520.

Simon, L., Le Moal, M., & Cardo, B. Self-stimulation in the dorsal pontine tegmentum in the rat. Behav. Biol., 1975, 13, 339-347.

Smith, N., & Coons, E. E. Temporal summation and refractoriness in hypothalamic reward neurons as measured by self-stimulation behavior. Science, 1970, 169, 782-785.

Snyder, S. H., Banerjee, S. P., Yamamura, H. I., & Greenberg, D. Drugs, neurotransmitters and schizophrenia. Science, 1974, 184, 1243-1253.

Stein, L. Effects of interactions of imipramine, chlorpromazine, reserpine and amphetamine on self-stimulation: Possible neuro-

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

physiological basis of depression. Recent Advances in Biological Psychiatry, 1962, 4, 288.

Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. Fed. Proc., 1964, 32, 836-849.

Stein, L. Psychopharmacological substrates of mental depression. In: International Symposium on Antidepressant Drugs, M. Dukes, Ed. Amsterdam: Excerpta Mecia Foundation, 1967. Pp. 130-140.

Stein, L. Chemistry of reward and punishment. In: Psychopharmacology. A Review of Progress (1957-1967), D. Efron, Ed. Washington: U.S. Govt. Printing Office, Public Health Service Publication, 1968, 1836, 105-123.

Stein, L. Norepinephrine reward pathways: Role in self-stimulation, memory consolidation and schizophrenia. Nebraska Symposium on Motivation, 1974.

Stein, L., & Wise, C. D. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. J. Comp. Physiol. Psychol., 1969, 67, 189-198.

Stein, L., & Wise, C. D. Possible etiology of schizophrenia: Progressive damage to the noradrenergic reward system by 6-hydroxydopamine. Science, 1971, 171, 1032-1037.

Steiner, S. S., Bodnar, R. J., Ackermann, R. F., & Ellman, S. J. Escape from rewarding brain stimulation of dorsal brainstem and hypothalamus. Physiol. Behav., 1973, 11, 589-591.

Steiner, S. S., & D'Amato, M. R. Rewarding and aversive effects of amygdaloid self-stimulation as a function of current intensity. Psychon. Sci., 1964, 1, 27-28.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Steiner, S. S., & Ellman, S. J. Relation between REM sleep and intracranial self-stimulation. Science, 1972, 177, 1122-1124.

Steiner, S. S., & Stokely, S. Methamphetamine lowers self-stimulation thresholds. Physiol. Psychol., 1973, 1, 161-164.

Stephens, D. N., & Herberg, L. J. Catecholamines and self-stimulation: Pharmacological differences between near- and far-lateral hypothalamic sites. Brain Research, 1975, 90, 348-351.

Stinus, L., & Thierry, A. M. Self-stimulation and catecholamines. II. Blockade of self-stimulation by treatment with alpha-methyltyrosine and reinstatement by catecholamine precursors' administration. Brain Research, 1973, 64, 189-198.

Stinus, L., Thierry, A. M., & Cardo, B. Self-stimulation and local injections of 6-hydroxydopamine into the rat brain: Enhanced behavioral depressive effects of alpha-methyltyrosine. Pharmacol. Biochem. Behav., 1975, 3, 19-23.

Szabo, I. Path neuron system of medial forebrain bundle as a possible substrate for hypothalamic self-stimulation. Physiol. Behav., 1973, 10, 315-328.

Szabo, I., Lenard, L., & Kosaras, B. Drive decay theory of self-stimulation: Refractory periods and axon diameters in hypothalamic reward loci. Physiol. Behav., 1974, 12, 329-343.

Szabo, I., & Milner, P. M. Electrode tip alignment and self-stimulation: Influence of anodal hyperpolarization. Physiol. Behav., 1973, 11, 581-583.

Szabo, I., Nad, E., & Szabo, C. Pole reversals and hypothalamic self-stimulation: Ascending spread of rewarding excitation. Physiol. Behav., 1972, 9, 147-150. (a)

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Szabo, I., Rozkowska, E., & Kolta, P. Influence of contingent amygdaloid stimulation on lateral hypothalamic medial forebrain bundle self-stimulation. Physiol. Behav., 1972, 9, 839-849.

Taylor, K. M., & Snyder, S. H. Amphetamine: Differentiation by d- and l-isomers of behavior involving brain norepinephrine and dopamine. Science, 1970, 168, 1487-1489.

Thallman, R. H. Self-stimulation in dorsal midbrain of the rat. Presented at Society for Neuroscience, New York, November 1975.

Ungerleider, L., & Coons, E. E. A behavioral measure of homosynaptic and heterosynaptic temporal summation in the self-stimulation of rats. Science, 1970, 169, 785 - 787.

Ungerstedt, U. Stereotaxic mapping of monoamine pathways of the rat brain. Acta physiol. Scand., Suppl., 1971, 367, 1-48.

Valenstein, E. S. Problems of measurement and interpretation with reinforcing brain stimulation. Psychol. Rev., 1964, 71, 415-437.

Valenstein, E. S., & Beer, B. Unipolar and bipolar electrodes in self-stimulation experiments. Amer. J. Physiol., 1961, 201, 1181-1186.

Valenstein, E. S., & Campbell, J. F. Medial forebrain bundle-lateral hypothalamic area and reinforcing brain stimulation. Amer. J. Physiol., 1966, 210, 270-274.

Wise, C. D., & Stein, L. Facilitation of brain self-stimulation by central administration of norepinephrine. Science, 1969, 163, 299-301.

Yeomans, J. S. Behavioral measurement of absolute refractory periods, relative refractory periods, and supranormal periods of neurons mediating self-stimulation with unequal current pulse pairs.

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Presented at Society for Neuroscience, New York, November 1975.

Zigmond, M. J., & Stricker, E. M. Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalmic lesions. *Science*, 1973, *182*, 717-719.

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TABLE 1

Comparison of Self-Stimulation Response Rates between Electrode Tips

Rat	Tip	Localization	Response rate	p Level
A. Hypothalamus				
60G	Lat.	Posterior medial forebrain bundle	82.3	$p > .1$
	Med.	Ventral tegmental area of Tsai	84.2	
61G	Lat.	Medial forebrain bundle	53.0	$p > .1$
	Med.	Perifornical area	48.4	
62G	Lat.	Dorsal medial forebrain bundle	99.6	$p \leq .1$
	Med.	Dorsal medial forebrain bundle	111.9	
67G	Lat.	Internal capsule	104.8	$p > .1$
	Med.	Lateral medial forebrain bundle	103.6	
69G	Lat.	H2 Fields of Forel	31.6	$p \leq .01$
	Med.	Dorso-medial medial forebrain bundle	50.2	
72G	Lat.	Dorsal perifornical area	43.1	$p \leq .001$
	Med.	Dorso-medial nucleus of hypothalamus	18.1	
75G	Lat.	Periventricular nuclei of hypothalamus	16.1	$p > .1$
	Med.	Periventricular nuclei of hypothalamus	15.8	
76G	Lat.	Anterior H1 Fields of Forel	64.2	$p > .1$
	Med.	Anterior H1 Fields of Forel	65.8	
77G	Lat.	Ventro-medial nucleus of hypothalamus	20.5	$p > .1$
	Med.	Ventro-medial nucleus of hypothalamus	18.2	
78G	Lat.	Dorso-medial medial forebrain bundle	61.9	$p > .1$
	Med.	Dorso-medial medial forebrain bundle	65.3	

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Rat	Tip	Localization	Response rate	p Level
82G	Lat.	H1 Fields of Forel	8.2	$p \leq .001$
	Med.	H1 Fields of Forel	23.0	
86G	Lat.	Medial medial forebrain bundle	71.4	$p \leq .01$
	Med.	Dorso-medial nucleus of hypothalamus	56.7	
89G	Lat.	Medial forebrain bundle	101.2	$p \leq .01$
	Med.	Medial medial forebrain bundle	74.9	
94G	Lat.	Internal capsule	42.8	$p > .1$
	Med.	Lateral medial forebrain bundle	48.7	
7H	Lat.	Perifornical area	61.2	$p \leq .01$
	Med.	Dorso-medial nucleus of hypothalamus	41.7	
8H	Lat.	Lateral medial forebrain bundle	48.4	$p \leq .05$
	Med.	Medial forebrain bundle	55.1	
11H	Lat.	Perifornical area	40.0	$p > .1$
	Med.	Perifornical area	37.7	
21H	Lat.	Medial forebrain bundle	98.9	$p > .1$
	Med.	Perifornical area	97.4	
23H	Lat.	Ventro-lateral to medial forebrain bundle	60.3	$p > .1$
	Med.	Ventral to fornix above optic tract	53.8	
30H	Lat.	TIP BAD	-	-
	Med.	Dorso-medial medial forebrain bundle	50.4	
31H	Lat.	Medial forebrain bundle	33.3	$p \leq .001$
	Med.	Fornix	22.0	
33H	Lat.	Dorso-medial perifornical area	9.0	$p \leq .1$
	Med.	Anterior hypothalamic nucleus	7.4	

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Rat	Tip	Localization	Response rate	p Level
34H	Lat.	Medial forebrain bundle	72.0	$p \leq .05$
	Med.	Dorsal perifornical area	60.7	
36H	Lat.	Lateral medial forebrain bundle	14.4	$p \leq .001$
	Med.	Medial forebrain bundle	63.9	
38H	Lat.	Medial forebrain bundle	64.4	$p > .1$
	Med.	Fornix	60.4	
42H	Lat.	Dorsal medial forebrain bundle	43.1	$p \leq .01$
	Med.	Perifornical area	28.0	
47H	Lat.	Medial medial forebrain bundle	100.3	$p \leq .01$
	Med.	Lateral fornix	70.1	
59H	Lat.	Medio-ventral to medial forebrain bundle	29.6	$p \leq .02$
	Med.	Anterior nucleus of hypothalamus	20.1	
60H	Lat.	Lateral internal capsule	27.9	$p \leq .001$
	Med.	Internal capsule	43.5	
63H	Lat.	Medial medial forebrain bundle	85.7	$p > .1$
	Med.	Lateral perifornical area	80.4	
75H	Lat.	Anterior & lateral to medial forebrain bundle	20.4	$p > .1$
	Med.	Anterior & lateral to medial forebrain bundle	19.0	
77H	Lat.	Ventro-lateral medial forebrain bundle	17.6	$p \leq .001$
	Med.		50.6	
80H	Lat.	Medial forebrain bundle	31.7	$p \leq .001$
	Med.	Perifornical area	11.3	
89H	Lat.	Lateral to medial forebrain bundle	16.0	$p \leq .001$
	Med.	Lateral medial forebrain bundle	26.3	

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Rat	Tip	Localization	Response rate	p Level
1I	Lat.	Post medial forebrain bundle	56.6	$p \leq .1$
	Med.	perifornical area	64.9	
6I	Lat.	Medial forebrain bundle	36.6	$p \leq .001$
	Med.	Perifornical area	17.8	
17I	Lat.	Lateral medial forebrain bundle	20.9	$p \leq .001$
	Med.	Medial forebrain bundle	51.5	
B. Locus Coeruleus				
61G	Lat.	Locus coeruleus	20.5	$p > .1$
	Med.	Locus coeruleus/dorsal tegmental nucleus	20.4	
67G	Lat.	Ventral cerebe-lum	0.7	$p \leq .001$
	Med.	Locus coeruleus/dorsal tegmental nucleus	9.6	
69G	Lat.	Floor IV ventricle above locus coeruleus	24.6	$p \leq .001$
	Med.	Floor IV ventricle above dorsal tegmental nucleus	2.2	
72G	Lat.	Dorsal locus coeruleus	47.8	$p \leq .05$
	Med.	Dorsal tegmental nucleu	29.9	
88G	Lat.	Locus coeruleus	12.2	$p \leq .001$
	Med.	Area between locus coeruleus and dorsal raphe in posterior midbrain	2.2	
10H	Lat.	Locus coeruleus/brachium conjunctivum	55.8	$p \leq .001$
10H	Med.	Medial locus coeruleus	21.5	
11H	Lat.	Locus coeruleus	12.9	$p \leq .05$
	Med.	Locus coeruleus	14.8	
30H	Lat.	Ventral cerebellum (lingua) at benue	59.0	$p \leq .05$
	Med.	of VII nerve		

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Rat	Tip	Localization	Response rate	p Level
31H	Lat.	Locus coeruleus	10.2	$p > .1$
	Med.	Dorsal tegmental nucleus	9.4	
33H	Lat.	Midbrain locus coeruleus	31.5	$p \leq .01$
	Med.	Midbrain locus coeruleus	19.2	
34H	Lat.	Anterior locus coeruleus	19.7	$p \leq .1$
	Med.	Dorsal noradrenergic bundle lateral to dorsal raphe	12.1	
38H	Lat.	Subcoeruleus	26.4	$p \leq .001$
	Med.	Medial to subcoeruleus	14.9	
42H	Lat.	Dorsal locus coeruleus/forsal tegmental nucleus	18.6	$p \leq .001$
	Med.	Dorsal tegmental nucleus	0.0	
60H	Lat.	Midbrain locus coeruleus	11.9	$p > .1$
	Med.	Midbrain locus coeruleus	13.8	
73H	Lat.	Dorsal noradrenergic bundle	12.2	$p > .1$
	Med.	Medial to dorsal noradrenergic bundle	10.1	
74H	Lat.	Lateral to dorsal noradrenergic bundle	8.2	$p \leq .001$
	Med.	Dorsal noradrenergic bundle	16.8	
77H	Lat.	Posterior midbrain ventral	17.9	$p \leq .1$
	Med.	to dorsal tegmental nuclei	20.6	
80H	Lat.	Lateral locus coeruleus	7.7	$p \leq .001$
	Med.	Locus coeruleus	17.3	
85H	Lat.	Dorsal raphe	33.2	$p \leq .001$
	Med.	Lateral to dorsal raphe	11.9	
93H	Lat.	Postmidbrain dorsal noradrenergic bundle	27.6	$p \leq .001$
	Med.	Medial to dorsal noradrenergic bunlde	16.9	

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Rat	Tip	Localization	Response rate	p Level
94H	Lat.	Post midbrain dorsal noradrenergic bundle	10.2	$p \leq .001$
	Med.	Medial to dorsal noradrenergic bundle	5.6	
6I	Lat.	Lateral to locus coeruleus	7.7	$p \leq .001$
	Med.	Locus coeruleus	19.4	
17I	Lat.	Lateral to locus coeruleus	32.8	$p \leq .001$
	Med.	Locus coeruleus	47.6	
C. Periaqueductal Midbrain Central Gray/Substantia Nigra				
76G	Lat.	Edinger-Westphal nucleus	48.0	$p > .1$
	Med.	Edinger-Westphal nucleus	48.6	
77G	Lat.	Ventral tegmental decussation	47.9	$p \leq .05$
	Med.	Ventral tegmental decussation	65.0	
78G	Lat.	Lateral dorsal tegmental decussation	21.7	$p > .1$
	Med.	Dorsal tegmental decussation	25.2	
82G	Lat.	Ventro-lateral to oculomotor nucleus dorsal to red nucleus	1.8	$p \leq .001$
	Med.	Ventro-lateral to oculomotor nucleus	30.9	
86G	Lat.	Dorsal tegmental decussation	53.1	$p \leq .001$
	Med.	Lateral to dorsal tegmental decussation	17.8	
7H	Lat.	Edinger-Westphal nucleus	3.1	$p \leq .01$
	Med.	Medial longitudinal fasciculus	0.7	
21H	Lat.	Nucleus linearis/dorsal tegmental decussation	57.8	$p \leq .001$
	Med.	Lateral to dorsal tegmental decussation	33.0	
47H	Lat.	Dorsal nucleus linearis	32.1	$p \leq .05$
	Med.	Dorsal nucleus linearis	36.2	

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Rat	Tip	Localization	Response rate	p Level
59H	Lat.	Ventral tegmental decussation	44.3	$p \leq .001$
	Med.	Lateral to ventral tegmental decussation	18.0	
63H	Lat.	Lateral to medial longitudinal fasciculus	17.7	$p \leq .001$
	Med.	Medial longitudinal fasciculus	47.3	
75H	Lat.	Fasciculus retroflexus	0.0	$p \leq .001$
	Med.	Medial to fasciculus retroflexus; dorsal to III ventricle	4.4	
89H	Lat.	Dorso-lateral to red nucleus	4.0	$p \leq .001$
	Med.	Dorso-medial to red nucleus	24.2	
75G	Lat.	Substantis nigra, pars compacta/lateral medial lemniscus	8.7	$p \leq .05$
	Med.	Substantis nigra, pars compacta	14.0	
89G	Lat.	Substantia nigra, pars reticulata	4.6	$p \leq .01$
	Med.	Substantia nigra, pars reticulata	1.0	
36H	Lat.	Substantia nigra, pars reticulata	55.2	$p \leq .001$
	Med.	Substantia nigra, pars compacta	81.6	
D. Septum				
73H	Lat.	Lateral septum	5.3	$p \leq .05$
	Med.	Medial septum	7.0	
74H	Lat.	Medial septum	10.1	$p \leq .01$
	Med.	Medial septum	13.0	
82H	Lat.	Ventro-lateral septum	11.4	$p > .1$
	Med.	Ventro-lateral septum	8.5	
85H	Lat.	Medial septum	14.0	$p \leq .05$
	Med.	Medial septum	17.2	

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Rat	Tip	Localization	Response rate	p Level
93H	Lat.	Ventro-medial septum dorsal	22.4	$p > .1$
	Med.	to Isles of Caleja	19.9	
94H	Lat.	Accumbens	18.2	$p \leq .01$
	Med.	Accumbens	14.6	
98H	Lat.	Diagonal Band of Broca	27.5	$p \leq .01$
	Med.	Accumbens		

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TABLE 2

Response Rate Differentiations between ICSS Placements

Site 1	Site 2	No.	Site 1 sig* site 2	Site 1 non-sig. site 2	Site 2 non-sig. site 1	Site 2 sig* site 1
A. Hypothalamic Placements						
1) Medial forebrain bundle	Perifornical area/medial hypothalamic area	14	7	5	2	0
2) Medial forebrain bundle	Lateral to medial forebrain bundle	11	6	3	2	0
3) Perifornical area	Medial hypothalamic area	8	3	4	1	0
B. Locus Coeruleus Placements						
1) Locus coeruleus/dorsal noradrenergic bundle	Dorsal tegmental nucleus/medial to dorsal noardrenergic bundle	13	9	3	1	0
2) Locus coeruleus/dorsal noradrenergic bundle	Lateral to locus coeruleus and dorsal noradrenergic bundle	5	3	1	0	1
C. Periaqueductal Midbrain Central Gray Placements						
1) Midline placements	Lateral to midline placements	12	10	2	0	0

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Site 1	Site 2	No.	Site 1 sig* site 2	Site 1 non-sig. site 2	Site 2 non-sig. site 1	Site 2 sig* site 1
D. Substantia Nigra Placements						
1) Substantia nigra, pars compacta	Substantia nigra, pars reticulata	3	3	0	0	0
E. Septum						
1) Medial septum	Lateral septum	1	1	0	0	0

* A greater or lesser difference is determined only if the difference is significant at the .05 level; if the difference does ot meet this criterion, then it is considered equal.

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TABLE 3

Within-Site Functions in Hypothalamic, Locus Coeruleus, Periaqueductal Midbrain Central Gray and Septal ICSS Electrode Sites: Determinants of Microamperage, Point of Inflection and R-Scores

Site	Number of functions	Number of animals	Microamperage (uA)	Point of inflection (msec)	R-score
Medial forebrain bundle/ perifornical area	34	24	482.4	1.02	.7943
Non-medial forebrain bundle/ perifornical area	22	12	468.6	1.03	.7235
Locus coeruleus	27	23	481.5	1.15	.8150
Periaqueductal midbrain/ substantia nigra	19	14	466.3	1.02	.7380
Septum	7	7	492.9	1.30	.8590

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TABLE 4

Within-Site Functions in Hypothalamic and Hindbrain ICSS Electrode Sites: Differences between Bipolar and Monopolar Stimulation as Measured by Microamperage, Point of Inflection and R-Scores

Site	Number of Functions	Microamperage		Point of Inflection		R-score	
		Bipolar Condition	Monopolar Condition	Bipolar Condition	Monopolar Condition	Bipolar Condition	Monopolar Condition
Hypothalamus	12	488.3uA	379.2uA	1.14 msec	0.92 msec	.7490	.6064
Periaqueductal midbrain	2	375.0uA	240.0uA	0.73 msec	0.91 msec	.5884	.6756
Locus coeruleus	3	515.3uA	283.3uA	1.09 msec	1.27 msec	.7843	.8823
Total	17	480.0uA	345.9uA	1.09 msec	0.98 msec	.7187	.6617

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TABLE 5

Electrode Placements for Animals tested for Neurophysiological Interactions

Rat	Hindbrain placement	Forebrain placement	C-HB, T-FB Response rate	C-FB, T-HB Response rate	p Level
A.	Locus coeruleus-crus cerebri/Fields of Forel group (N = 5)				
67G	Dorso-medial locus coeruleus	Internal capsule	97.5	47.7	$p \leq .01$
69G	Dorso-medial locus coeruleus	Dorso-medial lateral hypothalamus/Fields of Forel	54.1	34.6	$p \leq .01$
10H	Dorsal noradrenergic bundle/ brachium conjunctivum	Medial amygdaloid nucleur	30.3	29.3	$p > .05$
42H	Dorso-medial locus coeruleus	Anterior Fields of Forel	35/6	31.9	$p \leq .01$
60H*	Locus Coeruleus	Medial internal capsule	25.2	23.6	$p \leq .05$
B.	Locus coeruleus-medial forebrain bundle/perifornical group)N = 9)				
61G	Locus coeruleus	Medial forebrain bundle	18.4	18.1	$p > .05$
11H	Medial locus coeruleus	Fornix	57.0	59.3	$p \leq .05$
31H*	Locus coeruleus	Lateral hypothalamus/medial forebrain bundle	27.9	25.8	$p > .05$
33H*	Locus coeruleus	Dorso-medial perifornical area	19.7	19.6	$p > .05$
34H	Locus coeruleus	Medial forebrain bundle	35.3	34.2	$p > .05$

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38H*	Sub-coeruleus	Medial forebrain bundle	55.2	56.8	p .05
77H*	Locus coeruleus	" " "	48.2	51.7	$p > .01$
80H*	" "	" " "	44.8	45.0	$p \leq .05$
6I*	" "	" " "	52.4	50.8	$p > .05$
17I*	" "	" " "	49.7	52.4	$p > .05$

C. Substantia nigra-non-medial forebrain bundle groups (N = 2)

75G	Substantia nigra, pars compacta	Periventricular hypothalamic nucleus	11.3	8.4	$p \leq .01$
23H	Substantia nigra, pars reticulata	Ventro-lateral to medial forebrain bundle	35.9	31.2	$p \leq .01$

D. Substantia nigra-medial forebrain bundle group (N = 2)

89G	Substantia nigra, pars reticulata	Medial forebrain bundle	33.6	37.3	$p > .05$
36H	Substantia nigra, pars compacta/ventral tegmental area	Medial forebrain bundle	100.8	103.4	$p > .05$

E. Periaqueductal midbrain central gray-medial forebrain bundle/perifornical area group (N = 4)

78G	Dorsal tegmental decussation	Medial medial forebrain bundle	48.7	42.5	$p \leq .01$
7H	Medial longitudinal fasciculus/Edinger-Westphal nucleus	Fornix	47.8	42.2	$p \leq .01$

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47H	Dorsal nucleus linearis	Medial medial forebrain bundle	65.5	61.9	$p \leq .01$
63H	Medial longitudinal fasciculus	Medial medial forebrain bundle	87.1	72.0	$p \leq .01$
F.	Periaqueductal midbrain central gray-non-medial forebrain bundle group (N = 8)				
76G	Edinger-Westphal nucleus	Anterior Fields of Forel	43.9	44.8	$p > .05$
82G	Ventro-lateral oculomotor nucleus	Lateral Fields of Forel	15.6	17.4	$p \leq .05$
82G	Ventro-lateral oculomotor nucleus	Medial Fields of Forel	26.3	27.0	$p > .05$
86G	Ventral oculomotor nucleus/medial longitudinal fasciculus	Dorso-medial nucleus of hypothalamus	37.8	41.7	$p > .05$
21H	Nucleus linearis	Ventro-lateral medial forebrain bundle	81.2	80.2	$p > .05$
59H	Ventral tegmental decussation	Ventro-lateral medial forebrain bundle	59.7	57.7	$p > .05$
75H	Medial to fasciculus retroflexus/dorsal to III ventr.	Lateral medial forebrain bundle	42.9	49.1	$p \leq .01$
89H	Dorsal to red nucleus	Lateral medial borebrain bundle	32.8	33.0	$p > .05$

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G. Locus coeruleus-septum group (N = 5)

88G	Locus coeruleus	Lateral septum	21.5	16.5	$p \leq .01$
73H	Dorsal noradrenergic bundle	Medial septum	29.1	28.6	$p > .05$
85H	Dorsal raphe	Medial septum	34.6	36.0	$p > .05$
93H	Dorsal noradrenergic bundle	Medial septum	35.9	35.0	$p > .05$
94H	Dorsal noradrenergic bundle	Accumbens	17.5	17.9	$p > .05$

* NOTE:-These animals underwent Experiment V after Experiment III where lesions of the locus coeruleus were made.

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TABLE 6

Summary of Directionality of Interaction for Six Site Combinations

	Site Combination	N	Directionality	Relationship
A.	Locus coeruleus (LC)-crus cerebri/Fields of Forel (CC)	5	Directly ascending	C-LC, T-CC > C-CC, T-LC
B.	Locus coeruleus (LC)-medial forebrain bundle/perifornical area (MFB)	9	Bidirectional descending	C-LC, T-MFB ≤ C-MFB, T-LC
C.	Substantia nigra (SN)-non-medial forebrain bundle (NMFB)	2	Directly ascending	C-SN, T-NMFB > C-NMFB, T-SN
D.	Substantia nigra (SN)-medial forebrain bundle (MFB)	2	Bidirectional	C-SN, T-MFB = C-MFB, T-SN
E.	Periaqueductal midbrain central gray (PMCG)-medial forebrain bundle/perifornical area (MFB)	4	Directly ascending	C-PMCG, T-MFB > C-MFB, T-PMCG
F.	Periaqueductal midbrain central gray (PMCG)-non-medial forebrain bundle/perifornical area (NMFB)	8	Bidirectional descending	C-PMCG, T-NMFB ≤ C-NMFB, T-PMCG

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TABLE 7

Summary of Elimination of Response Decrements in Interaction Functions

by Strychnine and Picrotoxin

		Strychnine		Picrotoxin	
	N	C-hindbrain T-hypothalamus	C-hypothalamus T-hindbrain	C-hindbrain T-hypothalamus	C-hypothalamus T-hindbrain
Locus coeruleus/crus cerebri	2	-	-	-	2
Locus coeruleus/medial forebrain bundle-perifornical area	2	1	-	1	-
Substantia nigra/non-medial forebrain bundle	1	-	1	-	-
Substantianigra/medial forebrain bundle	1	-	1	-	-
Periaqueductal midbrain central gray/medial forebrain bundle	2	-	-	1	-
Periaqueductal midbrain central gray/non-medial forebrain bundle	2	-	1	1	-
Total	10	1	3	3	2

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TABLE 8

Multiplicative Effects of Strychnine and Picrotoxin over Saline Baseline for the Two Neurophysiological Interaction Conditions

		C-T Interval (msec)								
	Condition	.3	.5	.8	1.0	1.2	1.5	2.0	3,0	5.0
Picrotoxin:	C-hypothalamus, T-hindbrain	0.97	0.81	0.78	0.89	1,02	0.93	1.05	0.97	1.06
Picrotoxin:	C-hindbrain, T-hypothalamus	0.95	1,02	1.00	0.96	1.08	0.96	0.95	0.97	0.92
Strychnine:	C-hypothalamus, T-hindbrain	0.95	1.03	1.03	1.02	1.04	1.00	1.08	0.97	1.15
Strychnine:	C-hindbrain, T-hypothalamus	0.94	0.89	0.98	1.03	1.03	0.97	0.96	0.97	1.03

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Figure Legends

1. Schematic representation of differential response rate areas in the hypothalamus. Area A corresponds with the medial forebrain bundle; area B corresponds with the perifornical area and far-lateral hypothalamus. Area C corresponds with the dorsal and medial hypothalamus. Area A supported the highest ICSS response rates; area C supported the lowest. This section corresponds with Figure 35b in Konig and Klippel (1963).
2. Schematic representation of differential response rate areas in the dorsal pons. Area A corresponds with the locus coeruleus and sub-coeruleus, while area B corresponds with areas lateral and medial to the locus coeruleus which support lower ICSS response rates. This section was drawn from the slides of Palkovits and Jacobowitz (1974).
3. Schematic representation of differential response rate areas in the mesencephalon. Area A-1 corresponds with the midline structures of the periaqueductal midbrain central gray, while area A-2 corresponds with the pars compacta of the substantia nigra. Area B-1 corresponds with areas lateral to the periaqueductal midbrain central gray, while area B-2 corresponds with areas lateral or ventral to the pars compacta of the substantia nigra. Both parts of area B supported lower ICSS rates thatn both parts of area A. This section corresponds with Figure 49b in Konig and Klippel (1963).
4. Representative within-site pulse-pair functions of response

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rate to C-T interval for electrode placements localized in the periaqueductal midbrain central gray.

5. Representative within-site pulse-pair functions of response rate to C-T interval for electrode placements localized in the hypothalamus. Sections A and C are functions elicited from non-medial forebrain bundle/perifornical area placements, while sections B and D are functions elicited from medial forebrain bundle/perifornical area placements.
6. Representative within-site pulse-pair functions of response rates to C-T interval for electrode placements localized in the locus coeruleus.
7. Representative within-site pulse-pair functions of response rates to C-T interval for electrode placements localized in the septum.
8. Representative within-site pulse-pair function of response rate to C-T interval for Rat 67G with an electrode placement localized in the internal capsule of the hypothalamus. (Standard errors of the mean are indicated for each interval.)
9. Representative within-site pulse-pair function of response rate to C-T interval for Rat 67G with an electrode placement localized in the locus coeruleus. (Standard errors of the mean are indicated for each interval.)
10. Pulse-pair function of response rate to C-T interval for Rat 67G with the C pulse delivered to the hypothalamus and the T pulse deliverd to the locus coeruleus. The triangle on the ordinate represents the sum of response rates elicited by

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delivery of C pulses to the hypothalamus alone and delivery of T pulses to the locus coeruleus alone. (Standard errors of the mean are indicated for each interval.)

11. Representative functions of response rate to C-T interval with the pulse pair split between electrode placements localized in the locus coeruleus and crus cerebri/Fields of Forel. Triangles represent response rates elicited by the delivery of the C pulse to the locus coeruleus and the T pulse to the crus cerebri/Fields of Forel. Circles represent response rates elicited by the delivery of the C pulse to the crus cerebri/Fields of Forel and the T pulse to the locus coeruleus.
12. Schematic representation of the locus coeruleus placements of animals tested in the locus coeruleus/hypothalamus interaction combination. Triangles represent the locus coeruleus electrode placements for the locus coeruleus-crus cerebri/Fields of Forel group. Circles represent the locus coeruleus electrode placements for the locus coeruleus-medial forebrain bundle/perifornical area group. The first two sections correspond with Figures 55b and 57b in Konig and Klippel (1963). The last three sections were drawn from the slides of Palkovits and Jacobowitz (1974).
13. Schematic representation of the hypothalamic placements tested in the locus coeruleus/hypothalamus interaction combination. Triangles represent the hypothalamic electrode placements for the locus coeruleus-crus cerebri/Fields of Forel group. Circles represent the hypothalamic electrode placements for the locus coeruleus-medial forebrain bundle/perifornical area

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group. All enumerated sections correspond with those in Konig and Klippel (1963).

14. Representative functions of response rate to C-T interval with the pulse pair split between electrode placements localized in the locus coeruleus and medial forebrain bundle/perifornical area. Triangles represent response rates elicited by the delivery of the C pulse to the locus coeruleus and the T pulse to the medial forebrain bundle/perifornical area. Circles represent response rates elicited by the delivery of the C pulse to the medial forebrain bundle/perifornical area and the T pulse to the locus coeruleus.
15. Representative functions of response rate to C-T interval with the pulse pair split between electrode placements localized in the substantia nigra and hypothalamic areas either <u>outside</u> of the medial forebrain bundle/perifornical area (A and B) or <u>inside</u> of the medial forebrain bundle/perifornical area (C or D). Triangles represent response rates elicited by the delivery of the C-pulse to the substantia nigra and the T pulse to the hypothalamic placement. Circles represent response rates elicited by the delivery of the C pulse to the hypothalamic placement and the T pulse to the substantia nigra.
16. Schematic representation of the substantia nigra placements tested in the substantia nigra/hypothalamus interaction combination. Triangles represent the substantia nigra electrode placements for the substantia nigra-<u>non</u>-medial forebrain bundle group. Circles represent the substantia nigra electrode place-

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ments for the substantia nigra-medial forebrain bundle group. All enumerated sections correspond with those in Konig and Klippel (1963).

17. Schematic representation of the hypothalamic placements tested in the substantia nigra/hypothalamus interaction combination. Triangles represent the hypothalamic electrode placements for the substantia nigra-non-medial forebrain bundle group. All enumerated sections correspond with those in Konig and Klippel (1963).
18. Representative functions of response rate to C-T interval with the pulse pair split between electrode placements localized in the periaqueductal midbrain central gray and medial forebrain bundle/perifornical area. Triangles represent response rates elicited by the delivery of the C pulse to the periaqueductal midbrain central gray and the T pulse to the medial forebrain bundle/perifornical area. Circles represent response rates elicited by the delivery of the C pulse to the medial forebrain bundle/perifornical area. Circles represent response rates elicited by the delivery of the C pulse to the medial forebrain bundle and the T pulse to the periaqueductal midbrain central gray.
19. Schematic representation of the periaqueductal midbrain central gray placements tested in the periaqueductal midbrain central gray/hypothalamus interaction combination. Triangles represent the periaqueductal midbrain central gray electrode placements for the periaqueductal midbrain central gray-medial forebrain

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bundle/perifornical area group. Circles represent the periaqueductal midbrain central gray electrode placements for the periaqueductal midbrain central gray-non-medial forebrain bundle/perifornical area group. The square represents the red nucleus placement for the red nucleus-medial forebrain bundle animal. All enumerated sections correspond with those in Konig and Klippel (1963).

20. Schematic representation of the hypothalamic placements tested in the periaqueductal midbrain central gray/hypothalamus interaction combination. Triangles represent the hypothalamic electrode placements for the periaqueductal midbrain central gray-medial forebrain bundle/perifornical area group. Circles represent the hypothalamic electrode placements for the periaqueductal midbrain central gray-non-medial forebrain bundle periformical area group. All enumerated sections correspond with those in Konig and Klippel (1963).

21. Representative functions of response rate to C-T interval with the pulse pair split between electrode placements localized in the periaqueductal midbrain central gray and non-medial forebrain bundle/perifornical area. Triangles represent response rates elicited by the delivery of the C pulse to the periaqueductal midbrain central gray and the T pulse to the non-medial forebrain bundle/perifornical area. Circles represent response rates elicited by the delivery of the C pulse to the non-medial forebrain bundle/perifornical area and the T pulse to the periaqueductal midbrain central gray.

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22. Response rate to C-T interval function for an animal with the pulse pair split between electrode placements localized in the red nucleus and medial forebrain bundle. Triangles represent the response rates elicited by the delivery of the C pulse to the red nucleus and the T pulse to the medial forebrain bundle. Circles represent response rates elicited by the delivery of the C pulse to the medial forebrain bundle and the T pulse to the red nucleus. The thin line notates the response rate elicited by delivery of the C pulse to the medial forebrain bundle alone.
23. Representative functions of response rate to C-T interval with the pulse pair split between electrode placements localized in the locus coeruleus and either in the lateral septem (A) or medial septum (B). Triangles represent response rates elicited by the delivery of the C pulse to the locus coeruleus and the T pulse to the septum. Circles represent response rates elicited by the delivery of the C pulse to the septum and the T pulse to the locus coeruleus.
24. Schematic representation of the locus coeruleus placements tested in the locus coeruleus/septum interaction combination. Triangles represent the locus coeruleus electrode placements for the locus coeruleus-lateral septum group. Circles represent the locus coeruleus electrode placements for the locus coeruleus-medial septum group. The first two sections correspond with Figures 54b and 55b in Konig and Klippel (1963). The last three sections were drawn from the slides of Palkovits and Jacobowitz (1974).

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25. Schematic representation of the septal placements tested in the locus coeruleus/septum interaction combination. Triangles represent the septal electrode placements for the locus coeruleus-lateral septum group. Circles represent the septum electrode placements for the locus coeruleus-medial septum group. All enumerated sections correspond with those in Konig and Klippel (1963).

26. Representative functions of response rate to C-T interval with the pulse pair split between brainstem and hypothalamic electrode placements. In sections A and B, the C pulse was delivered to the brainstem electrode placement and the T pulse was delivered to the brainstem electrode placement. In all sections, circles represent response rates elicited by the delivery of appropriate pulse pairs after the administration of intraperitoneal injections of saline. In sections A and C, triangles represent response rates elicited by the delivery of appropriate pulse pairs after the administration of intraperitoneal injections of strychnine (.5 mg/ml/kg). In sections B and D, triangles represent response rates elicited by the delivery of appropriate pulse pairs after the administration of intraperitoneal injections of picrotoxin (.75 mg/ml/kg).

27. Within-site pulse pair function of response rate to C-T interval for the perifornical electrode placement of Rat 33H. In all sections, circles represent the baseline response rate elicited from each C-T interval before the locus coeruleus lesion. In section A, triangles represent the elicited response rates

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for days 1-5 after the locus coeruleus lesion; squares represent the elicited response rates for days 6-10 after the locus coeruleus lesion. In section B, triangles represent response rates for days 16-20 post-locus coeruleus lesion. In section C, triangles represent response rates for days 21-25 post-locus coeruleus lesion;squares represent response rates for days 26-30 post-locus coeruleus lesion.

28. Within-site pulse pair function of response rate to C-T interval for the medial forebrain bundle electrode placement of Rat 38H. In all sections, circles represent the baseline response rate elicited from each C-T interval before the locus coeruleus lesion. In section A, triangles represent the elicited response rates for days 1-5 after the locus coeruleus lesion; squares represent the elicited response rates for days 6-10 after the locus coeruleus lesion. In section B, triangles represent response rates for days 11-15 post-locus coeruleus lesion; squares represent response rates for days 16-20 post-locus coeruleus lesion. In section C, triangles represent response rates for days 21-25; squares 26-30. In section D, triangles represent response rates for days 31-35; squares 36-40.

29. Within-site pulse pair function of response rate to C-T interval for the medial forebrain bundle electrode placement of Rat 77H. In all sections, circles represent the baseline response rate elicited from each C-T interval before the locus coeruleus lesion. In section A, triangles represent the elicited response

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rates for days 1-5 after the locus coeruleus lesion; squares represent the elicited response rates for days 6-10 after the locus coeruleus lesion. In section B, triangles represent response rates for days 11-15 post locus coeruleus lesion; squares represent response rates for days 16-20 post locus coeruleus lesion. In section C, trianges represent response rates for days 21-25 post locus coeruleus lesion.

30. Within-site pulse pair function of response rate to C-T interval for the medial forebrain bundle electrode placement of Rat 31H. In all sections, circles represent the baseline response rate elicited from each C-T interval before the locus coeruleus lesion. In section A, triangles represent the elicited response rates for days 1-5 after the locus coeruleus lesion; squares represent the elicited response rates for days 6-10 after the locus coeruleus lesion. In section B, triangles represent response rates for days 11-15 post locus coeruleus lesion; squares represent response rates for days 16-20 post locus coeruleus lesion. In section C, triangles represent response rates for days 21-25 post locus coeruleus lesion; squares represent response rates for days 26-30 post locus coeruleus lesion.

31. Function of response rate to C-T interval with the pulse pair split between electrode placements localized in the locus coeruleus and perifornical area for Rat 33H. In sections A-1 and A-2, the C pulse was delivered to the perifornical area and the T pulse was delivered to the locus coeruleus; in sections B-1 and B-2, the C pulse was delivered to the locus coeru-

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leus and the T pulse was delivered to the perifornical area. In all sections, circles represent the baseline response rate elicited from the respective interaction conditions before the locus coeruleus lesion. In sections A-1 and B-1, triangles represent the respective elicited response rates for days 1-10 after the locus coeruleus lesion; squares represent the respective elicited response rates for days 11-20 after the locus coeruleus lesion. In sections A-2 and B-2, triangles represent the respective elicited response rates for days 21-30 after the locus coeruleus lesion.

32. Function of response rate to C-T interval with the pulse pair split between electrode placements localized in the locus coeruleus and medial forebrain bundle for Rat 38H. In sections A-1 and A-2, the C pulse was delivered to the medial forebrain bundle and the T pulse was delivered to the locus coreuleus. In sections B-1 and B-2, the C pulse was delivered to the locus coeruleus and the T pulse was delivered to the medial forebrain bundle. In all sections, circles represent the baseline response rate elicited from the respective interaction conditions before the locus coeruleus lesion. In sections A-1 and B-1, triangles represent the respective elicited response rates for days 1-10 after the locus coeruleus lesion; squares represent the respective elicited response rates for days 11-20 after the locus coeruleus lesion. In sections A-2 and B-2, triangles represent the respective elicited response rates for days 21-30 after the locus coeruleus lesion; squares represent the respec-

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tive elicited response rates for days 31-40 after the locus coeruleus lesion.

33. Function of response rate to C-T interval with the pulse pair split between electrode placements localized in the locus coeruleus and medial forebrain bundle for Rat 77H. In section A, the C pulse was delivered to the medial forebrain bundle and the T pulse was delivered to the locus coeruleus. In section B, the C pulse was delivered to the locus coeruleus and the T pulse was delivered to the medial forebrain bundle. In all sections, circles represent the baseline response rate elicited from the respective interaction conditions before the locus coeruleus lesion. In all sections, triangles represent the respective elicited response rates for days 1-10 after the locus coerulues lesion; squares represent the respective elicited response rates for days 11-20 after the locus coeruleus lesion.

34. Within-site pulse pair function of response rate to C-T interval for the internal capsule electrode placement of Rat 60H. In both sections, circles represent the baseline response rate elicited from each C-T interval before the locus coeruleus lesion. In section A, triangles represent the elicited response rates for days 1-5 after the locus coeruleus lesion; squares represent the elicited response rates for days 6-10 after the locus coeruleus lesion. In section B, triangles represent the elicited response rates for days 11-15 post locus coeruleus lesion.

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FIGURE 1

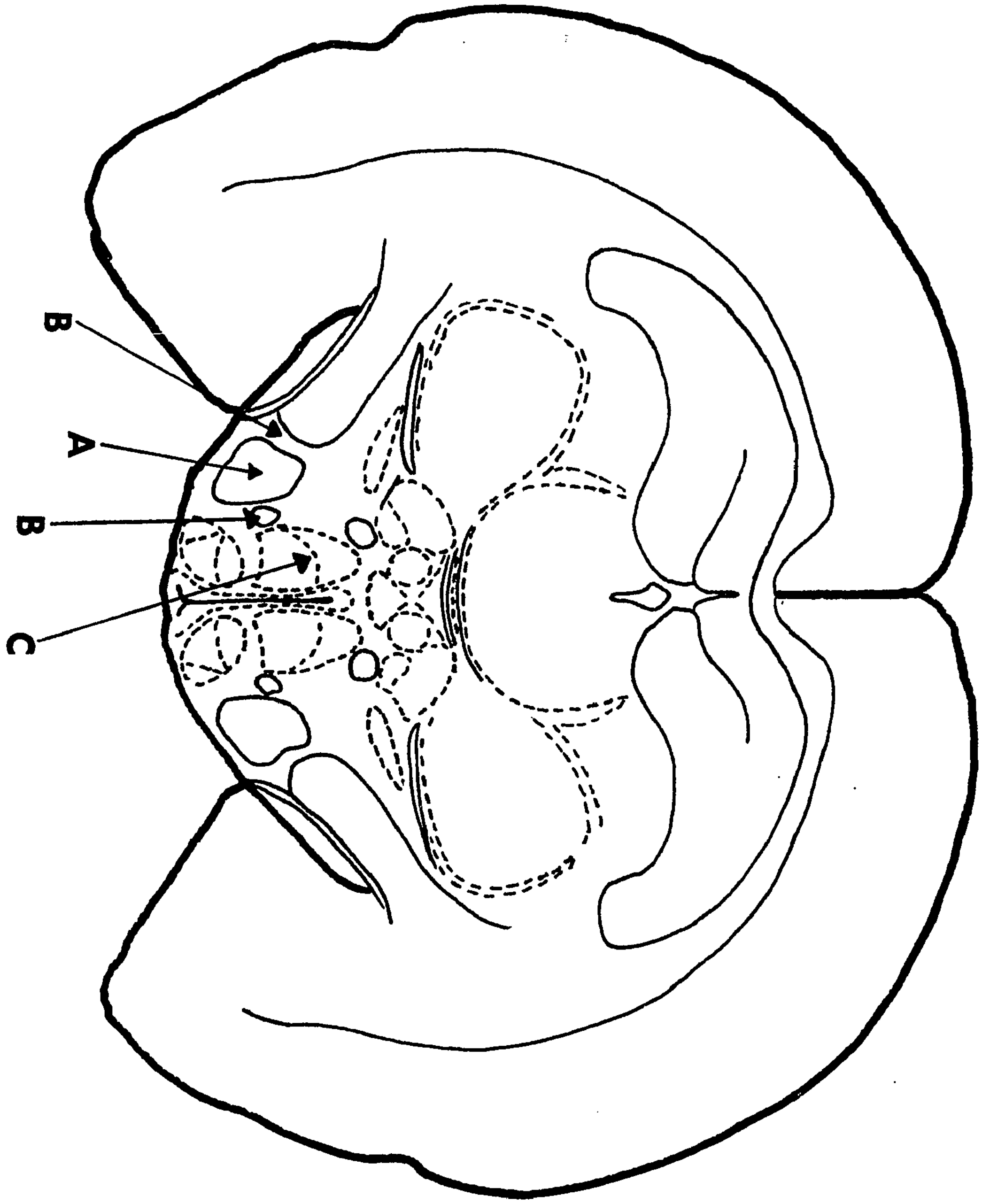

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FIGURE 2

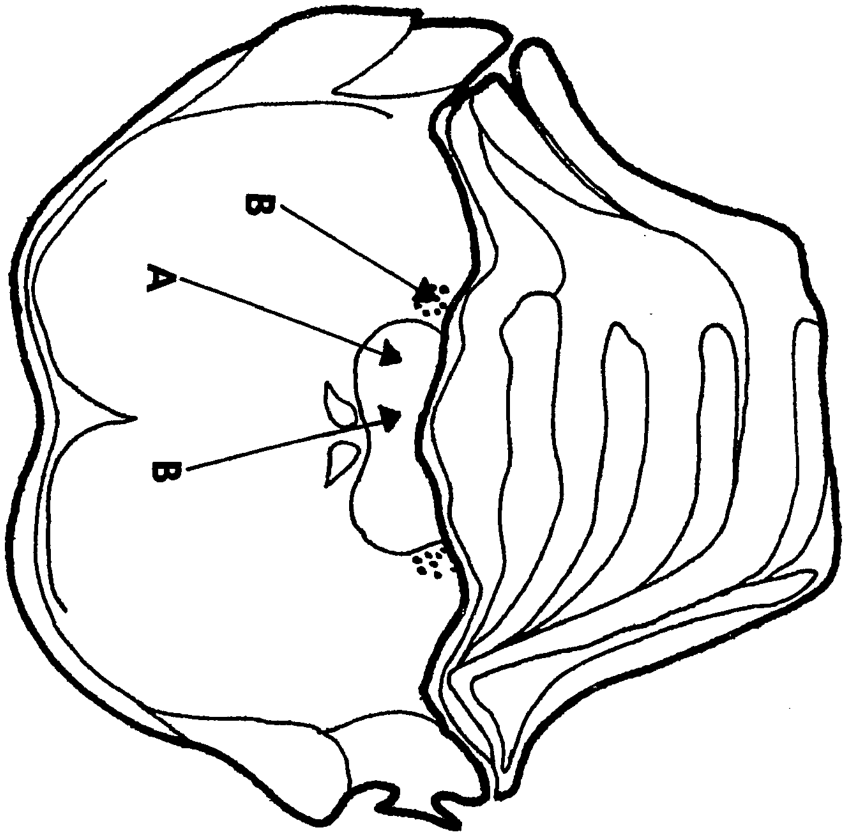

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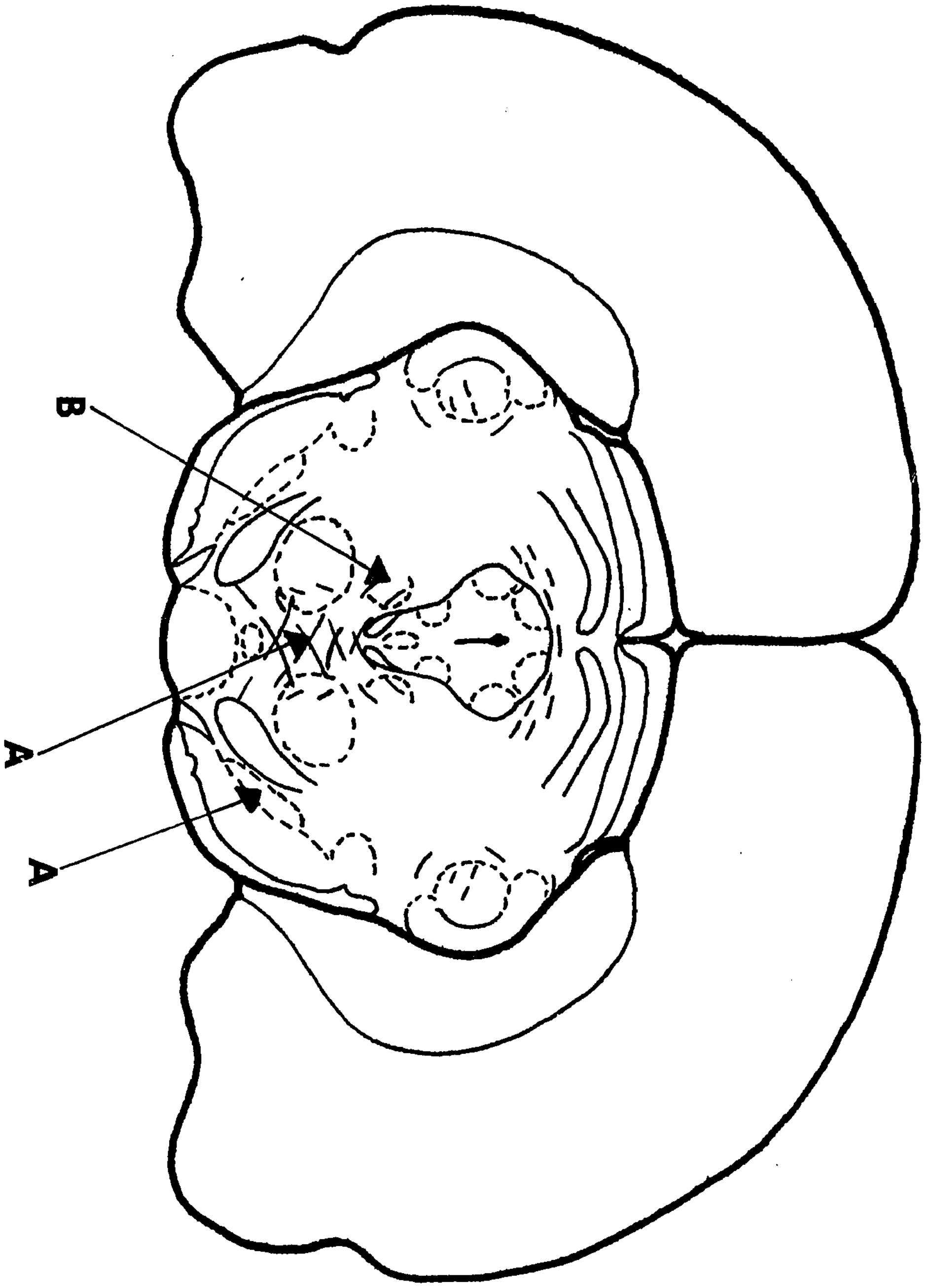
FIGURE 3



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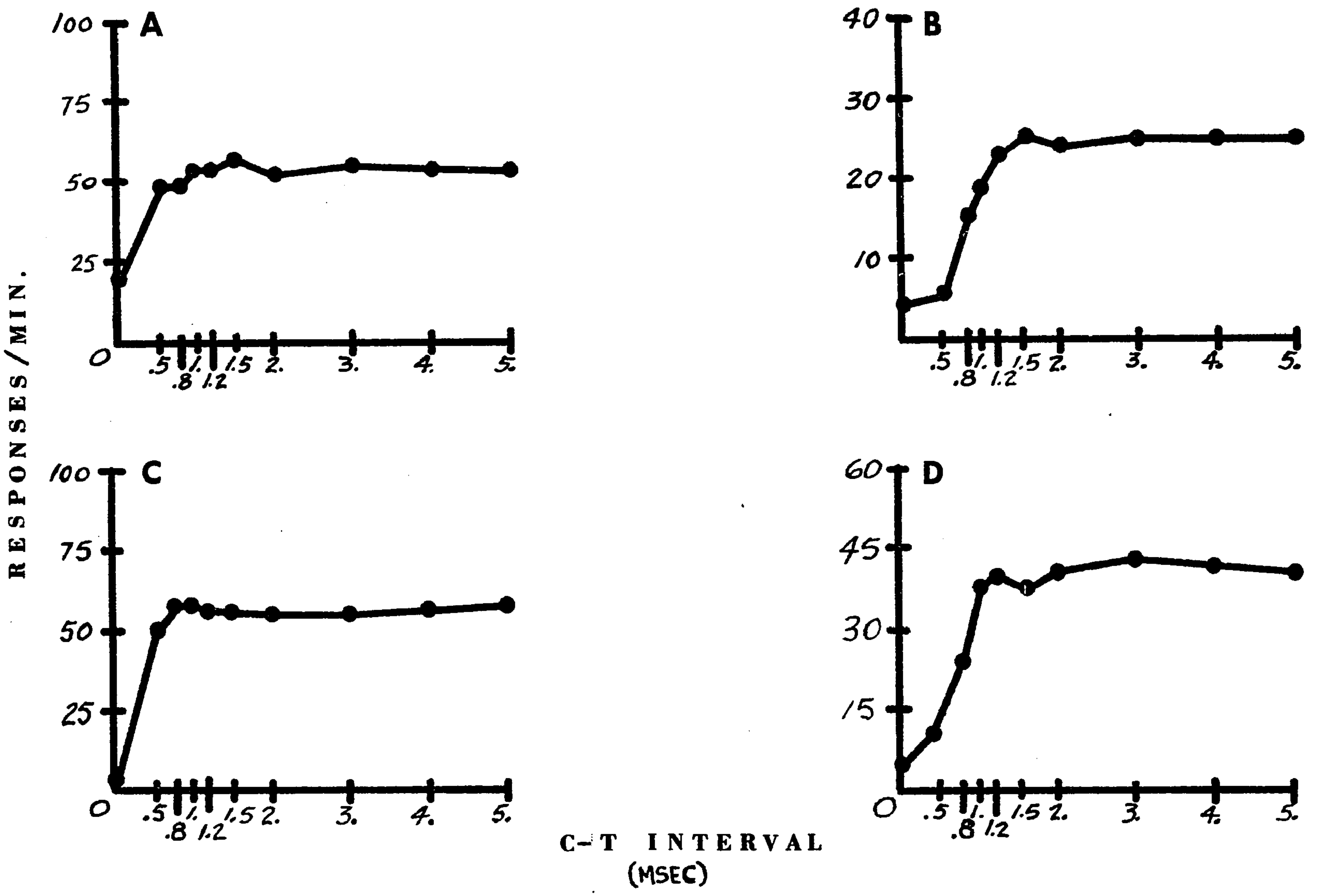

FIGURE 4

MIDBRAIN CENTRAL GRAY

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FIGURE 5

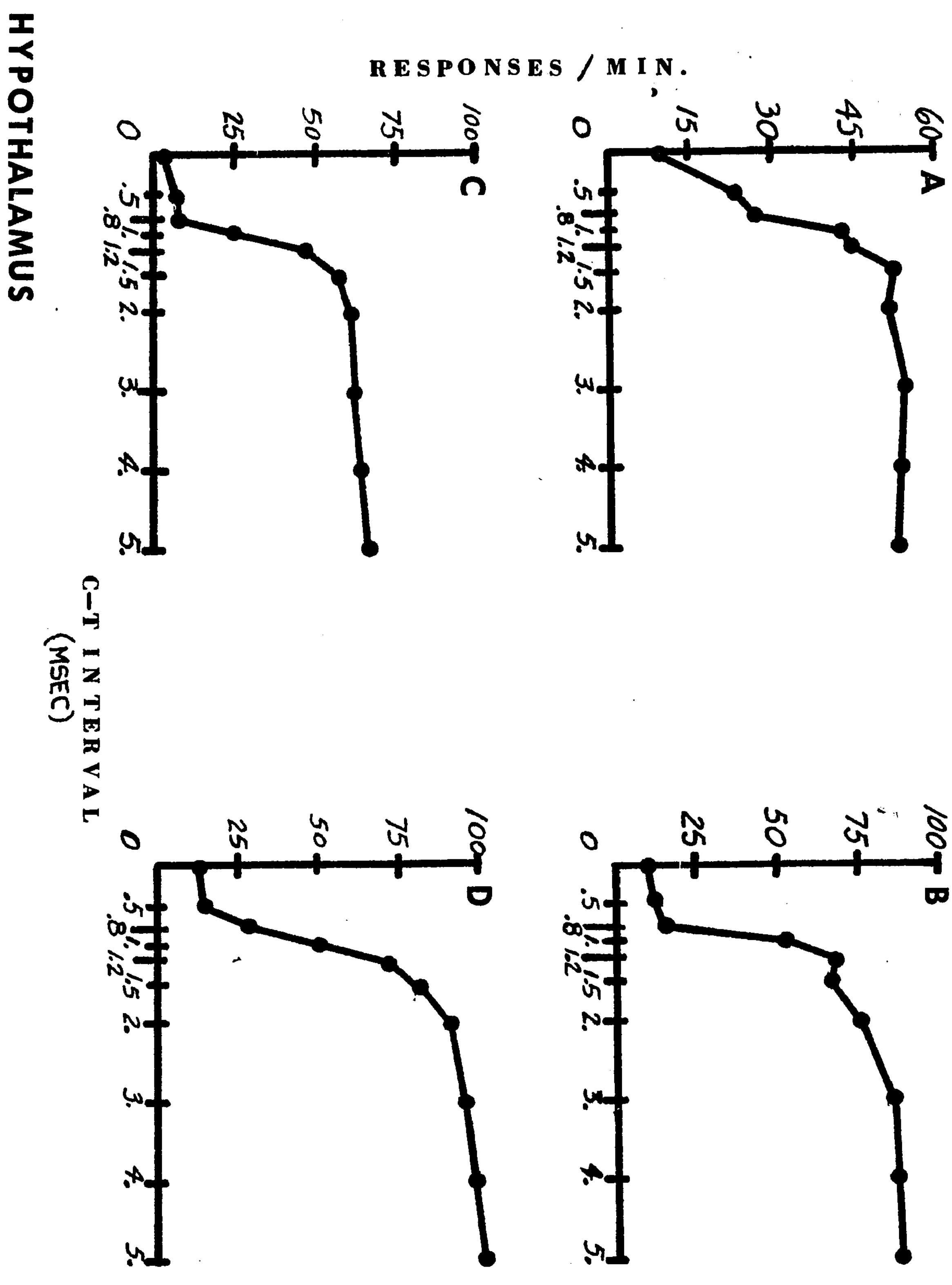

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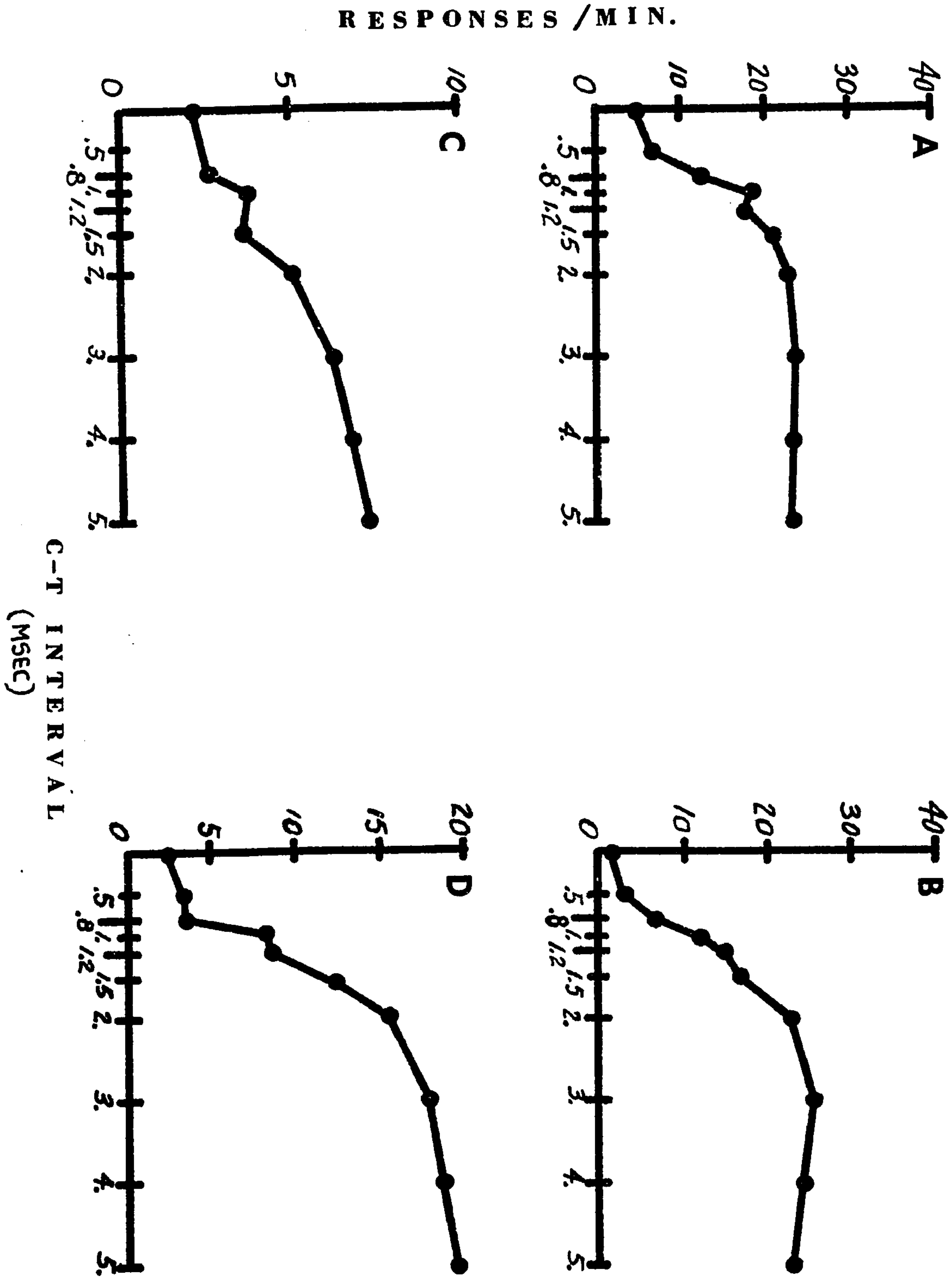
FIGURE 6



LOCUS COERULEUS

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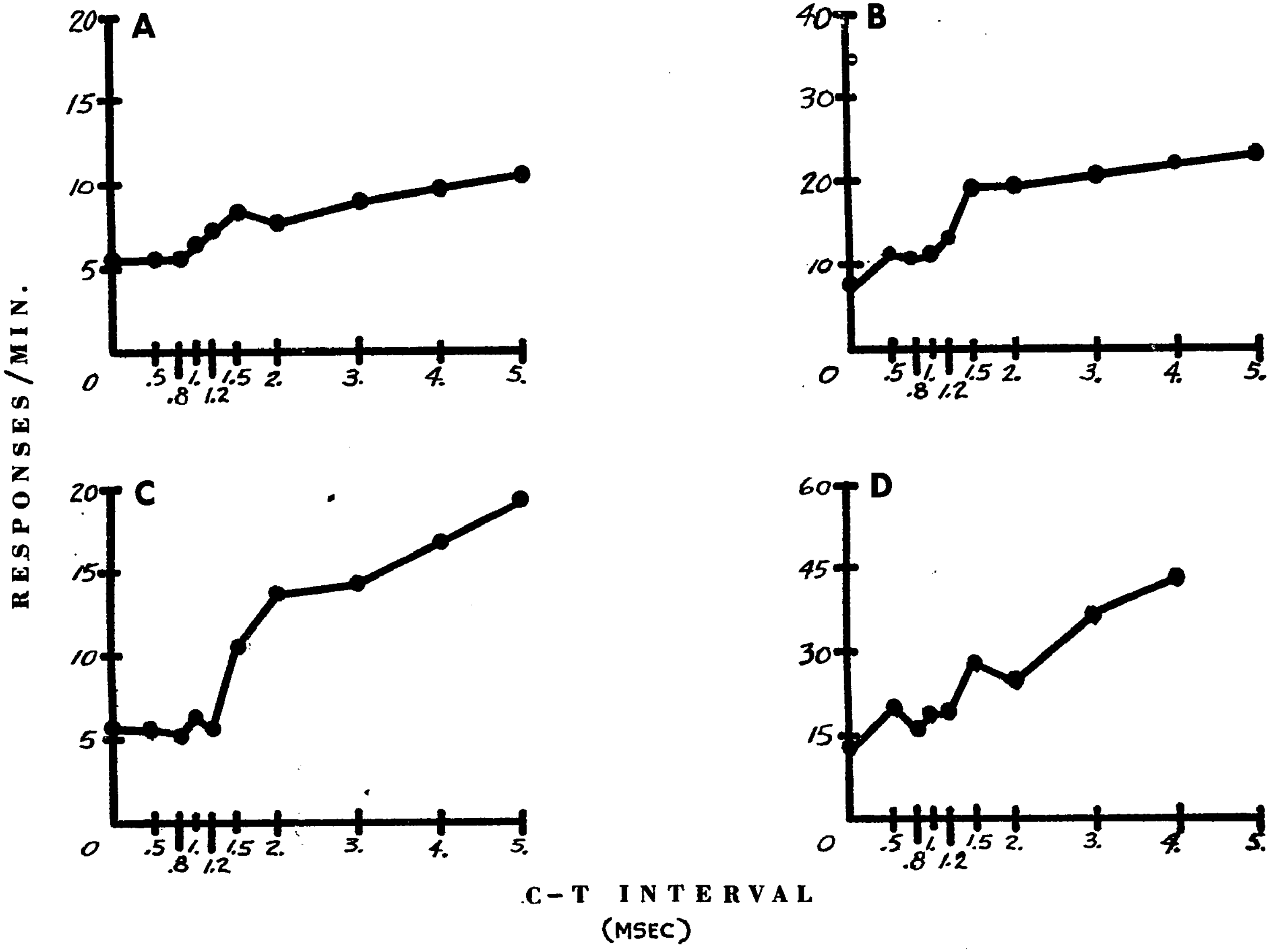

FIGURE 7

SEPTUM

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FIGURE 8

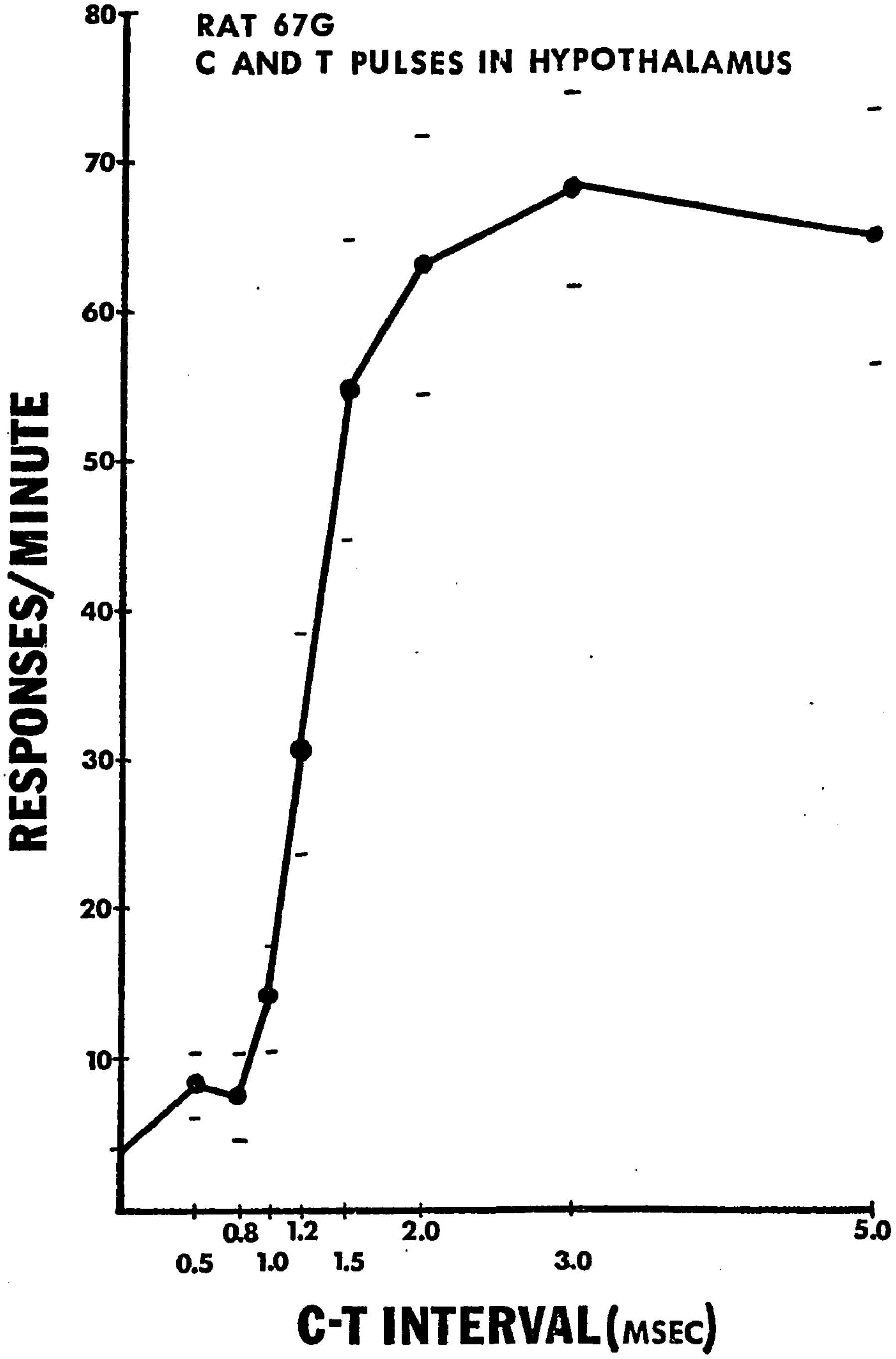

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FIGURE 9

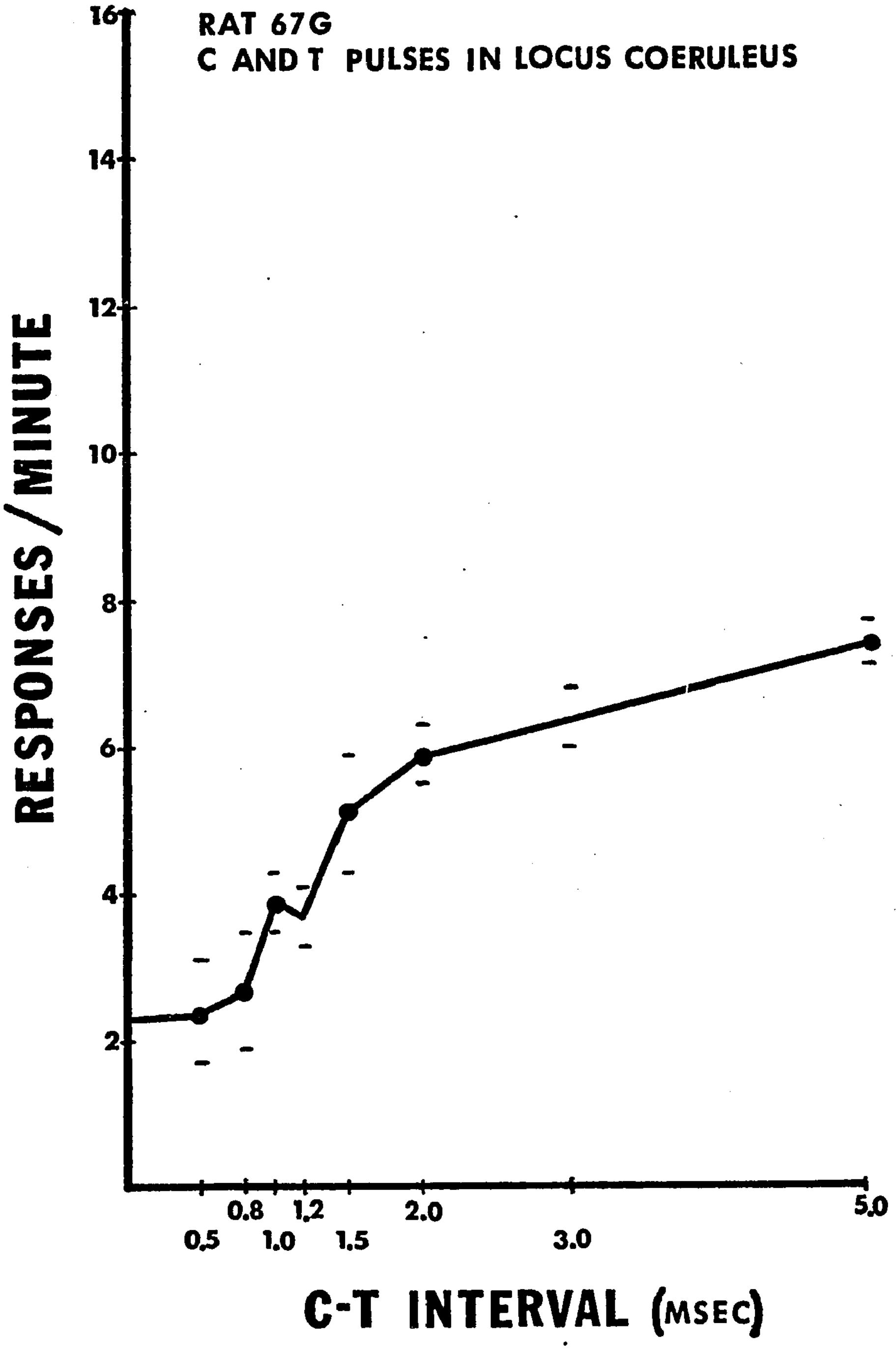

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FIGURE 10

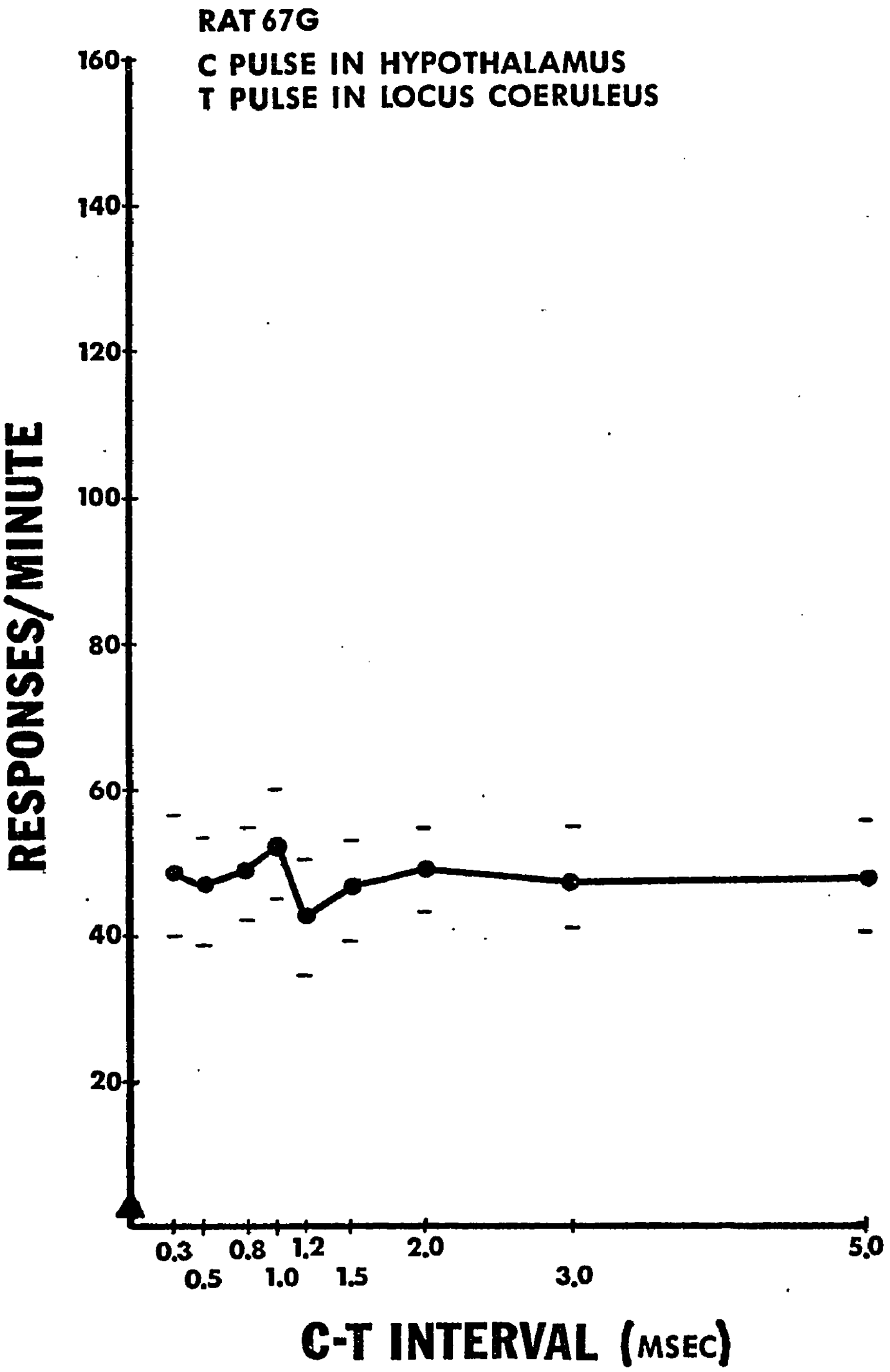

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FIGURE 11

LC-CC GROUP

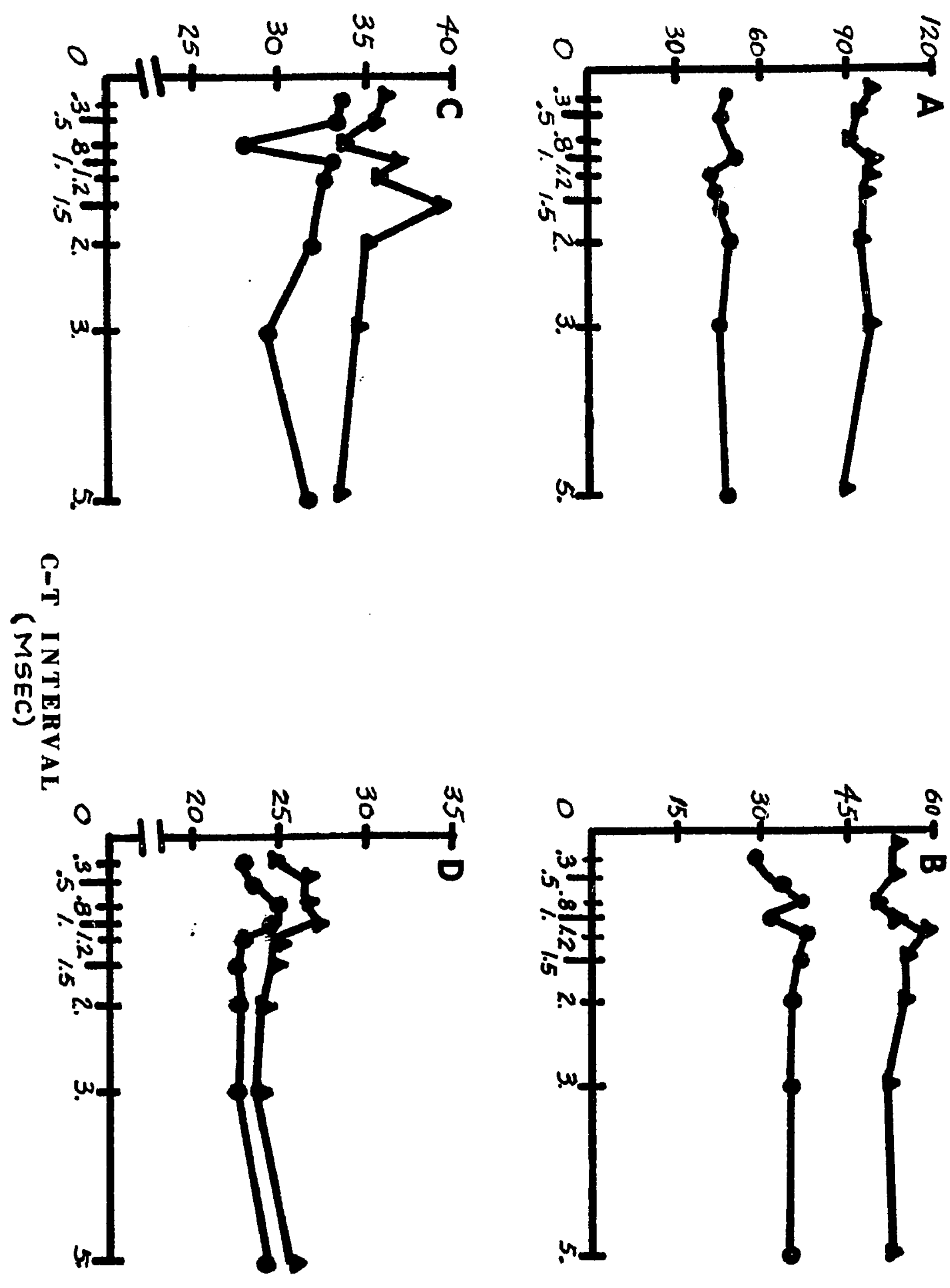

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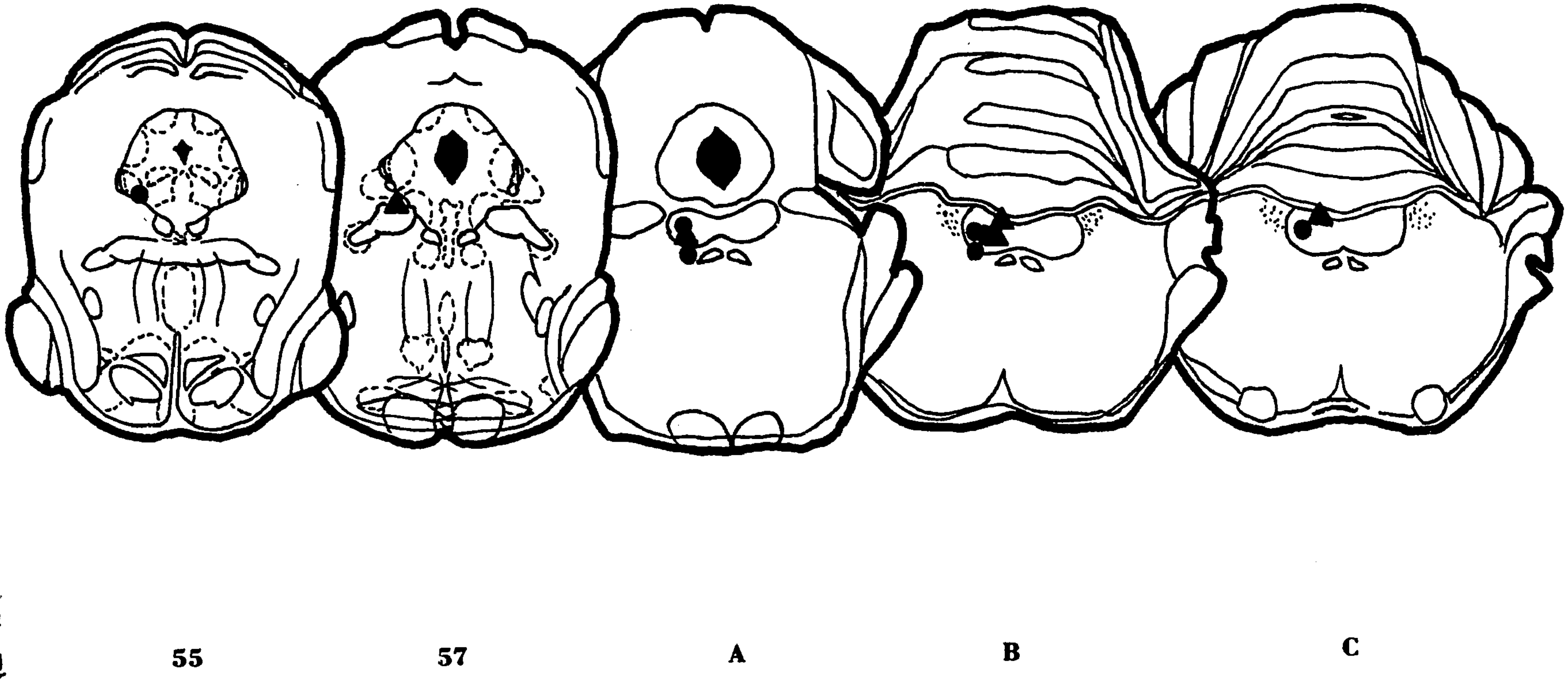
FIGURE 12



all LC

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FIGURE 13

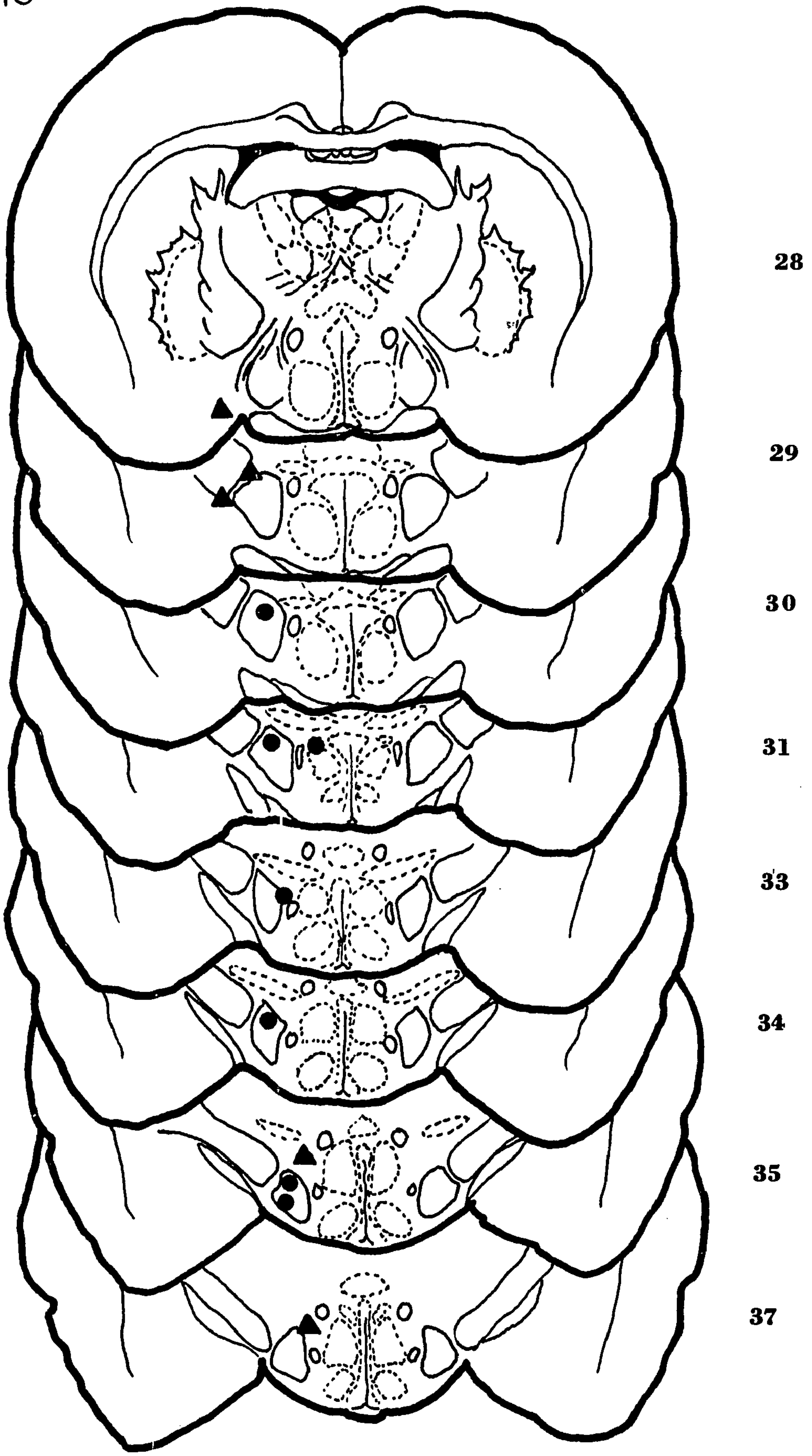

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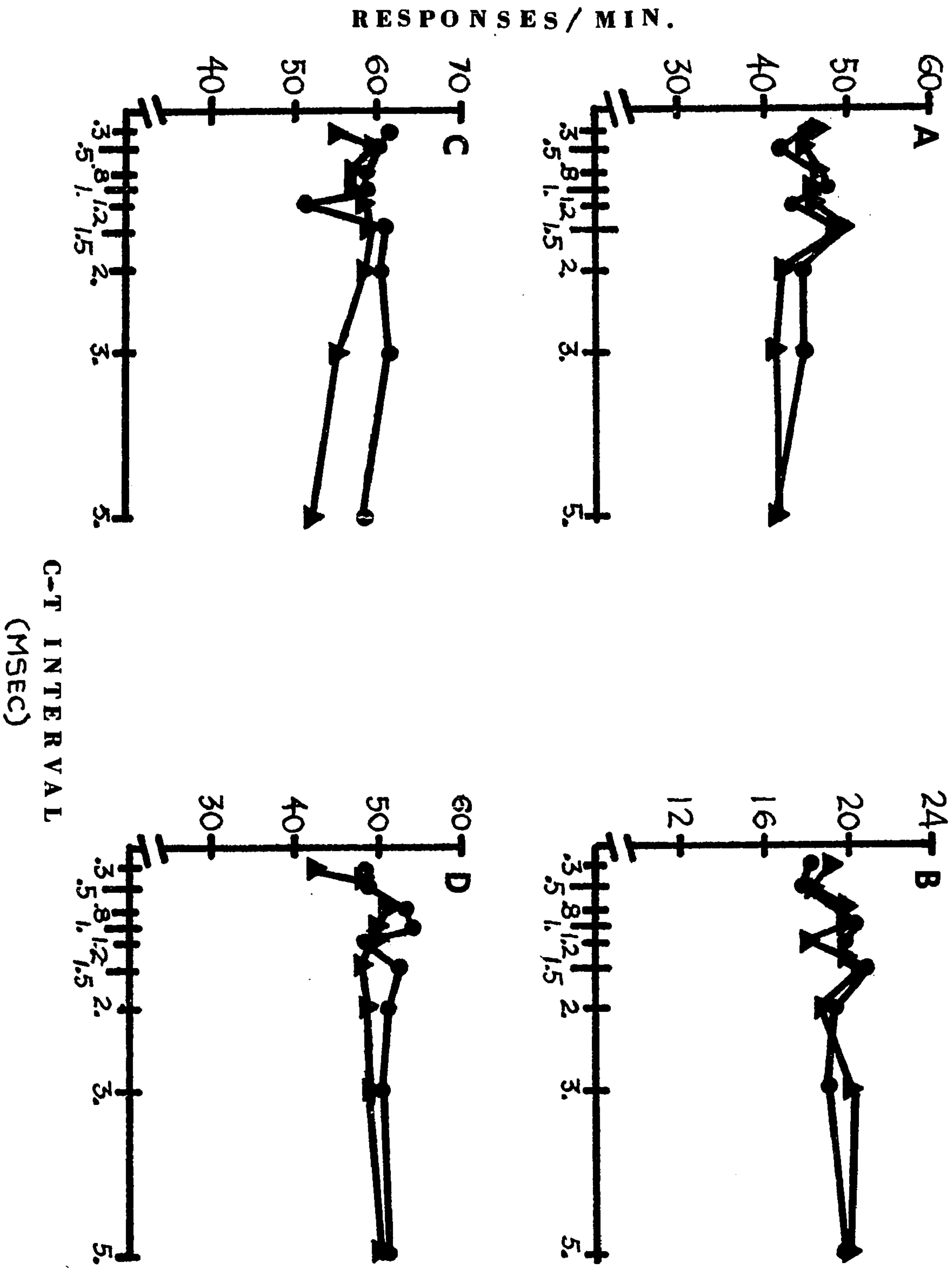
FIGURE 14



LC-MFB/PF GROUP

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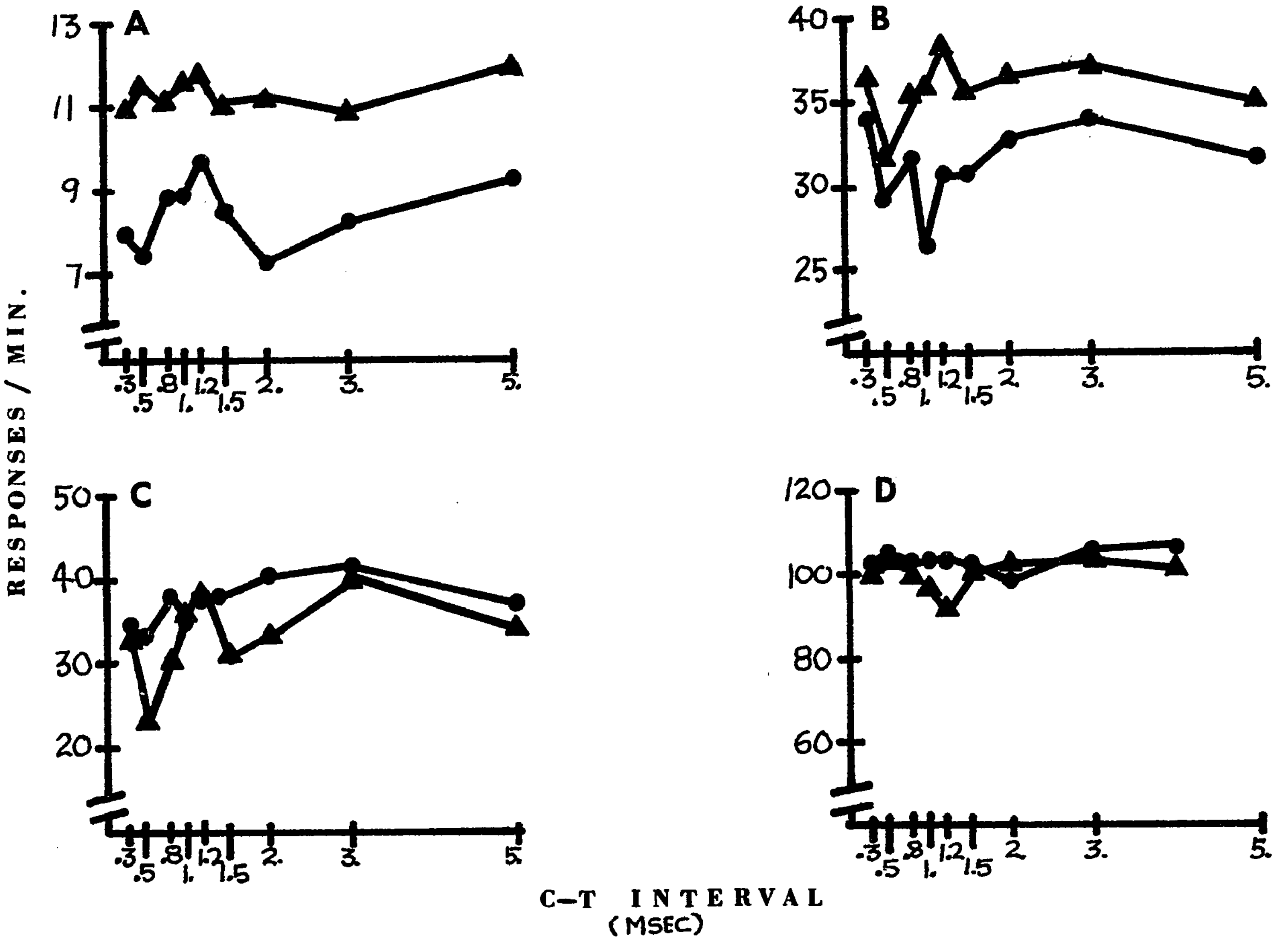

FIGURE 15

SN-NMFB(TOP) / SN-MFB(BOTTOM) GROUPS

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FIGURE 16

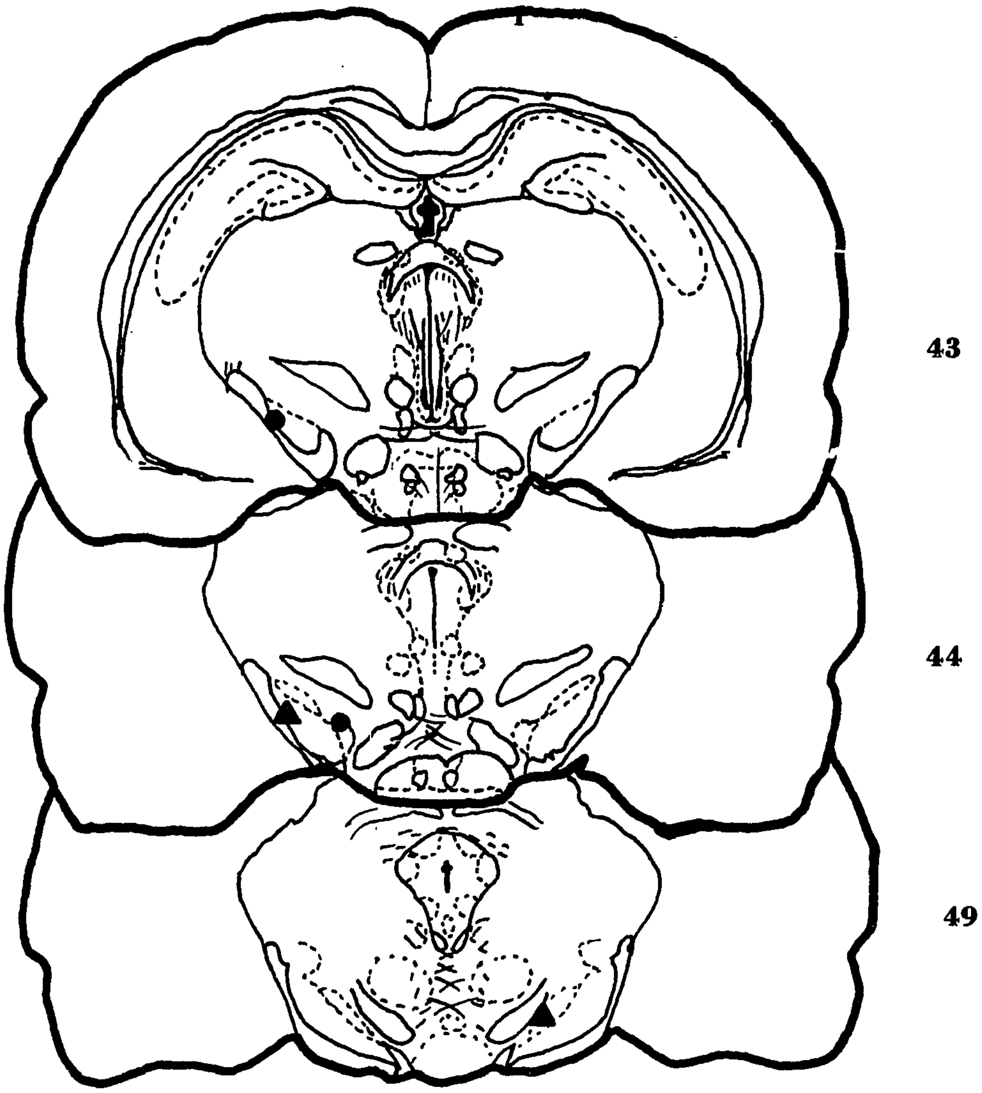

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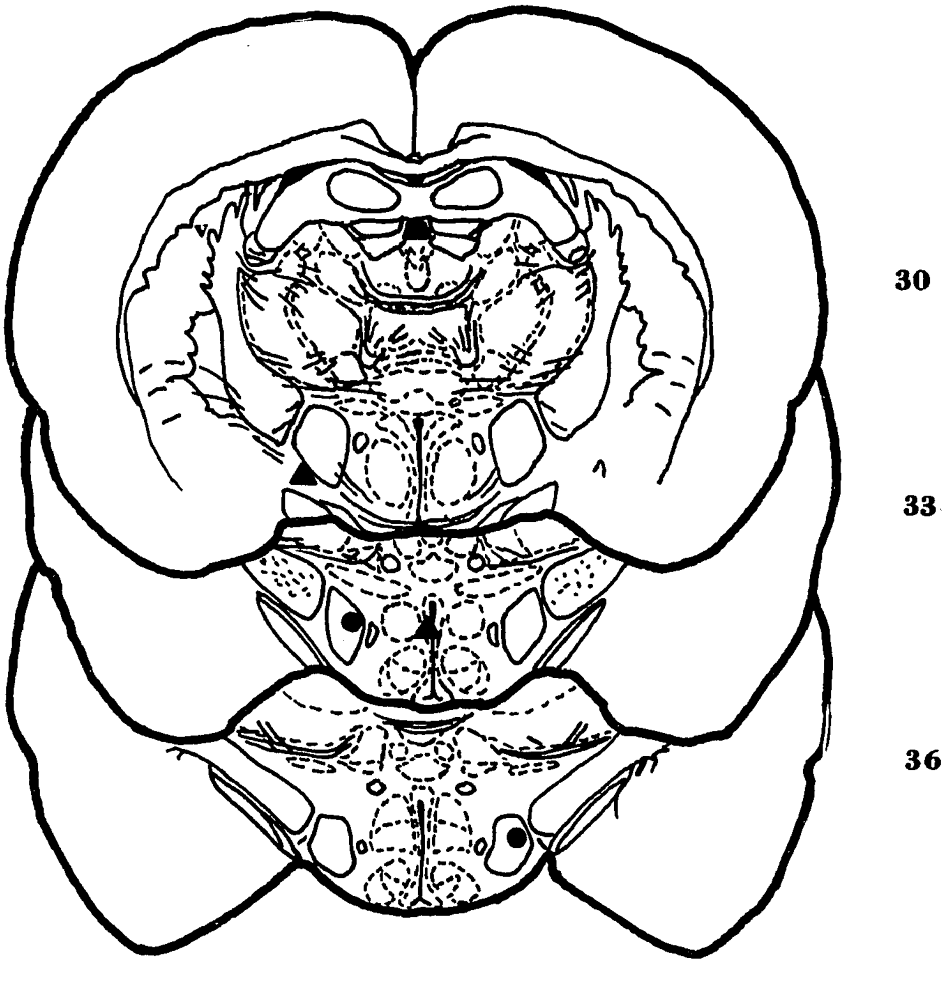
FIGURE 17



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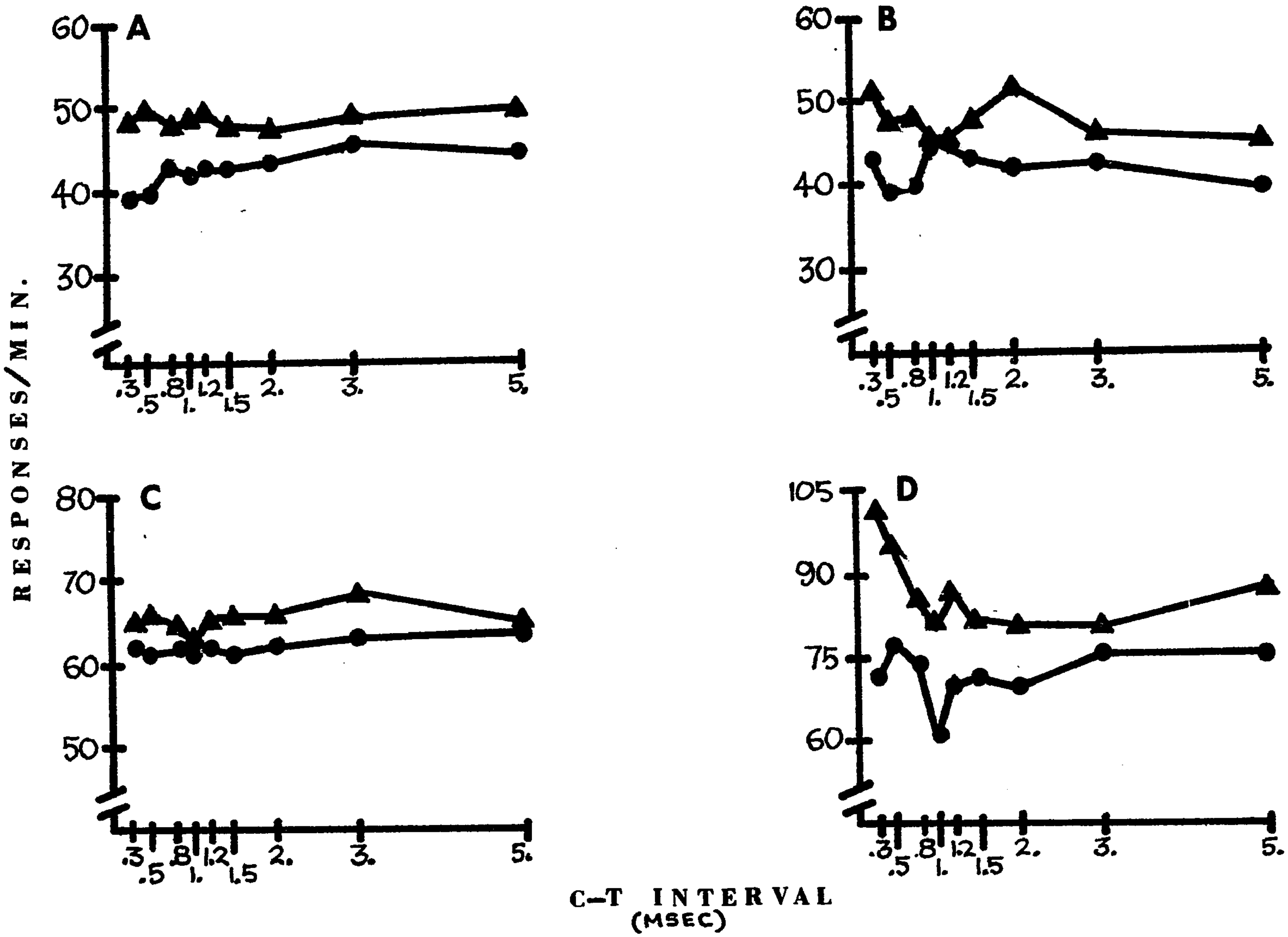

FIGURE 18

PMCG-MFB/PF GROUP

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FIGURE 19

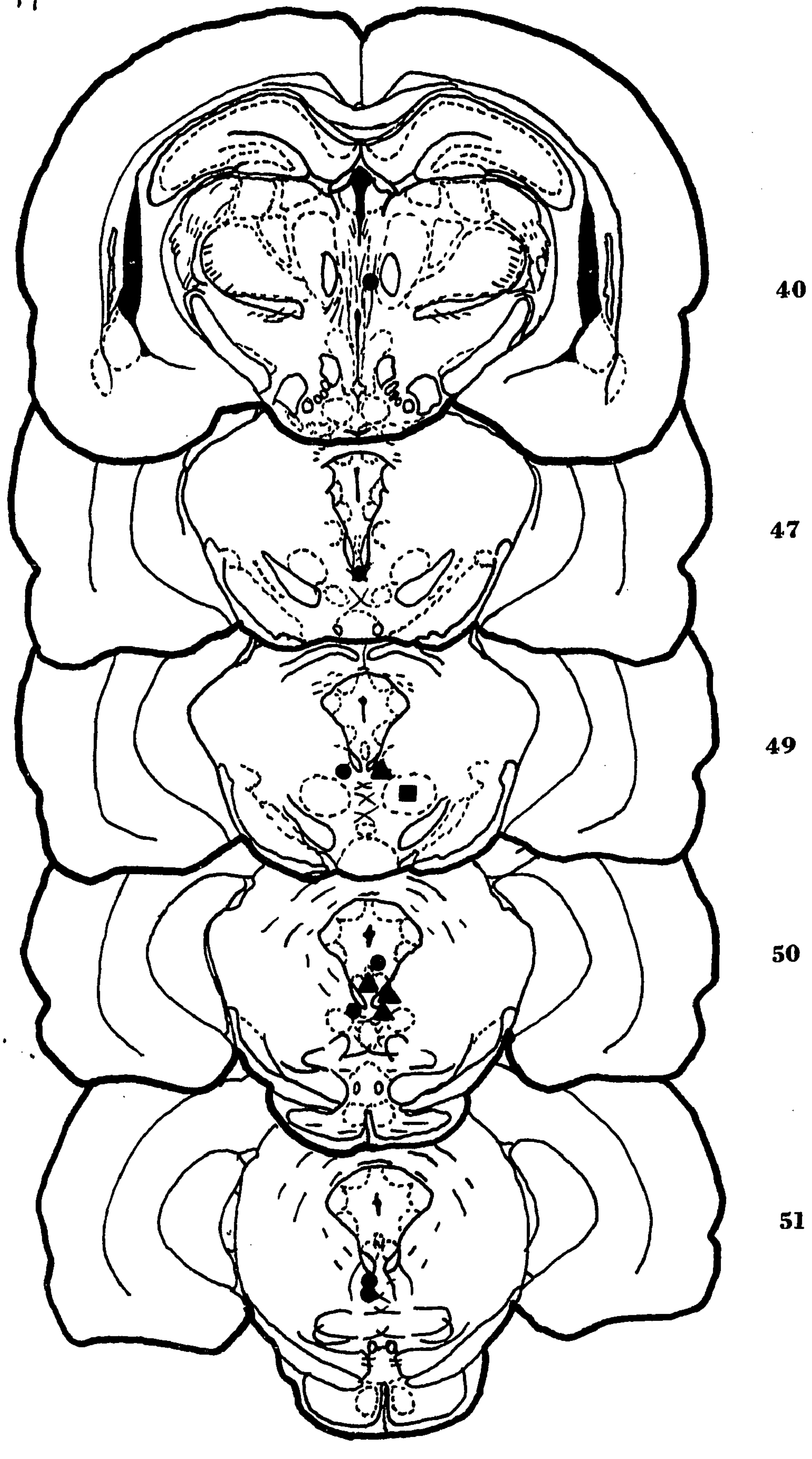

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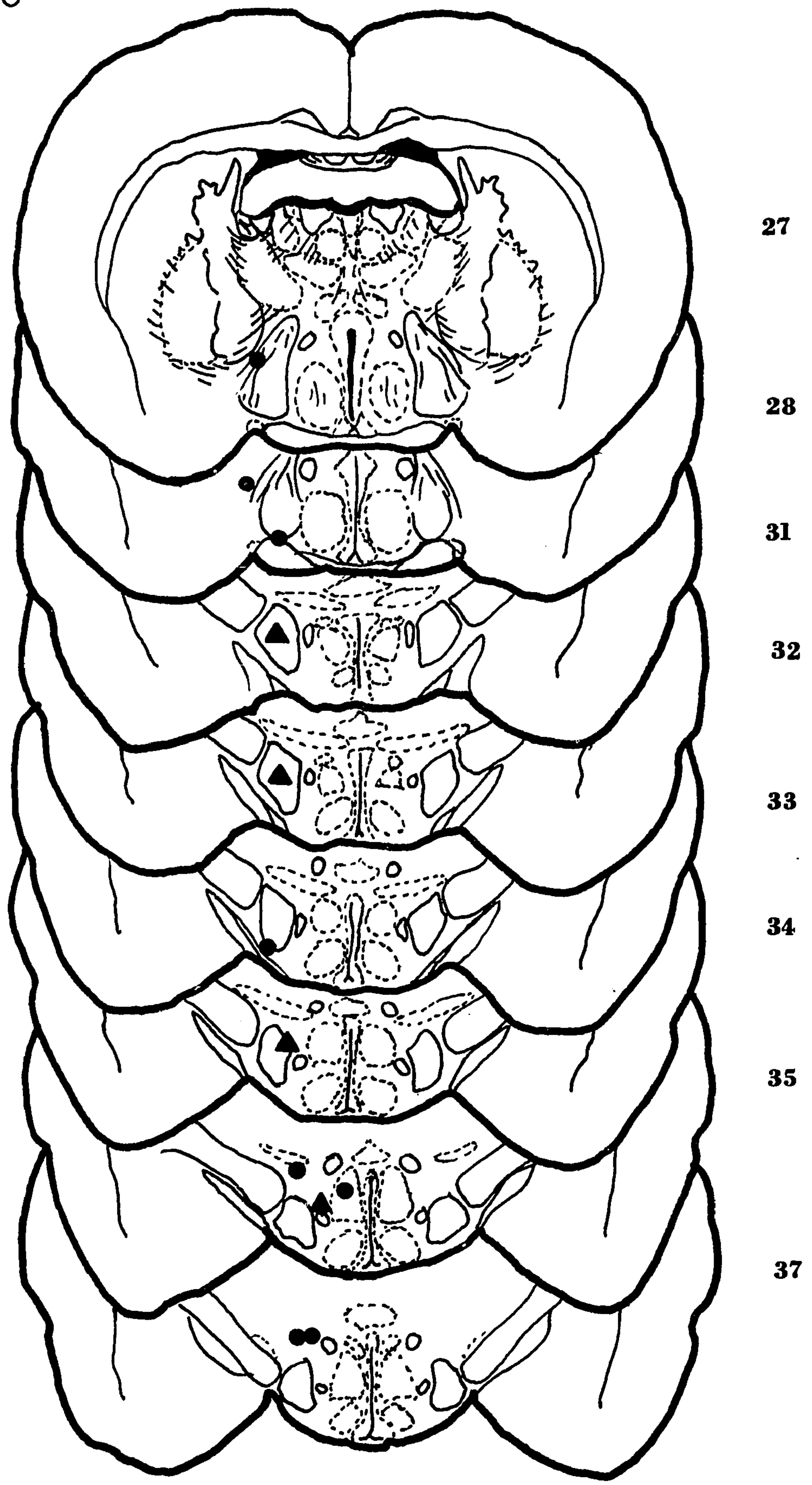
FIGURE 20



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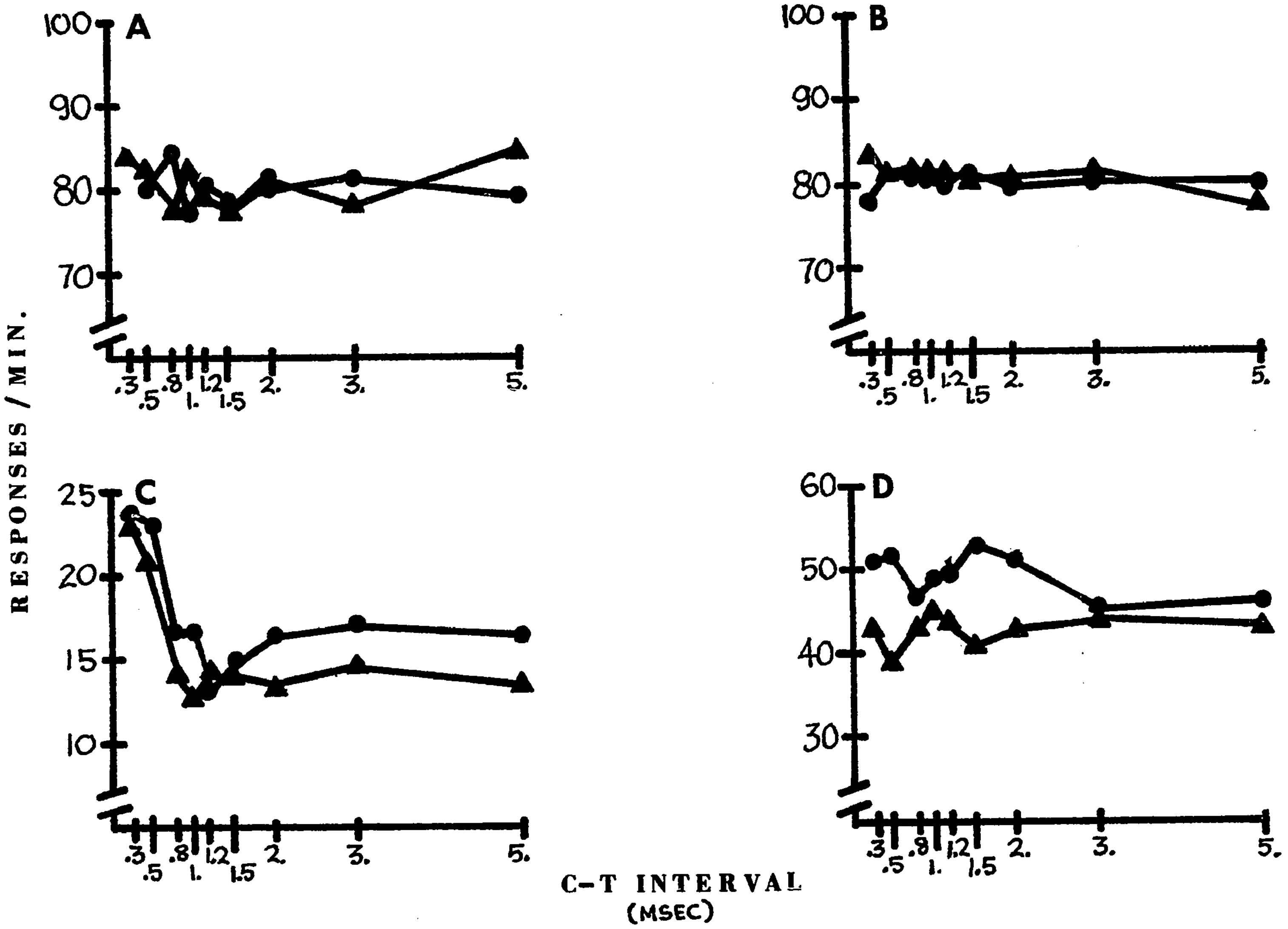

FIGURE 21

PMCG – NMFB GROUP

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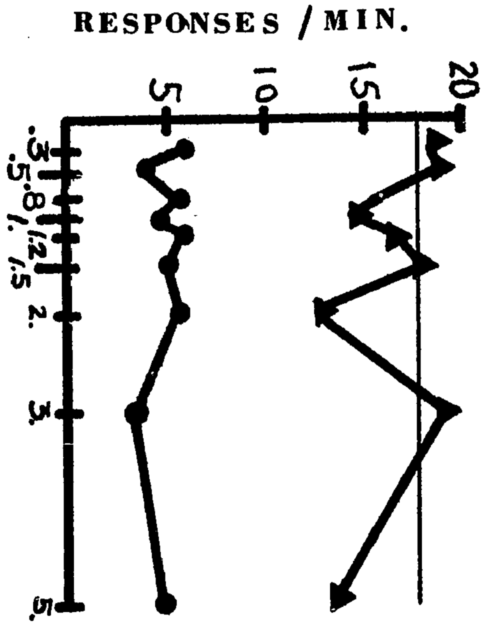
FIGURE 22



RED NUCLEUS-MFB ANIMAL

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FIGURE 23

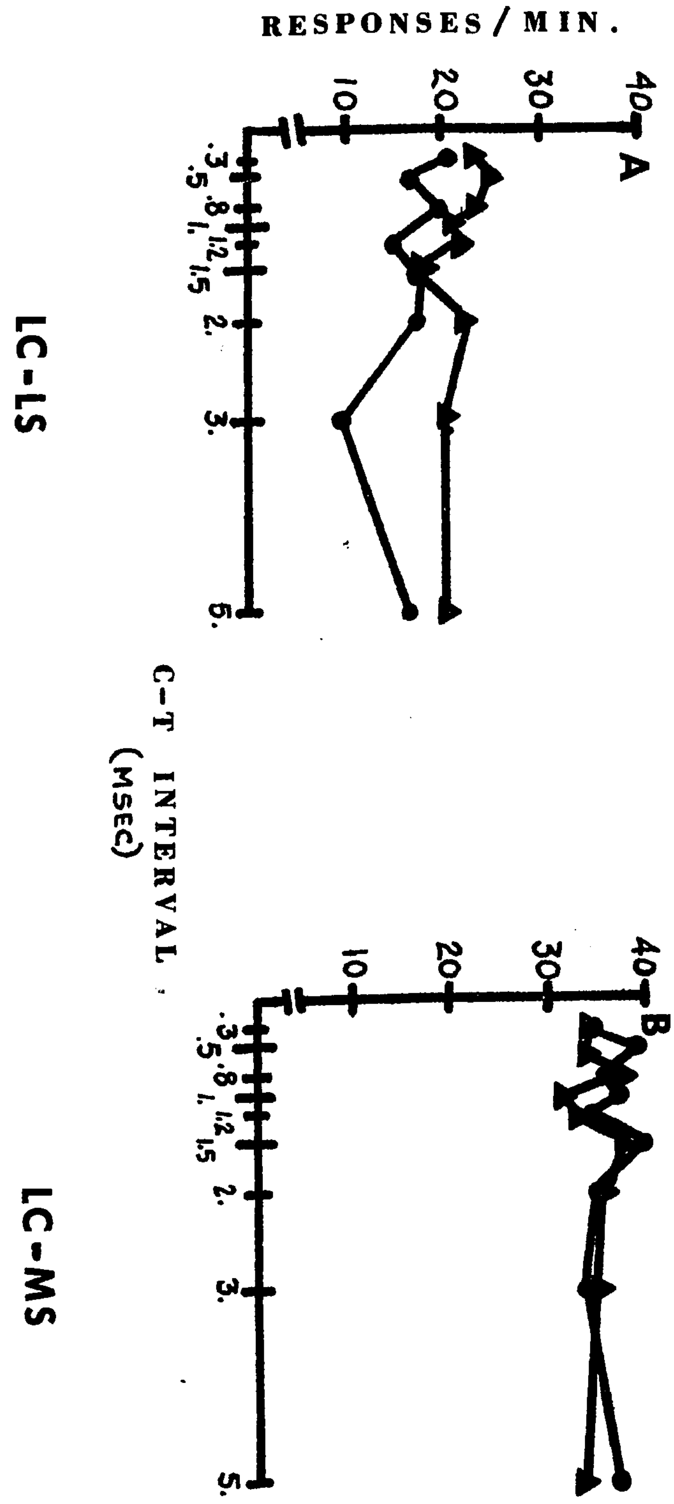

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FIGURE 24

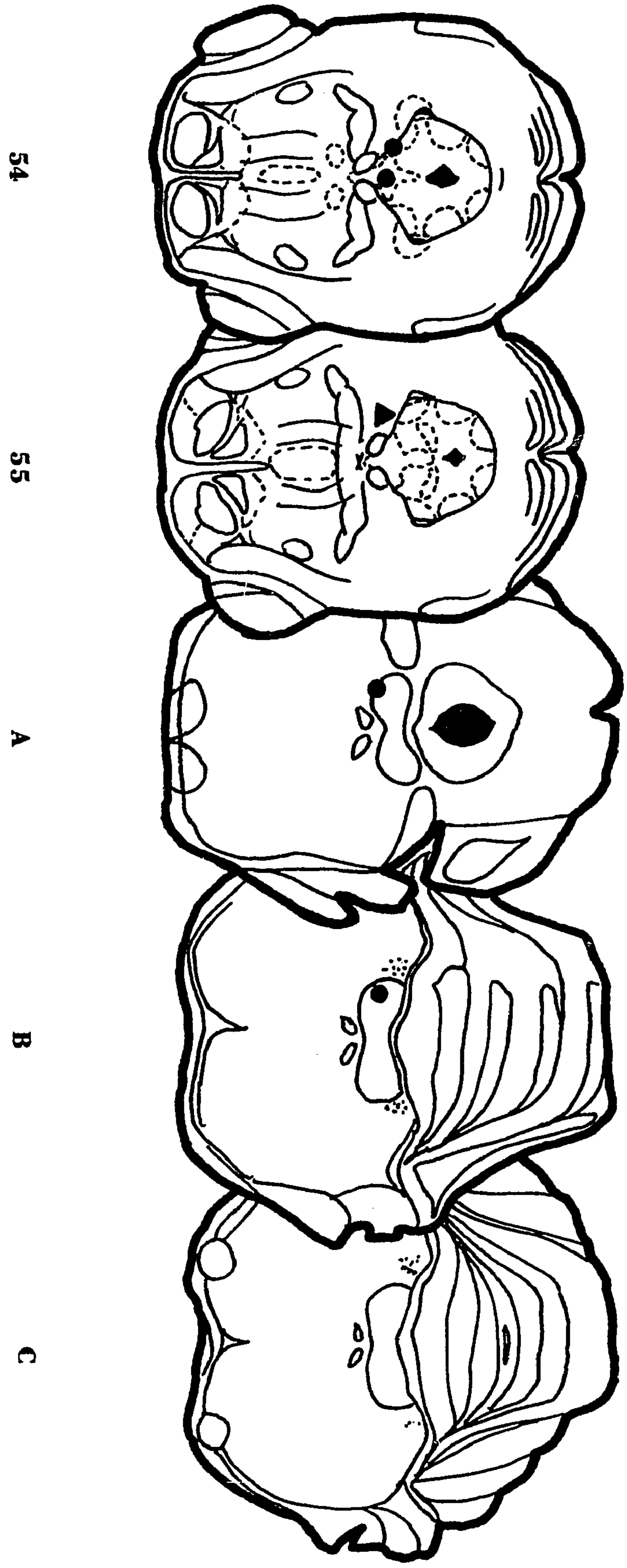

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FIGURE 25

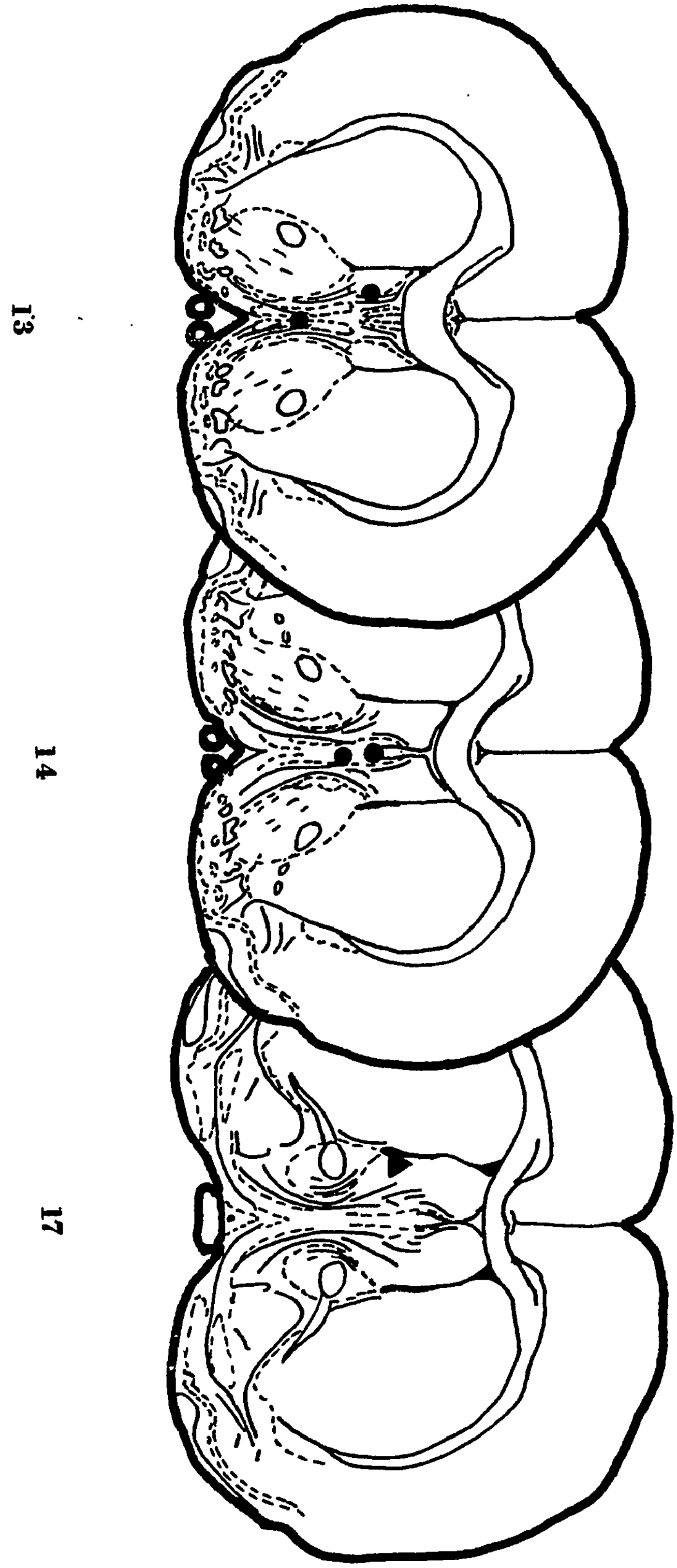

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FIGURE 26

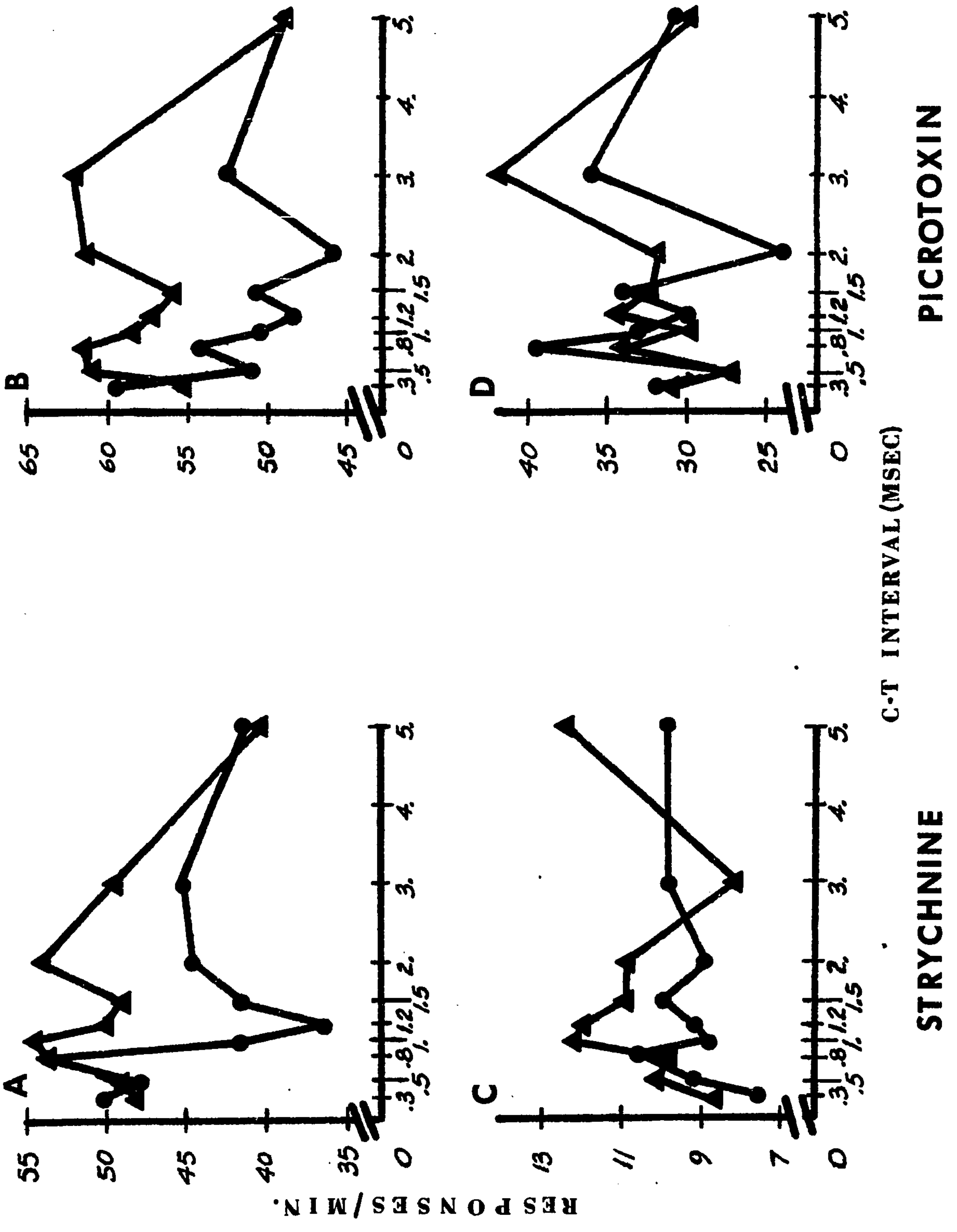

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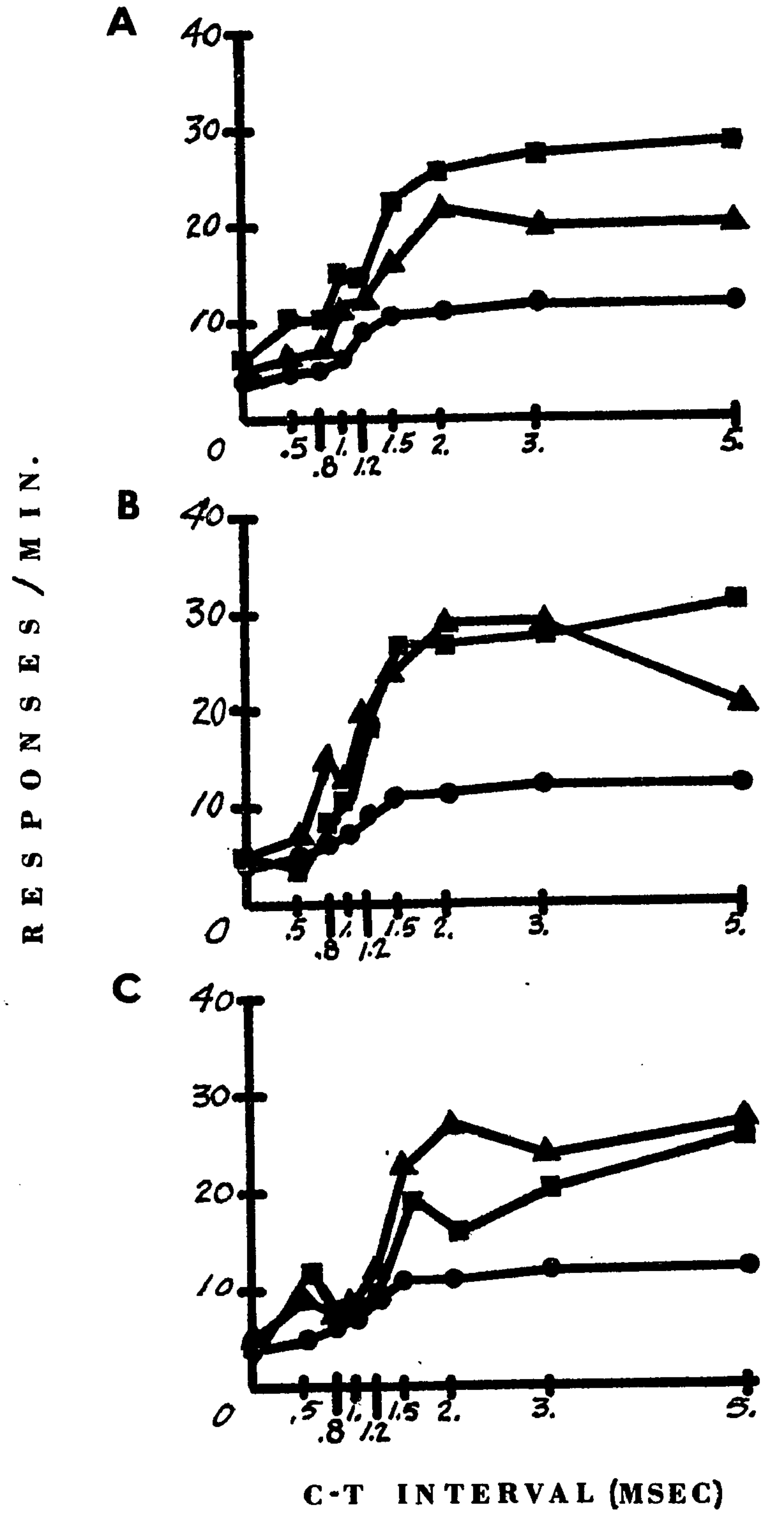
FIGURE 27



33H: C-T PF

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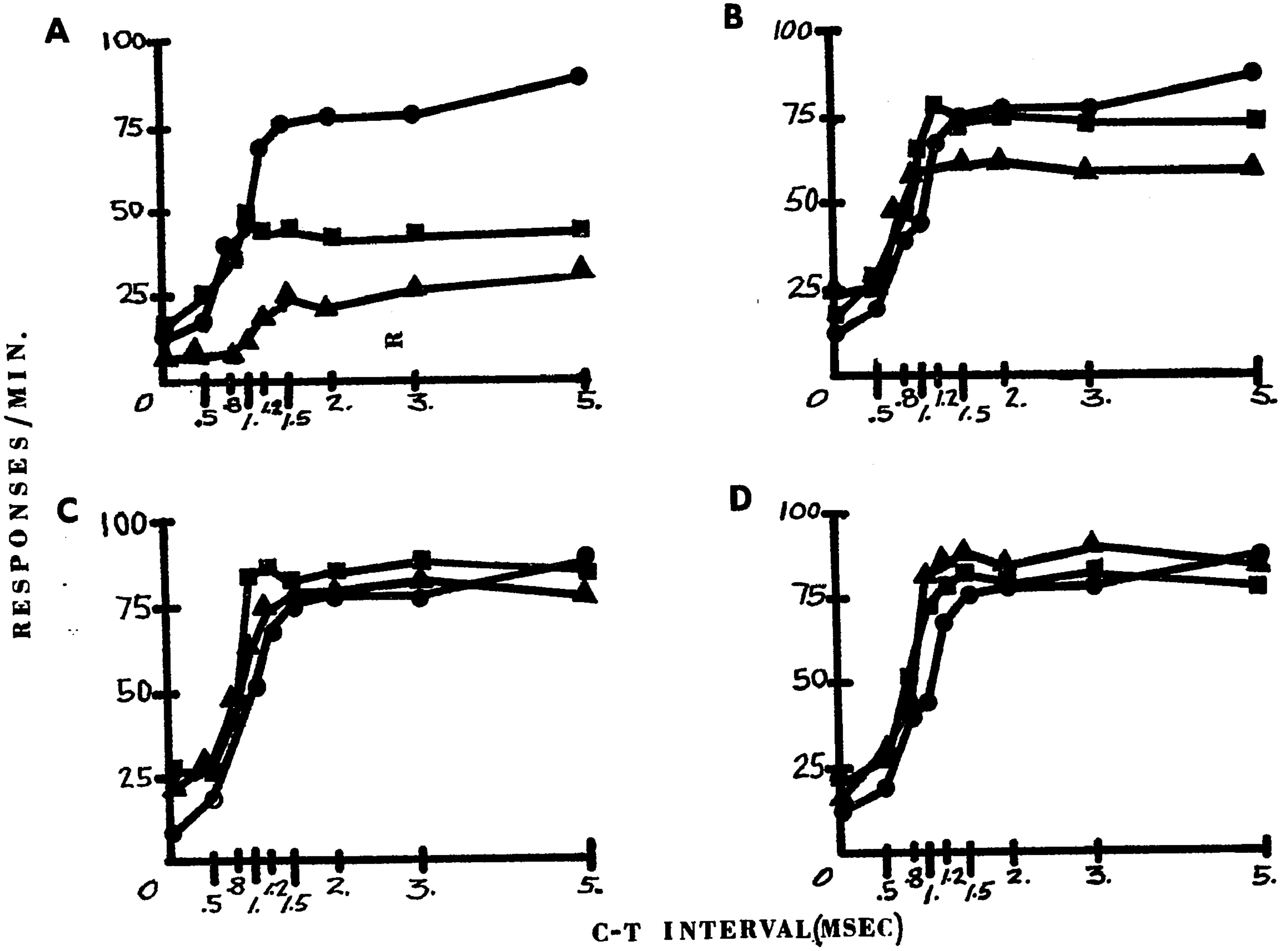

FIGURE 28

38H: C–T MFB

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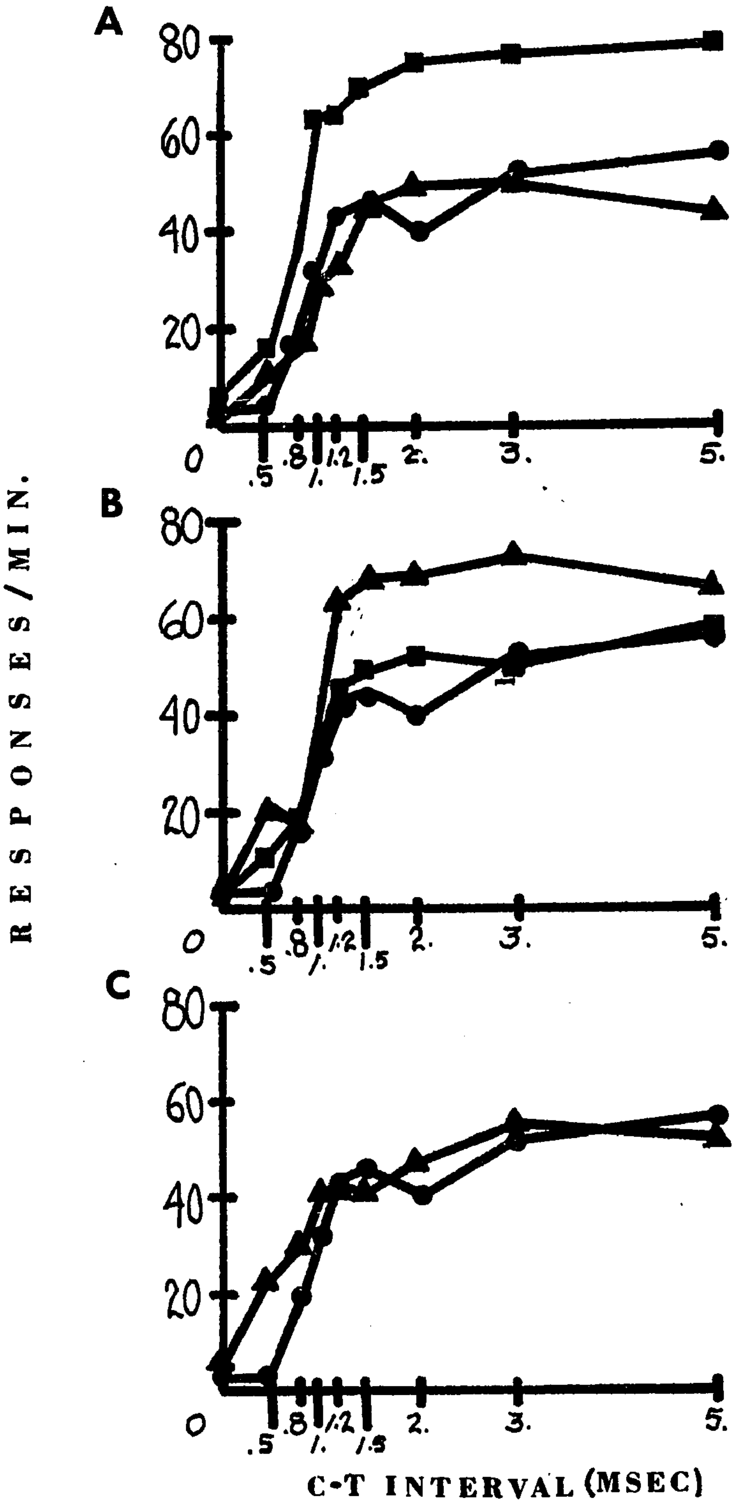
FIGURE 29



77H: C-T MFB

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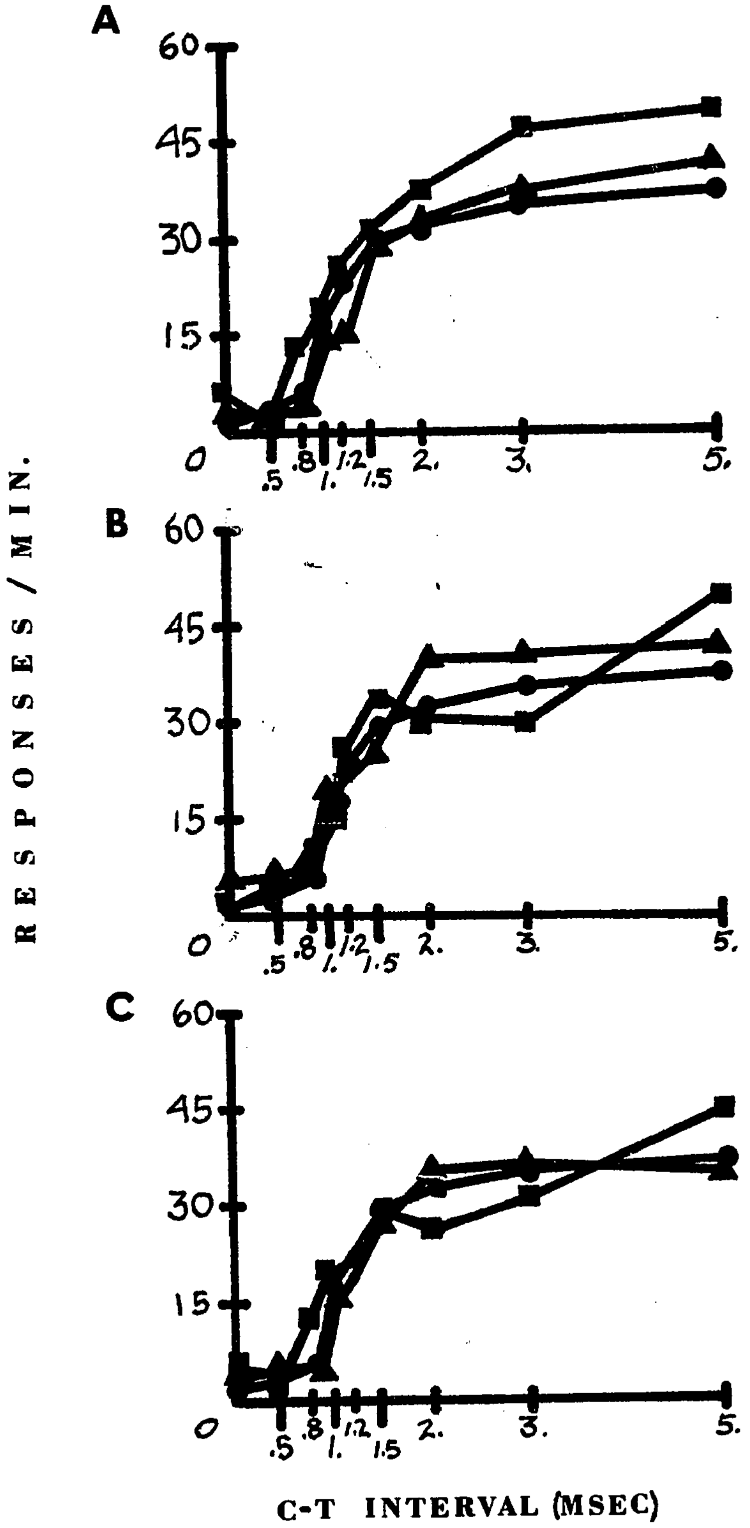
FIGURE 30



31H: C-T MFB

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FIGURE 31

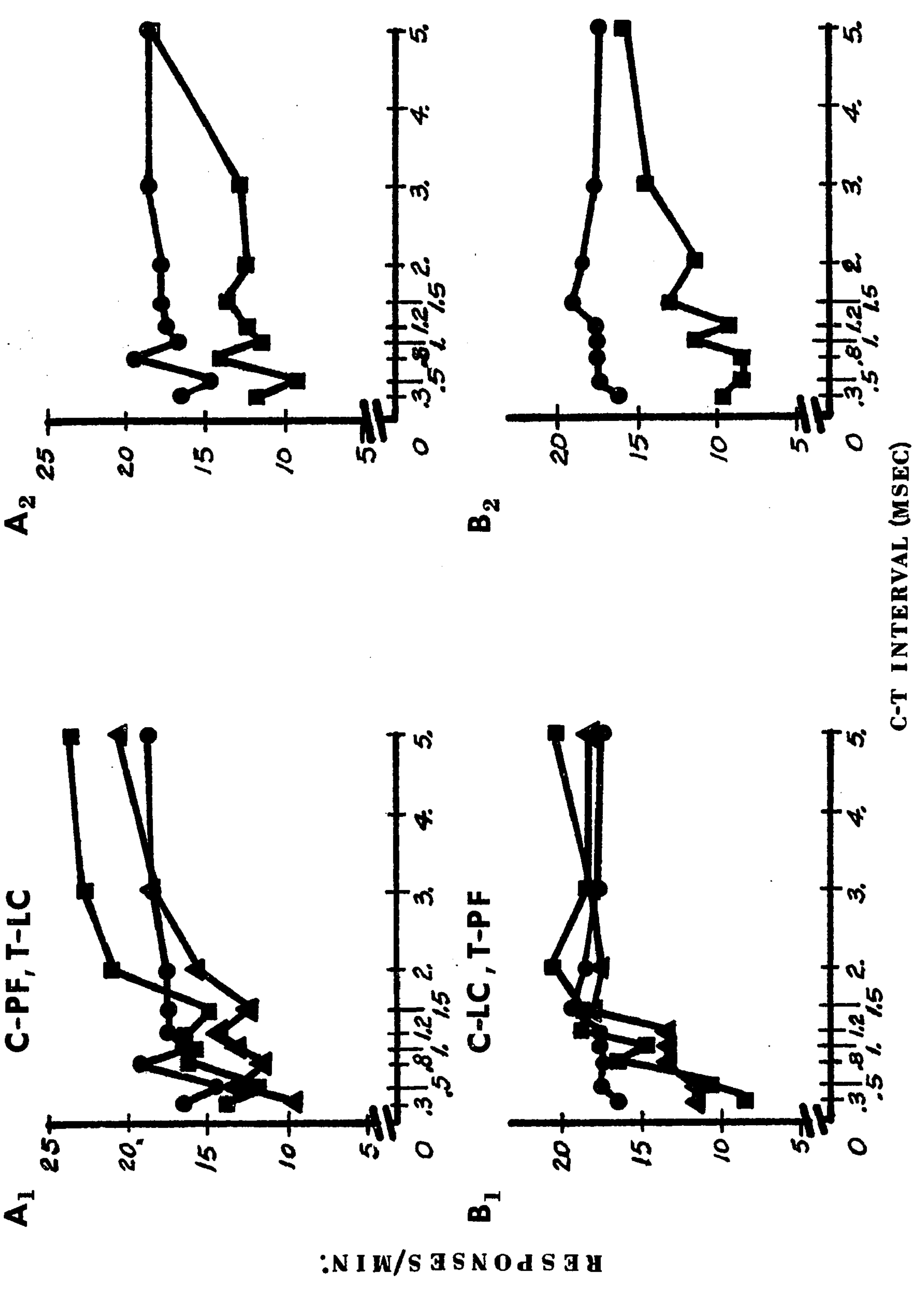

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FIGURE 32

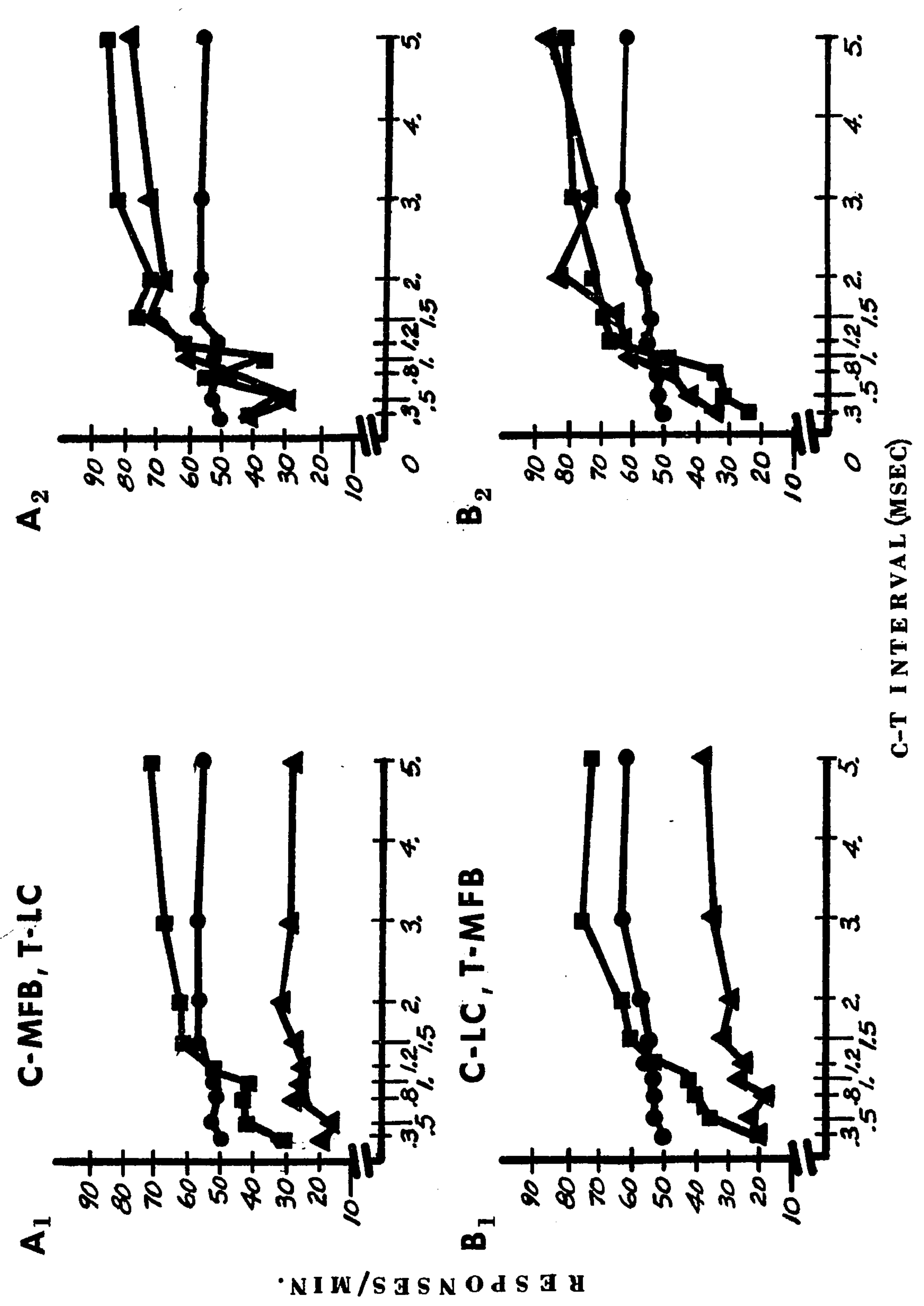

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FIGURE 33

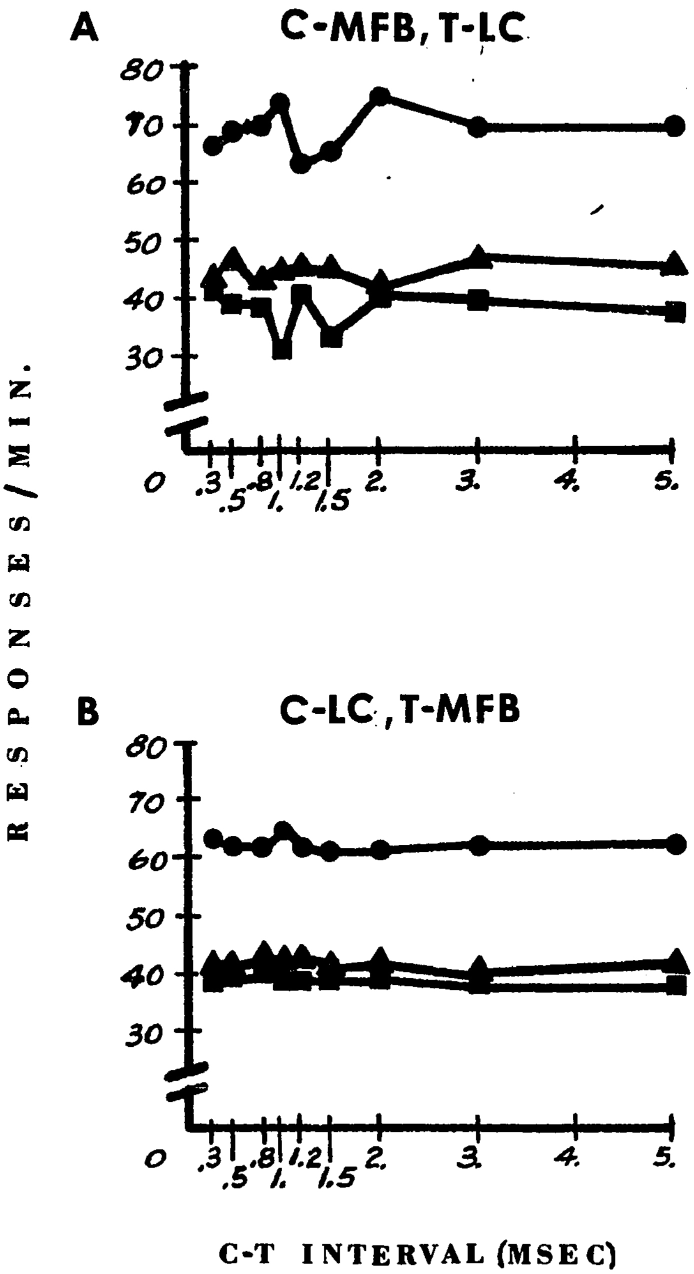

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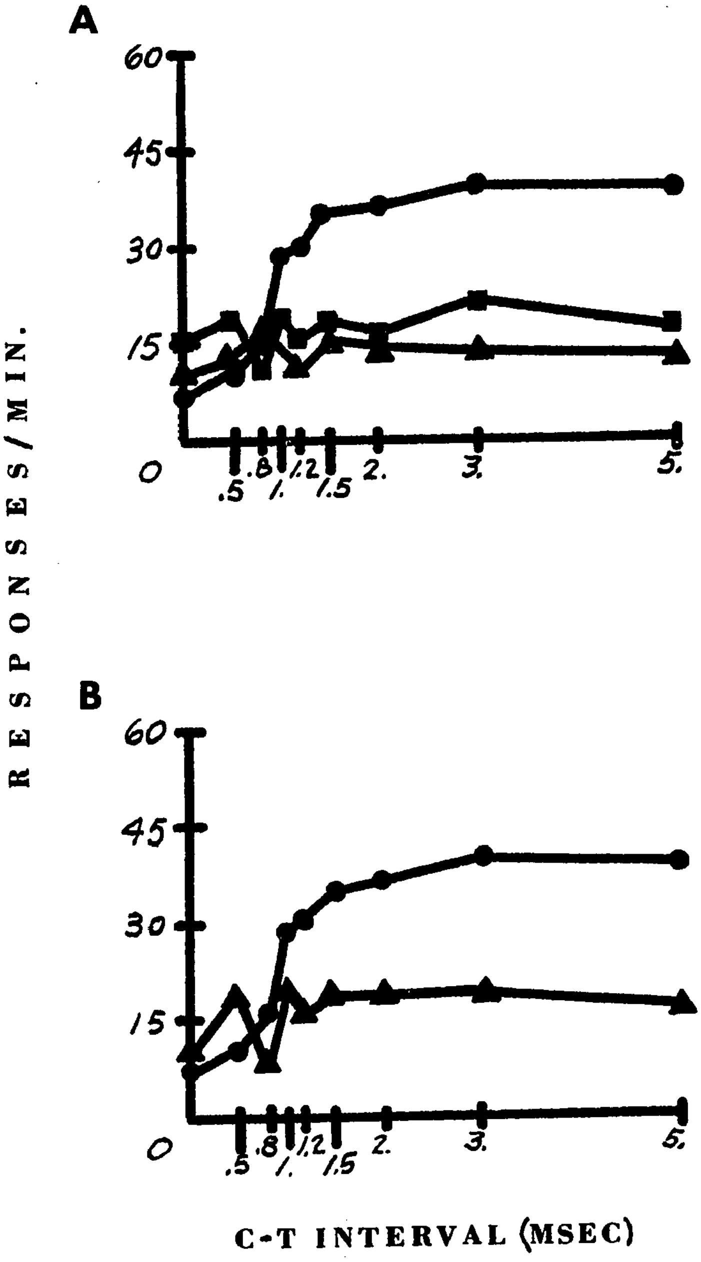
FIGURE 34



60H: C-T IC

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