SUBSTRATES OF REINFORCEMENT, AND INTERACTIONS AMONG HYPOTHALAMIC, PERIAQUEDUCTAL, AND PONTINE SELF-STIMULATION SITES IN THE ALBINO RAT

by

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Abstract

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Male Holtzman Sprague-Dawley albino rats were each implanted with two bipolar stainless steel electrodes, insulated except at the tips. One bipolar electrode was always in the lateral hypothalamic area; the other was in one of several tegmental areas: locus coeruleus or its emanating ascending projection, the dorsal noradrenergic bundle; midventral periaqueductal region and adjacent tegmentum including the oculomotor nuclei, Edinger-Westphal nuclei, medial longitudinal fasciculus, dorsal raphe nucleus, interstitial nucleus and nucleus linearis; lateral periaqueductal region and adjacent tegmentum, including the dorsal longitudinal fasciculus and nucleus Darkschewitsch; substantia nigra, pars compacta.

After post-surgical recovery, both electrode sites of

each animal were tested for self-stimulation. Each animal was shaped to bar-press for 250 msec trains of 60 Hz current delivered to one of its two electrode sites on a continuous reinforcement (FR:1) schedule. Most animals self-stimulated for current delivered to either electrode site (double-pressers); several animals self-stimulated for stimulation delivered to one electrode site, but not to the other (controls). A rate vs. current intensity function was obtained for each self-stimulation site. Then current intensities were chosen which would support subthreshold response rates (<10 responses per minute), and thereafter each day each animal was given the opportunity to self-stimulate, first for subthreshold stimulation delivered to its hypothalamic electrode site, then for subthreshold stimulation delivered to its tegmental electrode site, and finally for the same two intensities delivered simultaneously to both electrode sites. In all double-pressers, response rates obtained under simultaneous stimulation of hypothalamic and tegmental self-stimulation sites were significantly greater than the sum of the response rates obtained when each site was stimulated alone at the same intensities. In control subjects, having one self-stimulation site, simultaneous stimulation did not result in increased response rates.

The magnitude of interaction between hypothalamic and tegmental self-stimulation sites was estimated by delivering the same subthreshold intensity to one site

(constant site) and systematically reducing the intensity at the other site (varied site) until simultaneous stimulation no longer supported suprathreshold response rates. The difference between the varied site threshold intensity when stimulated alone, and the varied site threshold intensity when stimulated simultaneously with the constant site, was considered a measure of the magnitude of interaction between the two sites. The largest varied site threshold-reductions were obtained for midventral periaqueductal area/hypothalamus, and substantia nigra/hypothalamus site combinations; the smallest varied site threshold-reductions were obtained for locus coeruleus (dorsal noradrenergic bundle)/hypothalamus, and hypothalamus/contralateral hypothalamus site combinations.

According to Phillips & Fibiger (1973), norepinephrine-mediated self-stimulation is enhanced by d-amphetamine but not by l-amphetamine; dopamine-mediated self-stimulation is enhanced by both amphetamine isomers. Therefore, dorsal tegmental self-stimulation sites were tested under d- and l-amphetamine. Locus coeruleus (dorsal noradrenergic bundle) response rates were increased only by d-amphetamine; the same was true of the lateral periaqueductal area (dorsal longitudinal fasciculus) and hypothalamus, confirming norepinephrine-mediation of self-stimulation elicited from these areas. In contrast, midventral periaqueductal area response rates were increased by both amphetamine isomers, suggesting dopamine-mediation of

self-stimulation elicited from that area.

Finally, analysis of the previously obtained threshold-reduction data, together with the amphetamine data, revealed that small interactions occurred when areas having similar reactions to d- and l-amphetamine were simultaneously stimulated, while large interactions occurred when areas having dissimilar reactions to d- and l-amphetamine were simultaneously stimulated. These results imply that midventral periaqueductal self-stimulation is dopamine-mediated and that periaqueductal and pontine periventricular (i.e., locus coeruleus) self-stimulation areas interact with forebrain limbic structures. They also imply that dopamine-mediated self-stimulation areas interact more strongly with norepinephrine-mediated self-stimulation areas than do norepinephrine-mediated areas with each other.

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Finally, I wish to give public tribute to my wife, Ellen, who was an inspiration, critic, editor, typist, and both mother and father to our children during the course of this research. Were it not for her, this thesis would be an unfulfilled dream.

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The term "self-stimulation" refers to a phenomenon discovered by Olds and Milner (1954), who found that rats will emit at high rates those operant responses which result in delivery of small electric currents to certain areas of the brain. Olds (1956) went on to find that the brain areas which most consistently yielded self-stimulation behavior were those composing the limbic system (MacLean, 1949; Nauta 1958; Papez, 1937). Olds et al. (1960) found that of all limbic self-stimulation structures, the lateral hypothalamic area, through which courses the medial forebrain bundle, elicits the highest rates of self-stimulation behavior.

Nauta, employing his own degeneration silver staining technique (Nauta & Gygax, 1954), found that hypothalamic nuclei have descending projections throughout the midbrain (Nauta, 1956, 1958). Ventral midbrain nuclei (most prominently the ventral tegmental area of Tsai) receive most descending hypothalamic projections, but many hypothalamic projections also course throughout the dorsal midbrain, distributing terminals to several nuclei including Bechterew's raphe nucleus, the dorsal and ventral tegmental nuclei, and the periaqueductal gray area (Guillery, 1957; Nauta, 1958; Wolf & Sutin, 1966). Nauta (1958) argued that reciprocal connections between the hypothalamic and midbrain nuclei extend the limbic system to the posterior midbrain.

In 1962, Falck et al. developed a fluorescence histological technique which allows visualization of monoamine-containing neurons (serotonin, norepinephrine, dopamine)

in intact brain sections. Dahlstrom and Fuxe (1965), in a classic paper, described twelve catecholamine-containing nuclei and nine serotonin-containing nuclei throughout the brain. They classified catecholamine-containing nuclei as "A" nuclei, and serotonin-containing nuclei as "B" nuclei. The medulla oblongata contains the noradrenergic (Ungerstedt, 1971) nuclei A5, A6, and A7. Nucleus A6 has turned out to be of particular interest because this group of norepinephrine-containing neuron cell bodies is coincident with the locus coeruleus. The pons also contains serotonergic cell bodies of nuclei B3, B5, and B6. The mesencephalon contains nuclei A8, A9, and A10; all three of these catecholamine-containing nuclei are dopaminergic (Anden et al., 1964, 1965, 1966; Dahlstrom & Fuxe, 1965). Nucleus A9 is of particular interest because it is coincident with the pars compacta of the substantia nigra. The mesencephalon also contains serotonergic nuclei B7, B8, and B9. Nucleus B7 is the dorsal raphe nucleus of the periaqueductal gray area. Dahlstrom and Fuxe (1965) found the hypothalamic portion of the diencephalon to contain catecholaminergic nuclei A11 and A12; nucleus A11 was subsequently found to be composed of both noradrenergic and dopaminergic neurons, and nucleus A12, solely of dopaminergic neurons (Bjorklund & Nobin, 1973; Dahlstrom & Fuxe, 1965). Two additional hypothalamic dopaminergic nuclei, A13 and A14, were later described (Bjorklund & Nobin, 1973; Fuxe et al., 1969). In the following years, a succession of investigators (e.g., Lind-

vall & Bjorklund, 1974; Lindvall et al., 1974; Loizou, 1969; Maeda & Shimizu, 1972; Olson & Fuxe, 1972; Ungerstedt, 1971) systematically explored the brain and spinal cord for monoamine-containing neurons. These studies established that:

1. the serotonin-containing neurons concentrated in midline (raphe) nuclei throughout the midbrain, pons and medulla (B1-B12) project to various forebrain (telencephalon and diencephalon) nuclei by way of the medial forebrain bundle (Ungerstedt, 1971);

2. dopamine-containing neurons concentrated in ventral midbrain nuclei (A8, A9, and A10) project to various forebrain nuclei (caudoputamen, nucleus accombens, olfactory tubercle, amygdala, nucleus interstitialis, striae terminalis) by way of the "nigrostriatal" bundle, a fiber tract which courses through the hypothalamus on the lateral outskirt of the medial forebrain bundle (Arbuthnott et al., 1970; Lindvall & Bjorklund, 1974); and

3. norepinephrine-containing neurons concentrated in the pons and medulla (A1-A7) project to the forebrain in two distinct fiber tracts, the ventral noradrenergic bundle and the dorsal noradrenergic bundle (Olson & Fuxe, 1972; Ungerstedt, 1971). Fibers composing these bundles terminate in the midbrain (the periaqueductal region), the diencephalon (thalamus and hypothalamus), and telencephalon (hippocampus and cerebral cortex, amygdala) (Arbuthnott et al., 1970; Lindvall & Bjorklund, 1974; Ross & Reis, 1974; Ungerstedt, 1971). These noradrenergic bundles course through the diencephalon as a component of the medial forebrain bundle.

The neurons of nucleus A6 (locus coeruleus) are particularly ubiquitous. They project to the cerebellum, the dorsal midbrain, thalamus, hypothalamus, hippocampus and neocortex (Ross & Reis, 1974; Ungerstedt, 1971). Other neurons of nucleus A6 descend to the medulla (Ungerstedt, 1971). Thus, monoaminergic neurons, organized in dispersed but discrete nuclei in the brainstem, have divergent innervation to many

forebrain and midbrain limbic structures.

Dahlstrom and Fuxe (1965) and Stein (1968) noted the correspondence between ascending monoaminergic fibers and midbrain and forebrain limbic structures. Stein had particular interest in the ascending norepinephrine-containing fibers from brainstem catecholamine nuclei, derived from his conviction that norepinephrine is the primary mediator of self-stimulation behavior elicited from the lateral hypothalamic area (medial forebrain bundle).

Miller (1957) first reported that methamphetamine, known to be a peripheral releaser of norepinephrine (Burn & Rand, 1958), increases the rate of responding for electrical brain stimulation in rats. Stein (1964) suggested that amphetamines lower all "reward" thresholds. Therefore, the increased rate of responding could be accounted for by assuming that under the influence of amphetamine, ordinarily subthreshold rewarding stimuli are converted to suprathreshold stimuli. Stein and Ray (1960) had shown that amphetamine lowers self-stimulation thresholds. Steiner and Stokely (1973) later showed that methamphetamine not only lowers self-stimulation thresholds, but shifts the entire rate vs. intensity function to the left, i.e., toward lower intensities. Stein (1964) also reviewed pharmacological data which, he argued, suggested that the most important mechanism mediating the central stimulating effect of amphetamines is the release of catecholamines into their synapses. At that time, Stein guessed that the catecholamines which were most likely the

mediators of amphetamine's effects were norepinephrine or dopamine: norepinephrine, because "it occurs in high concentrations throughout the reward system"; dopamine, because "it occurs in high concentrations in the extrapyramidal system," which, according to Olds (1962), is perhaps the final common pathway for "the modulation of behavior by reinforcing stimuli." Stein (1964) also presented data indicating that d-amphetamine is at least five times more potent than l-amphetamine in eliciting self-stimulation behavior at subthreshold current intensity, and he cited evidence (Moore, 1963) indicating that d-amphetamine is three to five times as potent as l-amphetamine in depleting norepinephrine. Subsequently, Stein concluded that it is norepinephrine, specifically, which mediates all positive reinforcement, including positive reinforcement due to electrical self-stimulation (Stein, 1968); the evidence which led to this conclusion included data indicating that:

1. norepinephrine is released by amphetamine (Stein & Wise, 1967, 1969) and by electrical stimulation at highly rewarding, but not at marginally rewarding, neutral, or aversive sites (Stein, 1968; see also Arbuthnott et al., 1970, 1971; Dresse, 1966);

2. direct application of l-norepinephrine crystals to the amygdala reduces the behavior-suppresant effects of punishment, while dopamine has no effect (Stein et al., 1968);

3. intraventricular administration of l-norepinephrine increases electrical self-stimulation rates;dopamine sometimes also increases self-stimulation rates, but not in the presence of disulfiram or diethyldithiocarbamate (Wise & Stein, 1969). The latter finding is important because dopamine is contained in all catecholaminergic neurons; however, in noradrenergic neurons, the dopamine is converted to norepinephrine

by an enzyme, dopamine-β-hydroxylase. Both disulfiram and diethyldithiocarbamate block the conversion of dopamine into norepinephrine. Therefore, these drugs allow neurons whose transmitter is dopamine to continue functioning, but render noradrenergic neurons transmitterless.

4. apomorphine, thought to be an effective dopamine substitute (agonist) at dopaminergic post-synaptic receptor sites, suppresses self-stimulation (Stein & Wise, 1973) and other "reward" behaviors (De Oliviera & Graeff, 1972) over a wide dose range.

Of all the norepinephrine-containing nuclei, the locus coeruleus has drawn the most attention from various investigators.

Jouvet (1972), an eminent sleep neurophysiologist, discovered that in cats, the locus coeruleus is necessary for the initiation and maintenance of rapid eye movement (REM) sleep phenomena. REM sleep is characterized by low voltage, mixed frequency cortical electroencephalographic (EEG) activity, by rapid conjugated eye movements, and by markedly reduced skeletal muscle tonus. Jouvet and other workers transected the brains of cats at various levels (Hobson, 1965; Jouvet, 1962; Villablanca, 1966). Whenever the plane of transection was anterior to the locus coeruleus, cortical EEG no longer showed the characteristic low voltage mixed frequencies during sleep episodes (sleep EEG now consisted exclusively of "slow" waves [Hobson, 1965]); however, there were periodic episodes of skeletal muscle atonia, accompanied by REM's, but these episodes were no longer correlated with cortical EEG and the rapid eye movements were aberrant. Only the eye muscles subserved by the motor nuclei between the transection and the locus coeruleus contracted; eye

muscles subserved by motor nuclei anterior to the transection remained relaxed during REM episodes (Villablanca, 1966).

If instead, the plane of transection was at the caudal third of the locus coeruleus, there followed the usual EEG and REM indicators of REM episodes during sleep, but the skeletal muscle atonia characteristic of REM periods failed to occur (Jouvet, 1962).

If the locus coeruleus was destroyed, all indicators of REM episodes failed to appear, though waking-slow-wave sleep cycles remained essentially normal (Jouvet, 1962).

In the meantime, the successive fluorescence studies described above (e.g., Dahlstrom & Fuxe, 1965; Lindvall & Bjorklund, 1974; Ungerstedt, 1971) demonstrated that, in rats, the locus coeruleus has far-reaching projections. Ascending axons of locus coeruleus neurons extend from their origins in the pons all the way to the cerebral cortex and other telencephalic structures; along the way, collaterals from these axons innervate the cerebellum, the periaqueductal region, several thalamic nuclei, medial aspects of the hypothalamus, and the amygdala. It was found that one of the two major ascending noradrenergic pathways, the dorsal noradrenergic bundle of Ungerstedt, is composed almost exclusively of axons ascending from the locus coeruleus (Lindvall & Bjorklund, 1974).

Steiner and Ellman (1972) had discovered a reciprocal relationship between hypothalamic self-stimulation and REM

sleep. They found that depriving rats of REM sleep resulted in higher self-stimulation rates accompanied by reduced self-stimulation thresholds. They also found that allowing rats to self-stimulate reduced significantly the amount of REM rebound which normally occurs when a REM-sleep-deprived animal is subsequently allowed undisturbed sleep. Steiner and Ellman reasoned that their results were indicative of interactions between the neurophysiological mechanisms subserving hypothalamic self-stimulation and the neurophysiological mechanisms subserving REM sleep phenomena.

Recall that the mediation of hypothalamic self-stimulation by norepinephrine had already been established (Wise & Stein, 1969), and that amphetamine had been shown to increase hypothalamic self-stimulation rates (Miller, 1957) and reduce self-stimulation thresholds (Stein, 1964; Stein & Ray, 1960; Steiner & Stokely, 1973); Steiner and Ellman (1972) were now showing that REM sleep deprivation has the same effect on hypothalamic self-stimulation as amphetamine, i.e., REM sleep deprivation was acting as if it was increasing the availability of norepinephrine at hypothalamic synapses. Also recall that the locus coeruleus is essential to REM sleep phenomena (Jouvet, 1972), and is composed almost exclusively of norepinephrine-containing neurons which send fibers to (Loizou, 1969; Maeda & Shimizu, 1972; Olson & Fuxe, 1972), and receive fibers from (Mizuno & Nakamura, 1970), the hypothalamus. Therefore, their suggestion that structures mediating REM sleep interact with structures

mediating self-stimulation was supported by much convergent, albeit circumstantial, evidence.

An important piece of evidence was added when Crow et al. (1972) and Farber et al. (1972) independently discovered that the locus coeruleus is itself a self-stimulation site. Ritter and Stein (1973) confirmed these findings, and they also showed that self-stimulation behavior elicited by locus coeruleus electrode placements is enhanced by d-amphetamine, like self-stimulation elicited from hypothalamic (Miller, 1957) and midbrain periaqueductal (Margules, 1969) electrode placements.

The discovery that the locus coeruleus supports self-stimulation madepossible a straightforward test of Steiner and Ellman's hypothesis that the locus coeruleus and the hypothalamus interact. Albino and Lucas (1962) had demonstrated that in rats having self-stimulation electrodes in the septum and in the ventral midbrain, near-simultaneous stimulation of the two electrode sites yielded response rates which were significantly higher than the sum of the response rates elicited when either electrode site was stimulated alone. Similar results were reported for other pairs of limbic self-stimulation sites (German & Holloway, 1973; Szabo et al., 1972; Ungerleider & Coons, 1970). Thus, if the locus coeruleus does in fact interact neurophysiologically with the hypothalamus or other limbic structures, that interaction ought to be detectable as enhanced self-stimulation rates under the condition of simultaneous stimulation of both

structures. Therefore, for this thesis, simultaneous stimulation interactions between the hypothalamus and the locus coeruleus and other dorsal and ventral tegmental self-stimulation areas were studied.

Recall that Stein found d-amphetamine to be at least five times more effective as an enhancer of hypothalamic self-stimulation behavior than l-amphetamine (Stein, 1964; Wise & Stein, 1974). Coyle and Snyder (1969) found d-amphetamine to be ten times more potent than l-amphetamine in inhibiting catecholamine uptake by synaptosomes obtained from brain regions (non-striatal) where norepinephrine is the predominant catecholaminergic transmitter. However, they found d- and l-amphetamine to be equipotent inhibitors of catecholamine uptake by synaptosomes obtained from the corpus striatum, a forebrain nucleus which is rich in dopaminergic terminals. Taylor and Snyder (1970, 1971) confirmed these findings in vivo. In addition, they showed that in rats, d-amphetamine is 10 times more potent than l-amphetamine in evoking locomotor activity, but only twice as potent in evoking gnawing behavior. To Taylor and Snyder, this disparity in differential potency of the d- and l- isomers of amphetamine in evoking locomotor activity as compared to gnawing behavior supports the suggestion that these two behaviors are mediated by different transmitters: locomotor activity by norepinephrine (Carlson & Lindqvist, 1967; Gunne & Reis, 1963; Sulser et al., 1968); gnawing behavior by dopamine (Anden et al., 1967; Ernst, 1967; Scheel-Kruger &

Randrup, 1967). They also suggested that the great difference in potency of the two amphetamine isomers in noradrenergic areas, but not in dopaminergic areas, could be useful in elucidating the functions of dopaminergic tracts (Taylor & Snyder, 1970). Subsequently, Phillips and Fibiger (1973) sought to apply Snyder and his co-worker's findings in order to show that self-stimulation elicited from the substantia nigra (A9), a dopaminergic midbrain nucleus, is mediated primarily by dopamine rather than norepinephrine. They reported that for electrodes in the substantia nigra, the d- and l- isomers of amphetamine were equipotent enhancers of self-stimulation rates, while for electrodes in the hypothalamus, d-amphetamine was a seven to ten times more potent enhancer of self-stimulation than l-amphetamine; the latter was a replication of Stein's earlier findings for the hypothalamus. Phillips and Fibiger suggested that differential enhancement of self-stimulation could be used to help determine which catecholaminergic transmitter, norepinephrine or dopamine, mediates self-stimulation at other brain areas, equipotent enhancement indicating dopaminergic mediation, differential enhancement indicating noradrenergic mediation.

Therefore, in this thesis, the d- and l-amphetamine drug technique was applied to the locus coeruleus and other dorsal and ventral tegmental self-stimulation areas in order to determine the primary catecholaminergic mediator at each site.

Finally, data obtained from the simultaneous stimulation experiments were compared with the data obtained from the d- and l-amphetamine experiments to see if the size of obtained interactions is systematically related to the combinations of transmitters mediating the interaction.

Rationale

A number of theorists have suggested that various aspects of behavior are attributable to neuronal "systems"; such systems can be defined along neuroanatomical (e.g., limbic system), neurophysiological, or neurochemical criteria.

Olds et al. (1960) noted the existence of two "systems" yielding self-stimulation, dorsal and ventral, which seemed to correspond with Hess' (1957) parasympathetic areas. They also reported a large "region" between the two self-stimulation "systems" which yielded "negative" results and which seemed to correspond with Hess' sympathetic areas. Stein (1968) suggested a laterally place "reward" system running among midbrain and forebrain structures and mediated by norepinephrine, and a more medially placed "punishment system mediated by acetylcholine (Margules & Stein, 1969); he later substituted serotonin for acetylcholine in the punishment system (Wise et al., 1973). Deutsch (1964) correlated behavioral measures with self-stimulation "refractory periods," and proposed a theory comprising two kinds of neurons mediating motivated behavior: short refractory period "reward" neurons, and longer refractory

period "drive" neurons. Crow (1973), like Deutsch, postulated "drive" and "reward" systems. However, unlike Deutsch, who defined his system neurophysiologically, Crow defined his system neuroanatomically and neurochemically. Crow suggested that there are two self-stimulation systems: a ventral ascending "drive" system which is associated with olfactory pathways and is mediated primarily by dopamine, and a dorsal ascending "reward" system associated with gustatory pathways and mediated by norepinephrine. Crow's suggestion that dopamine-containing neurons can support self-stimulation behavior received support from Phillips and Fibiger's (1973) study which confirmed that the substantia nigra, composed of dopaminergic neurons, supports self-stimulation behavior (see also Albino & Lucas, 1962; Olds & Peretz, 1960; Routtenberg & Malsbury, 1969), and that the d- and l- isomers of amphetamine affect substantia nigra self-stimulation differently from the way they affect hypothalamic (noradrenergic) self-stimulation. More recently, Farber et al. (1975) have shown that the dopaminergic "system" and the noradrenergic "system" are in fact interrelated. They found that locus coeruleus lesions abolish self-stimulation from hypothalamic electrodes placed among dopaminergic "nigrostriatal" fibers, but do not affect self-stimulation from hypothalamic electrodes placed more medially, in the medial forebrain bundle. Farber et al. also found that locus coeruleus lesions abolished self-stimulation elicited from the substantia nigra despite the

fact that there are no direct connections between them (see Ross & Reis, 1974). These results suggest that there may well be several distinct neuronal systems mediating self-stimulation, and that at least one of them comprises both noradrenergic and dopaminergic neurons.

Interaction studies like the one here described can serve to clarify the relationships among self-stimulation sites. For example, one might expect to observe large behavioral enhancements when structures belonging to two separate but interacting self-stimulation systems are stimulated simultaneously; smaller, yet significantly large behavioral enhancements when two structures belonging to the same self-stimulation system are stimulated simultaneously; and no enhancement at all when the two simultaneously stimulated structures belong to systems which are physiologically independent.

Likewise, strategic application of drug techniques like the d- and l-amphetamine screen utilized in this study can provide the rational basis for differentiating into distinct entities, fields of neurons which otherwise appear homogeneous, and for considering together as coherent physiological entities, far flung neuronal groups which at first glance appear to have little in common.

Method

Subjects and Surgical Procedure

Sixty male albino Holtzman Sprague-Dawley rats (375-500 gm) were anesthetized with 2 ml/kg Equithesin (Jensen) and stereotaxically (Kopf) implanted with either 2 or 3 bipolar electrodes (Plastic Products #MS-303-.018"-.312"-SS .010"), each electrode made of two intertwined strands of stainless steel wire (.34 mm diameter) completely insulated except at the tips. The electrode tips are .05-.10 mm apart. Electrodes were fastened to the skull with dental cement, which was anchored to the skull by means of three stainless steel screws. After the dental cement had dried, the incision was closed with sutures, and the animal returned to its home cage where it had access to food and water ad libitum.

In subjects inplanted with two bipolar electrodes, electrodes were aimed at one of the following site combinations:

a. (1) locus coeruleus (dorsal noradrenergic bundle)
   (2) hypothalamus

b. (1) substantia nigra
   (2) hypothalamus

c. (1) periaqueductal midbrain central gray area
   (2) hypothalamus

d. (1) left hypothalamus
   (2) right hypothalamus

e. (1) substantia nigra
   (2) locus coeruleus (dorsal noradrenergic bundle).

In subjects implanted with three bipolar electrodes, they were aimed at one of the following site combinations:

a. (1) locus coeruleus (dorsal noradrenergic bundle)
(2) periaqueductal midbrain central gray area
(3) hypothalamus

b. (1) locus coeruleus (dorsal noradrenergic bundle)
(2) substantia nigra
(3) hypothalamus

c. (1) locus coeruleus (dorsal noradrenergic bundle)
(2) left hypothalamus
(3) right hypothalamus

Hypothalamic coordinates were: (a) 4.2-4.4 mm posterior to bregma, (b) 1.5 mm lateral to the sagittal suture, and (c) 8.7 mm from the top of the skull at the intersection of coordinates (a) and (b). Locus coeruleus (dorsal noradrenergic bundle) coordinates were: (a) 1.5-2.0 mm posterior to lambda, (b) 1.0 mm lateral to a line extrapolated posteriorly from the sagittal suture, and (c) 7.0 mm from the top of the skull at the intersection of coordinates (a) and (b). Substantia nigra coordinates were: (a) 2.0 mm anterior of lambda, (b) 2.0 mm lateral to the sagittal suture, and (c) 8.2 mm from the top of the skull at the intersection of coordinates (a) and (b). Periaqueductal midbrain central gray area coordinates were: (a) .6 mm anterior of lambda, (b) 1.5 mm lateral to the sagittal suture, (c) 6.8 mm from the top of the skull at the intersection of coordinates (a) and (b), and (d) angled at 12°-16° towards the mid-sagittal plane. The incisor bar was set at -5.0 mm. In animals implanted with three bipolar electrodes, locus coeruleus and hypothalamic electrodes were ipsilateral to each other, while the substantia nigra electrodes were contralateral to the other two. Periaque-

ductal central gray area electrodes entered the brain contralateral to hypothalamic and locus coeruleus electrodes, but their tips were angled towards the mid-sagittal plane.

Apparatus and Preliminary Testing

After recovery from surgery (10 days), each animal was placed in an operant conditioning chamber and shaped to lever-press by the method of successive approximations. The chambers, constructed of Plexiglas and stainless steel, were 20 x 20 x 23 cm. A 1.5 x 5.5 cm retractable lever (Scientific Prototype RL-200B) was located 4 cm above the grid floor on one wall of the chamber. A force of .2 N was sufficient to depress the lever and constituted a response. Electromechanical and solid-state switching circuitry in an adjacent room monitored subjects' behavior, recorded minute-by-minute response rates, and controlled contingencies of reinforcement. Reinforcements were pulses of electrical stimulation delivered on a continuous reinforcement schedule, and passed through either one or two of the animal's bipolar electrodes, depending upon the experimental condition. Stimulation consisted of biphasic sinusoidal 60 Hz waves. Train duration was held constant at 250 msec. Current intensity was held constant within trials by placing a 100,000 ohm resistor in series with the animal, and varied between trials with a variable voltage transformer, according to the demands of the experiment. Wave form and stimulus intensity were continuously monitored by

observing the voltage drop across a 1,000 ohm resistor in series with the subject, on a Hewlett-Packard 1200B differential input cathode ray oscilloscope.

Animals were shaped for a minimum of 15 successive daily sessions at a variety of current intensities (5-200 uA) in each site. If, after 15 daily sessions in each site, a rat failed to self-stimulate from any site, it was discarded. Animals which did self-stimulate from at least one of their sites were continued in testing on the following schedule to determine rate-intensity functions for each site. Each day, each rat was allowed to self-stimulate from one site throughout a 42-minute session which was divided into six 7-minute periods; changes in current intensity occurred during a 1-minute time-out which separated each successive 7-minute period. Data from the first two minutes of each 7-minute period were disregarded in order to diminish any "carryover" effects (D'Amato, 1970, p. 121) in the analysis of data. The mean response rates over the last five minutes of each 7-minute period were recorded, and constituted the dependent variable in all conditions of the experiment. During the first 7-minute period, stimulation intensity was sufficiently low so that the animal's mean response rate over the last five minutes of the period was below an arbitrarily defined response threshold of 10 responses per minute (subthreshold). The second, third, fourth, and fifth intensities, presented in ascending order, were chosen such that they

sustained self-stimulation behavior at rates approaching or reaching highest responding; ascertaining peak response rates was always attempted. Responses during the final 7-minute period resulted in no stimulation (extinction). The resulting rate-intensity functions, averaged over five days, were determined for each electrode site in each rat.

Histology

Animals used in these experiments were subsequently killed with an overdose of Equithesin (2 ml) and perfused with .9% normal saline solution, followed by 10% formalin solution. Frozen serial sections were cut at 40 microns thickness on a Spencer 860 sliding microtome. The sections were then stained with luxol fast blue and cresyl violet (Kluver & Barrera, 1953), and electrode locus determined by comparing the stained sections with the photographs and diagrams in the rat brain atlas of Konig and Klippel (1963).

Experimental Procedure

Experiment 1: Simultaneous Stimulation. Animals demonstrating self-stimulation behavior in at least two electrode sites were termed double pressers; animals demonstrating self-stimulation behavior in only one electrode site were controls. After determination of a rate-intensity function for each electrode site, the double pressers began the following schedule, represented in Table 1 for a typical animal.

Every day, each double presser had the opportunity to lever-press during a 70-minute session which was divided into 10 7-minute periods. As before, data from the first two minutes were disregarded, and the last five minutes of each 7-minute period recorded.

During the first, third, fifth, and seventh 7-minute periods, lever-pressing resulted in no stimulation (extinction) in order to further reduce any contrast effects carried over from the preceding stimulation period. During Period 2, lever-pressing resulted in delivery of subthreshold stimulation to one electrode site (single stimulation); during Period 4, lever-pressing resulted in delivery of subthreshold stimulation to the alternate electrode site (single stimulation); during Period 6, lever-pressing resulted in simultaneous delivery of subthreshold stimulation to both sites (simultaneous stimulation) at the same intensities as in the earlier periods (2 and 4); during Period 8, lever-pressing resulted in delivery of subthreshold stimulation to the electrode site which had elicited the higher response rate during the earlier single stimulation periods. During Period 9, lever-pressing led to delivery of a stimulation intensity previously determined to elicit peak response rates for one electrode site; during Period 10, lever-pressing led to delivery of an intensity previously determined to elicit peak response rates for the alternate electrode site. Monitoring peak response rates for each site served three purposes:

1. to discern any shifts in rate-intensity functions,
2. to compare these peak response rates with the response rate elicited by the simultaneous stimulation condition, and
3. to maintain behavior in the face of the many extinction periods comprised by this paradigm.

The procedure was modified for controls (Table 2); each animal was tested in a 56-minute session divided into eight 7-minute periods. During Periods 1, 3, and 5, lever-pressing resulted in no stimulation as in the above paradigm. During Periods 2 and 6, lever-pressing led to delivery of stimulation which had previously elicited response rates just above threshold at the subject's only self-stimulation site (10-25 responses/min.). During Period 4, lever-pressing led to simultaneous stimulation of (1) the same intensity as in Period 2 to the self-stimulation site, and (2) one of a variety of current intensities (5-200 uA) to the site which did not support self-stimulation. During Period 7, lever-pressing led to delivery of the same stimulation intensity to the non-self-stimulation site as in Period 4; this was done to insure that this site did not support self-stimulation following the simultaneous stimulation condition. During Period 8, lever-pressing led to delivery to the self-stimulation site of the stimulation intensity previously determined to elicit peak response rates. This paradigm was repeated over approximately 40 days; the current intensity delivered to the non-self-

stimulation site was changed from day to day.

Experiment 2: Simultaneous Stimulation Thresholds. Most rats having simultaneous stimulation interactions in Experiment 1 were continued into Experiment 2.

After subthreshold intensities which support simultaneous stimulation interactions were determined for each site, a modified psychophysical method of limits was employed to determine stimulation intensity thresholds under the simultaneous stimulation condition. Stimulation intensity was held constant at one site and varied at the alternate site. With one exception (Rat 7E), in all interactions involving the hypothalamus as one of the two sites, the hypothalamic site was the one at which current intensity was held constant (constant site), and the alternate site the one at which current intensity was systematically varied (varied site). When simultaneous stimulation resulted in suprathreshold response rates (>10 responses/min.), current intensity in the varied site was reduced over successive days in steps of 1.4 uA per day until simultaneous stimulation no longer supported suprathreshold response rates. This procedure was then reversed and current intensity at the varied site increased over successive days in steps of 1.4 uA per day until suprathreshold response rates were once again attained during the simultaneous stimulation condition, and then increased further until suprathreshold response rates were attained at the varied site. For each varied site at least two alternate descending and ascending

sequences of intensities were run, and an overall varied site (alone) and simultaneous stimulation threshold determined for each pair of sites. The difference between the intensity at which the varied site supported self-stimulation when it alone was stimulated and the intensity at which the varied site supported self-stimulation when paired with the constant site stimulation was taken as a measure of the magnitude of interaction between the two electrode sites.

Experiment 3: d- and l-Amphetamine. Naive rats and 10 rats continuing from Experiment 2 entered the following drug paradigm which consisted of a series of six 3-day sequences. Days one and three of each 3-day sequence served as pre- and post-drug saline controls; on these days, only saline solution (1 ml of .9% normal saline solution/kg body weight) was injected intraperitoneally 30 minutes before the self-stimulation session. On the second day of each 3-day sequence, animals were injected intraperitoneally 30 minutes before the self-stimulation session with either d- or l-amphetamine sulfate (Smith, Kline & French) dissolved in .9% normal saline solution. The dosage was 1 mg amphetamine sulfate/kg of body weight; the concentration was 1 mg amphetamine sulfate/ml of .9% normal saline solution.

For the first 10 animals, each day, 30 minutes post-injection, the five current intensities composing the rate-intensity functions of either one or both of the rat's two

electrode sites was presented in an ascending interdigitated order, i.e., delivery of the current was alternated between the animal's two self-stimulation electrode sites every 7 minutes in an ABBAABBAAB manner. Responses during the final 7-minute period resulted in no stimulation (extinction). For each site, intensity was held constant throughout each 7-minute period; current intensity was changed between successive 7-minute periods. The purpose of the interdigitated procedure was to allow comparison between tegmental and hypothalamic responses to d- and l-amphetamine and to insure that both electrode sites were tested equally in the same time interval and under the same conditions. After it became apparent that the hypothalamic results were in accord with previously published reports (Phillips & Fibiger, 1973; Stein, 1964) (Figure 16), testing of hypothalamic sites was discontinued. Thereafter, standard single site rate-intensity functions were run under the same drug schedule.

Each animal in this experiment received both d- and l-amphetamine administered alternately in successive 3-day sequences in an ABBAAB manner. The order of drug presentation was counterbalanced across animals. Thus, each animal received 18 injections: three of d-amphetamine sulfate at 1 mg/kg; three of l-amphetamine sulfate at 1 mg/kg, and 12 of .9% normal saline. All comparisons were made between drug days (day 2) and pre-drug saline days (day 1). Data from post-drug saline days (day 3) were disregarded in

order to exclude drug carryover effects. Thus, all drug days were separated by at least two consecutive saline days.

In addition, eight animals were tested in the above procedure under a variety of drug doses for d-amphetamine (0.25, 0.5, 1.0, 2.0 mg/kg) and l-amphetamine (0.5, 1.0, 2.0 mg/kg) in order to determine if observed differential responding to d- and l-amphetamine is dose-dependent. These animals also received 18 injections per dose, as described above. Drug doses were presented in random order.

Response rates under both isomers were compared for each electrode site, and a determination made as to whether each electrode site responded to d- and l-amphetamine as do hypothalamic electrode sites (large response rate enhancement under d-amphetamine, little or no enhancement under l-amphetamine), or as do substantia nigra electrode sites (moderate response rate enhancements under d-amphetamine or l-amphetamine). Sites which exhibited a significantly greater response enhancement under d-amphetamine as compared to l-amphetamine were grouped into Drug Category I, while sites which exhibited nearly equal response enhancement under both isomers were grouped into Drug Category II. Then the microscopic slides of each electrode locus were examined to determine if Drug Category I electrode loci and Drug Category II electrode loci could be systematically differentiated neuroanatomically.

Results

Experiment 1: Simultaneous Stimulation

Twenty-seven animals completed Experiment 1: in 25 animals one pair of sites was tested and in two animals two pairs of sites were tested. In 24 of the 29 combinations, both sites supported self-stimulation. In the remaining five combinations, only one of the two sites supported self-stimulation. Table 3 summarized the data collected in Experiment 1. Table 7 shows the electrode locus for each site in each animal.

The data for each site combination were analyzed separately; for each of the 24 double presser combinations, the mean response rate under simultaneous stimulation was significantly higher than the sum of the two single-site conditions (correlated difference score t-tests, $p \leq .05$). By contrast, for each of the five control combinations, there was no significant difference between the simultaneous stimulation condition and the sum of the two single-site conditions regardless of the intensity delivered to the self-stimulation site (correlated difference score t-tests $p > .05$). This confirms Ungerleider and Coons' (1970) finding that both electrodes of a pair must be located in areas supporting self-stimulation to obtain response enhancement. If one of the electrode sites does not support self-stimulation, then simultaneous stimulation of both sites will not result in response rate enhancement. The lack of neurophysiological interaction in control combin-

ations was independent of the control site's locus. No significant interaction was found when either a tegmental electrode site was the control site and the hypothalamus was the self-stimulation site (e.g., Rat 22E) or vice versa (Rat 43F). Therefore, the control electrode site combinations indicate that the source of any interaction is neurophysiological and not merely the result of passive spread of current.

Figure 1 illustrates the differential effects of simultaneous stimulation of a double presser animal (3E) vs. a control animal (22E). Rat 3E, shown on the left, self-stimulates from both electrode sites, while Rat 22E, shown on the right, self-stimulates from the hypothalamus but not from the tegmentum. Rat 3E, the double presser, shows clear enhancement in response rate when both sites are stimulated at intensities which yield sub-threshold response rates when either site is stimulated alone at the same intensities. It is equally clear that no matter what current intensity was employed in the non-stimulation site, the control animal (22E) did not show enhancement in response rate during the simultaneous stimulation condition. Rat 22E further demonstrates that enhancements under simultaneous stimulation are contingent upon both electrode sites' capability of sustaining self-stimulation behavior. During the experiment, there were two days when Rat 22E inexplicably had enhanced response rates in the simultaneous stimulation condition; however, on the same days, Rat 22E pressed at

high rates for stimulation delivered to its non-stimulation site. Then, after these two days, Rat 22E never again self-stimulated from the non-stimulation site, nor demonstrated enhancements under simultaneous stimulation.

An enumeration of the 24 double presser combinations follows (see Table 3):

a) locus coeruleus (dorsal noradrenergic bundle)/ hypothalamus: n=6

b) periaqueductal midbrain central gray area/ hypothalamus: n=5

c) substantia nigra/hypothalamus: n=5

d) hypothalamus/contralateral hypothalamus: n=4

e) interpeduncular nucleus/locus coeruleus: n=1

f) interpeduncular nucleus/hypothalamus: n=1

g) locus coeruleus/substantia nigra: n=2

The five control animals (Table 3) had their non-stimulation electrodes located in the following areas (see Table 7):

a) one near the hypothalamus (Rat 43F)

b) one near the periaqueductal midbrain central gray area (Rat 8F)

c) one near the substantia nigra (Rat 79F)

d) two near the locus coeruleus (Rats 22E and 23E)

To repeat, no control animal demonstrated enhancement in response rate under the simultaneous stimulation condition, despite their being tested under a range of intensities which normally support self-stimulation and despite the close proximity of these non-stimulation sites to self-stimulation

sites.

In those interactions which occurred between two self-stimulation sites, the response rates elicited under simultaneous stimulation at threshold intensities were comparable to peak response rates for each site. As can be seen in Table 4, in eight instances simultaneous stimulation at threshold intensities elicited response rates that were higher than one of the site's peak intensity response rate. In five instances, simultaneous stimulation of two sites at threshold intensities elicited response rates that approached (90 percent) peak responding for one of the sites. In 11 instances, simultaneous stimulation of two sites at threshold intensities elicited response rates which, although well below peak responding, were substantially higher than the sum of the response rates elicited by stimulating each site alone. In fact, in every instance, simultaneous stimulation of two self-stimulation sites at threshold intensities elicited response rates that were greater than the sum of the response rates elicited by stimulation at either site alone.

Experiment 2: Simultaneous Stimulation Thresholds

In 16 of 18 animals completing Experiment 2, one pair of electrode sites was tested; two animals (74E and 41E) were tested in two site combinations (Table 5).

The twenty site combinations for which simultaneous stimulation thresholds were obtained follow (the varied site appears first, and the constant site appears second) (see

Table 5):

a) locus coeruleus (dorsal noradrenergic bundle)/ hypothalamus: n=6

b) midbrain periaqueductal gray/hypothalamus: n=4

c) substantia nigra/hypothalamus: n=4

d) hypothalamus/contralateral hypothalamus: n=4

e) interpeduncular nucleus/hypothalamus: n=1

f) locus coeruleus/interpeduncular nucleus: n=1

For every electrode site combination, the varied site threshold intensity was lower in the simultaneous stimulation condition than when the varied site was stimulated alone. Thus, in every case, in the simultaneous stimulation condition suprathreshold responding (>10 responses per minute) was maintained with varied site intensities well below that required when varied site stimulation was presented alone.

For the twenty electrode combinations, the magnitude of the difference between the threshold intensities in the simultaneous stimulation condition and the varied-site-alone condition varied between 1.6 uA and 42.4 uA.

This data is presented in two series of figures, Figures 2 through 10, and Figures 11 through 15. Figures 2 through 10 show the differences in threshold intensity between the varied-site alone condition and simultaneous stimulation condition as a function of threshold determinations. Each threshold determination represents a set of conditions in which the varied site intensity was systematically reduced

(odd-numbered threshold determinations) or increased (even-numbered threshold determinations), according to a modified method of limits (D'Amato, 1970). Each threshold determination provided a varied-site-alone threshold and a simultaneous stimulation threshold, which are the ordinate values in these figures. Figures 11 through 15 show, for representatives of various site combinations, differences in response rates between the varied-site-alone and simultaneous stimulation condition as a function of the number of microamps below the threshold intensity of the varied site (deviation from threshold). The important data in these figures are the intensities at which rate of responding drops below ten responses per minute.

Figures 2 and 3 show varied-site-alone vs. simultaneous stimulation thresholds across threshold determinations for the locus coeruleus (dorsal noradrenergic bundle)/hypothalamus group. This group had varied site threshold reductions ranging from 1.6 to 8.5 uA (Table 5). Figure 11 shows, for a representative animal (74E), the overall mean decrease in response rates under both single-site stimulation and simultaneous stimulation conditions as the varied site intensity was gradually reduced, and then increased over successive days.

Figures 4 and 5 show threshold differences between the simultaneous stimulation and varied-site-alone condition for the periaqueductal gray/hypothalamus site combinations. Threshold reductions for these site combinations ranged

between 3.4 to 14.4 uA (Table 5; Figures 12 and 13).

For the substantia nigra/hypothalamus site combinations, reductions in simultaneous stimulation thresholds ranged between 3.2 and 16.5 uA (Table 5; Figures 6, 7 and 14).

Reductions in simultaneous stimulation thresholds for hypothalamus/contralateral hypothalamus site combinations ranged between 1.6 and 12.4 uA (Table 5; Figures 8, 9 and 15).

In the single animal (Rat 41E) which had an electrode in the interpeduncular nucleus, there were large interactions with both the hypothalamus (9.2 uA threshold reduction), and with the locus coeruleus (42.4 uA threshold reduction, Table 5; Figure 10). The latter threshold reduction (42.4 uA) was the largest obtained in these experiments.

The results of this experiment will be discussed further in the next section in conjunction with the results of Experiment 3.

Experiment 3: d- and l-Amphetamine

Data from 26 electrode sites were obtained in Experiment 3 (Table 6). Sites were assigned to Drug Category I when d-amphetamine, but not l-amphetamine, produced large increases in self-stimulation rates. Sites were assigned to Drug Category II when d- and l-amphetamine were nearly equipotent in increasing self-stimulation rates. In every instance, dorsal tegmental Drug Category II electrodes

(designated "mid-ventral periaqueductal group") (10 electrodes, Figures 20 and 21) were subsequently found to be localized in the mid-ventral portions of the midbrain periaqueductal and pontine periventricular areas, and adjacent tegmentum, including such structures as the oculomotor nuclei, the Edinger-Westphal nuclei, the dorsal raphe nucleus, the interstitial nuclei, and the medial longitudinal fasciculus. Dorsal tegmental Drug Category I electrodes were subsequently found to be located in one of three areas:

a) the locus coeruleus and the adjacent tegmentum which contains the main ascending coerulean projection, the dorsal noradrenergic bundle (designated "locus coeruleus [dorsal noradrenergic bundle]" group) (10 electrodes ; Figure 20);

b) the dorso-lateral portions of the midbrain periaqueductal gray area and adjacent tegmentum, including such structures as the nucleus Darkschewitsch and the dorsal longitudinal fasciculus (designated "lateral periaqueductal group") (5 electrodes ; Figure 21); and

c) the interpeduncular nucleus (one electrode; Figure 21).

The proportional effects of d- or l-amphetamine (1 mg/kg body weight) as compared to saline on rate-intensity functions obtained from the mid-ventral periaqueductal group, the locus coeruleus (dorsal noradrenergic bundle) group, and the lateral periaqueductal group are shown in Figures 17 and 18. Each data point in these functions is the mean ratio, calculated across animals, of response rates under drug conditions compared to response rates under saline conditions. This ratio was obtained for each animal

by assigning its saline response rate a value of one and its drug response rate a value proportionate to the ratio between drug response rate and saline response rate.

Both Figure 17 and Table 6 clearly show that for the mid-ventral periaqueductal group, there is no significant difference in response enhancement between d- and l-amphetamine (correlated difference score *t*-test, $p > .05$). In contrast to these results, for the locus coeruleus (dorsal noradrenergic bundle) group response enhancement under d-amphetamine is significantly greater than response enhancement under l-amphetamine (correlated difference score *t*-test, $p \leq .05$) (Table 6, Figure 17). Similarly, for the lateral periaqueductal group, response enhancement under d-amphetamine is significantly greater than response enhancement under l-amphetamine (correlated difference score *t*-test, $p \leq .05$) (Table 6, Figure 18). Moreover, upon ranking individual sites' drug-to-saline response-rate ratios, it is found that Drug Category I sites (lateral periaqueductal area, locus coeruleus [dorsal noradrenergic bundle]) had significantly greater response enhancements under d-amphetamine than did Drug Category II sites (mid-ventral periaqueductal area) (Mann-Whitney U test, $p \leq .05$); conversely, Drug Category II sites had significantly greater response enhancements under l-amphetamine than Drug Category I sites (Mann-Whitney U test, $p \leq .05$). To summarize, as a group, mid-ventral periaqueductal sites displayed moderate and virtually equal response enhancement under both amphet-

amine isomers while both the locus coeruleus (dorsal noradrenergic bundle) and lateral periaqueductal sites displayed a large response enhancement under d-amphetamine and almost no response enhancement under l-amphetamine.

Ten hypothalamic electrode sites were tested under d- and l-amphetamine. All but one (Rat 68F) displayed a large response enhancement under d-amphetamine and almost no response enhancement under l-amphetamine (Figure 22). Rat 68F had an anterior hypothalamic electrode which reacted nearly equally to both amphetamine isomers (Table 6).

Eight electrode sites (three mid-ventral periaqueductal, five locus coeruleus [dorsal noradrenergic bundle]) were tested across several dosages of d- and l-amphetamine. Figure 19 displays the proportional drug effect plotted against dosage. It can be seen that the results shown in Figures 17 and 18 for 1 mg/kg of d- or l-amphetamine remain essentially the same; across all doses, Drug Category I sites show differential responding under d- and l-amphetamine, while Drug Category II sites show virtually equal responding under d- and l-amphetamine.

Lateral periaqueductal and mid-ventral periaqueductal electrode sites can also be differentiated according to the magnitude of their maximal saline response rates (Table 6); peak response rates elicited from mid-ventral periaqueductal electrodes were significantly higher than peak response rates elicited from the lateral periaqueductal electrodes

(Mann-Whitney U test, $p \leq .05$). However, there was no significant difference between mid-ventral periaqueductal sites and locus coeruleus (dorsal noradrenergic bundle) sites (Mann-Whitney U test, $p > .05$) although the group mean response rate for the mid-ventral periaqueductal sites is somewhat higher. No significant difference (Mann-Whitney U test, $p > .05$) in individual mean response rates was obtained between the locus coeruleus (dorsal noradrenergic bundle) sites and the lateral periaqueductal sites, although the group mean response rate for the locus coeruleus (dorsal noradrenergic bundle) group is higher.

The single electrode which was located within the interpeduncular nucleus produced peak response rates which were comparable to those of the mid-ventral periaqueductal sites. By contrast, the interpeduncular electrode responded to d- and l-amphetamine like the Drug Category I sites, i.e., there was a significantly greater response to d-amphetamine than to l-amphetamine.

In summary, self-stimulation electrodes located in four general areas were tested under d- and l-amphetamine:

1. the locus coeruleus (dorsal noradrenergic bundle) group which produced moderate response rates which were greatly enhanced by d-amphetamine, but not by l-amphetamine (Drug Category I);

2. the lateral periaqueductal group which produced low rates of responding, and which responded to d- and l-amphetamine in a manner similar to the electrodes in the locus coeruleus (dorsal noradrenergic bundle) group (Drug Category I);

3. a mid-ventral periaqueductal group which produced high response rates which were equally enhanced by both amphetamine isomers (Drug Category II); and

4. the single interpeduncular nucleus placement which produced high response rates which were greatly enhanced by d-amphetamine, but not by l-amphetamine (Drug Category I).

A post hoc and Speculative Analysis of Experiments 2 and 3

After completion of Experiment 3, the results of Experiments 2 and 3 were analyzed together in light of several recent studies. Electrode site combinations were categorized into three groups (A, B, and C) on the basis of the response of each electrode's locus under d- and l-amphetamine (Table 5).

Group A encompasses site combinations in which both electrodes were located in areas in which response rates are greatly enhanced by d-amphetamine, but not by l-amphetamine. Ten electrode site combinations were placed in Group A on the basis of this and past studies' indicating that self-stimulation elicited from electrodes in the following areas is greatly enhanced by d-amphetamine, but not l-amphetamine:

1. the locus coeruleus (dorsal noradrenergic bundle) (Ellman et al., 1975 a, b; Phillips, Brooke & Fibiger, 1975; the present study);

2. the ventral tegmental nucleus (Ellman et al., 1975 a, b; the present study);

3. the dorsal longitudinal fasciculus (the present study);

4. the lateral hypothalamic area (medial forebrain bundle) (Ellman et al., 1975 a; Phillips & Fibiger, 1973);

5. the periformical area at the level of the hypothalamic dorsomedial nucleus (Ellman et al., 1975 a; Phillips & Fibiger, 1973); and

6. the crus cerebri/nigrostriatal bundle area (Farber et al., 1975).

All six locus coeruleus (dorsal noradrenergic bundle)/hypothalamus combinations, one lateral periaqueductal gray area/hypothalamus combination, and three of the four hypothalamus/contralateral hypothalamus combinations were sorted into Group A. These combinations had a mean threshold reduction of 4.5 uA with a range of 1.6-8.5 uA (Table 5). It should be noted that even though this group of site combinations shows small threshold reductions, the simultaneous stimulation response rates are quite high for varied site intensity values lying between the varied site threshold and the simultaneous stimulation threshold. It should also be noted that this group, Group A, comprises the electrode site combination which were the least distant from each other (hypothalamus/contralateral hypothalamus) and the most distant from each other (hypothalamus/locus coeruleus).

Group B encompasses site combinations in which one electrode was located in an area (in these instances, the lateral hypothalamus) which, based on the present and previous studies, was known to have response enhancements under d-amphetamine, but not l-amphetamine (Drug Category I electrode sites) while the second electrode was in one of the areas which, based on the present and previous studies (Phillips & Fibiger, 1973; Ellman et al., 1975 b).

known to have response rate enhancements under both amphetamine isomers (Drug Category II electrode sites). The latter (Drug Category II) electrodes were located in the mid-ventral periaqueductal area, and in the pars compacta of the substantia nigra. Also included in the latter drug category was a single hypothalamic electrode (Rat 68F, Tables 4 and 5) located in the anterior nucleus of the hypothalamus, medial to the fornix (Table 6). Rat 68F had electrodes in the lateral hypothalamic area and in the anterior hypothalamic nucleus. Unfortunately, this animal lost its electrodes after only one dose each of d- and l-amphetamine (d-amphetamine: 1 mg/kg; l-amphetamine: 0.5 mg/kg); however, while the lateral hypothalamic electrode showed a typical hypothalamic response in that response rates were greatly enhanced by d-amphetamine but not by l-amphetamine, the anterior hypothalamic electrode showed an atypical hypothalamic response in that response rates were greatly enhanced by both d- and l-amphetamine (Table 6), despite the fact that a low l-amphetamine dose (0.5 mg/kg) was administered. The two hypothalamic electrodes of Rat 68F were tested under drug in the "interdigitated" procedure described in the Method section. Therefore, the differentiation between its two hypothalamic electrodes under l-amphetamine is all the more striking because the anterior hypothalamic nucleus was responsive to l-amphetamine at the same time that the lateral hypothamus was not. Under simultaneous stimulation conditions, the two hypothalamic

electrodes of Rat 68F had a threshold reduction of 12.4 uA, nearly double the next highest reduction (6.4 uA, Rat 66F, Table 5) among hypothalamus/contralateral hypothalamus site combinations. Group B site combinations had a mean threshold reduction under simultaneous stimulation of 12.8 uA (range: 9.1-16.0 uA). If the odd contralateral hypothalamic site combination (Rat 68F, described above) is placed in Group B, there is no overlap between Groups A and B in threshold reduction magnitudes and, obviously, there is a significant difference ($p \leq .05$) between them on the Mann-Whitney U rank order test. The inference drawn from these results is that interactions between sites which are dissimilar in their reactivity to d- and l-amphetamine are greater than the interactions between sites which are similar in their reactivity to d- and l-amphetamine. It is important to note that Group A comprises both the least and the most distant site combinations. In addition, the total current delivered to Group A and Group B site combinations did not differ significantly (Mann-Whitney U test, $p > .05$). Both these facts give considerable difficulty to current-spread explanations of the observed response rate enhancements.

Group C comprises site combinations in which one of the two electrode sites has not been tested under d- and l-amphetamine in previous studies.

In one of these site combinations (Rat 80F), one electrode was located in the pars reticulata of the sub-

stantia nigra, as opposed to the pars compacta of the substantia nigra, the locus of all the other substantia nigra electrodes. Unfortunately, drug data was not obtained for this animal. As can be seen (Table 5) the threshold reduction for the hypothalamus/substantia nigra, pars reticulata site combination is considerably lower than the other hypothalamus/substantia nigra site combinations. This site combination was excluded from Group B because of its unique placement, together with the lack of drug data from this or any other study on electrode placements in the pars reticulata, substantia nigra.

The other two Group C site combinations were in the same animal (Rat 41E) which had three electrodes: hypothalamus, interpeduncular nucleus, and locus coeruleus. Its two site combinations were: hypothalamus/interpeduncular nucleus, and locus coeruleus/interpeduncular nucleus (Table 4). The hypothalamus/interpeduncular nucleus combination had a moderate threshold reduction under <u>simultaneous stimulation</u> (9.2 uA); the locus coeruleus/interpeduncular nucleus had by far the largest threshold reduction under <u>simultaneous stimulation</u> (42.4 uA). The interpeduncular nucleus electrode site was tested under d- and l-amphetamine (Table 6) and it had a much greater response to d-amphetamine than to l-amphetamine. These combinations were excluded from Group A because this was the only electrode tested which was in the interpeduncular nucleus, which is quite distant from any of the other loci studied

in these experiments.

Discussion

Periaqueductal Self-Stimulation

Previous studies have established the midbrain tegmentum and the locus coeruleus as self-stimulation areas (Crow et al, 1972; Ellman et al., 1974; Olds & Peretz, 1960; Huang & Routtenberg, 1971; Ritter & Stein, 1973; Routtenberg & Malsbury, 1969). By contrast, until recently, stimulation of periaqueductal and periventricular areas was thought to produce predominantly aversive effects (Olds & Olds, 1962; Margules & Stein, 1969; Routtenberg & Olds, 1966; Spiegel et al., 1954). However, Ball (1972) demonstrated reliable self-stimulation elicited from ventromedial hypothalamic electrodes in rats, and Valenstein (1965) reported that although stimulation of the periaqueductal region was thought to be generally aversive, reliable escape responding could be elicited only from dorsal periaqueductal electrode placements; ventral periaqueductal electrodes failed to reliably elicit escape responding. Valenstein did not test for self-stimulation from periaqueductal electrodes; Olds and Olds (1962) had previously concluded that periaqueductal electrodes do not support self-stimulation. However, Cooper and Taylor (1967) demonstrated that caudal thalamic periventricular, and rostroventral periaqueductal electrodes do support self-stimulation, although animals with such placements were difficult to shape and showed behavioral signs of fear and pain. Steiner et al. (1973) and Ellman et al. (1974)

demonstrated self-stimulation from rostrodorsal periaqueductal electrode sites; they also replicated Cooper and Taylor's rostroventral periaqueductal self-stimulation findings. Self-stimulation elicited from caudoventral periaqueductal sites (near the dorsal raphe nucleus [B7]) was reported by Margules (1969); this finding was also replicated and extended to other caudoventral periaqueductal sites (Ellman et al., 1974; Steiner et al., 1973; Wolfle et al., 1971). By contrast, although Liebman et al. (1973) placed electrodes throughout the periaqueductal gray, they failed to obtain self-stimulation for periaqueductal electrode sites other than in the region of the dorsal rsphe nucleus. On the basis of their incorrect results, they suggested a rostral-caudal differentiation of the periaqueductal gray, the rostral periaqueductal gray being primarily aversive, the caudal periaqueductal gray being primarily rewarding.

However, a number of studies have shown that within "limbic" structures the rewarding or aversive properties of stimulation are more dependent on the reinforcement contingencies than on the locus of stimulation. Generally, animals will work to receive short trains of relatively high intensity stimulation, and will also work to escape long trains of comparatively low levels of stimulation; both acquisition and escape behaviors can almost invariably be elicited from the same electrodes, often at the same intensity (Steiner et al., 1973). Therefore, before a stimulation site is characterized as aversive, it must be

carefully and patiently tested for self-stimulation, no matter how aversive long trains of passive stimulation may appear. This is particularly true for periaqueductal sites, in which passive stimulation elicits vocalizations (Fernandez de Molina & Hunsperger, 1962) and postures (Spiegel et al., 1954; Skultety, 1963) which are associated universally with aversive stimuli. However, in all the studies performed in this laboratory during the last five years (Ellman et al., 1974; Steiner et al., 1973; the present study) no electrode placed within the periaqueductal region has failed to support self-stimulation behavior. Granting that initial "shaping" of naive rats is generally more difficult with periaqueductal electrodes than with hypothalamic electrodes, periaqueductal area electrodes are eventually just as reliable as hypothalamic electrodes in eliciting self-stimulation behavior.

Experiment 1: Simultaneous Stimulation

The significant enhancement of response rates under simultaneous stimulation conditions is taken as evidence of neurophysiological interaction between the structures stimulated. Therefore, the results of Experiment 1 indicate that the hypothalamus interacts with: the locus coeruleus, the midventral periaqueductal area, the dorsolateral periaqueductal area, substantia nigra, the interpeduncular nucleus, and with the contralateral hypothalamus (the latter finding is a replication of earlier findings by Ungerleider & Coons, 1970). The results of Experiment 1 also indicate

interactions between the locus coeruleus and: the substantia nigra, the midbrain periaqueductal region, and the interpeduncular nucleus.

Several lines of evidence indicate that these are true neurophysiological interactions and not simply artifacts resulting from spread of current from one electrode to another. First, bipolar electrodes were utilized; therefore, current was far more likely to flow towards the opposing pole of the same electrode rather than through the relatively high resistance of several millimeters of tissue to another electrode. Second, only pairs of sites in which both electrodes independently support self-stimulation behavior yielded response rate enhancements. The only exceptions to this generalization were animals 7E and 89F which were only intermittent and erratic self-stimulators. However, these animals seemed interested in the stimulation and could easily be shaped to approach the bar with pontine stimulation, indicating that the stimulation was rewarding. For those animals in which one electrode was clearly non-rewarding (22E, 23E, 43F, 8F, 79F), response rate enhancement did not occur, even when high current intensities were delivered to the non-rewarding electrode. Animal 22E is particularly instructive in this respect. The pontine electrode of this animal failed to support self-stimulation on all days except two, and only on those days was there a behavioral enhancement under the <u>simultaneous stimulation</u> condition. Ungerleider and Coons (1970) have previously

reported the necessity for both of two electrodes' being rewarding in order to obtain behavior enhancement under near-simultaneous stimulation of contralateral hypothalamic sites.

Third, the smallest interactions were obtained from the most distant site combinations (hypothalamus/locus coeruleus) and from the least distant site combinations (hypothalamus/contralateral hypothalamus). Total delivered current was approximately the same for all site combinations; therefore, if behavioral enhancement were a product of spread of current, we would expect the latter group, bilateral hypothalamic combinations, to have the highest behavioral enhancement, since, unlike action potentials, passively spreading currents diminish in intensity with increasing distance through a resistant medium.

Fourth, in many interaction studies (Albino & Lucas, 1962; German & Holloway, 1973; Szabo et al., 1972; Ungerleider & Coons, 1970) stimulation is near-simultaneous but not exactly simultaneous, i.e., delivery of stimulation to the second self-stimulation site occurs a short interval after stimulation to the first site has been completed. Therefore, spread of current is ruled out in such studies because there is no potential on the electrodes of one site while the other site is stimulated. In this study, stimulation was delivered simultaneously; however, subsequent studies (Bodnar et al., in progress) testing locus coeruleus/hypothalamus and periaqueductal gray area/hypo-

thalamus interactions with a near-simultaneous stimulation technique have replicated the essential findings of this study.

Fifth, animals often exhibit high response rates under simultaneous stimulation as the varied site current intensity is reduced over days (Figures 11-15), only to cease responding abruptly within one or two intensity decrements (Figures 12, 14).

Sixth, Routtenberg and Olds (1966) were able to replicate inhibitory effects of continuous dorsal midbrain tegmentum stimulation on hypothalamic self-stimulation (Olds & Olds, 1962) by applying carbachol instead of electrical stimulation to the dorsal midbrain.

Therefore, we can conclude that interactions between two simultaneously stimulated sites are a consequence of summation and/or potentiation of the physiological sequelae of stimulating two self-stimulation sites. This summation and/or potentiation might occur at either or both stimulation sites, and such a model would require axonal projections from one stimulated locus to the other. It is also possible that summation and/or potentiation might occur at third sites, and such a model requires axonal projections from both stimulated sites to any suspected third, summating, locus. Evidence concerning these neuroanatomical inferences are discussed in the following section.

Neuroanatomical Substrates of Interaction

That these results, together with results from previous

near-simultaneous stimulation studies (Albino & Lucas, 1962; German & Holloway, 1973; Szabo et al., 1972; Ungerleider & Coons, 1970) would suggest that all forebrain limbic structures are neurophysiologically and therefore neuroanatomically interralated is not surprising since forebrain limbic structures have well-established and relatively direct neuroanatomical connections (Nauta, 1958; Papez, 1937). However, the anatomical relationships among the hypothalamus, locus coeruleus, periaqueductal gray area and substantia nigra have not been as well established, undoubtedly because the tracts interconnecting brainstem structures are generally more diffuse than forebrain tracts. However, it is important to bear in mind that the diffuseness of a tract does not necessarily indicate a lack of specificity in its connections, or physiological and behavioral insignificance (Ramon-Moliner & Nauta, 1966). A striking confirmation of this point is that of the pathways mediating pain (Melzack, 1973). These pathways are quite diffuse and therefore difficult to trace, yet no one would argue that they do not have a specific and critically important role in regulating behavior.

Be that as it may, the diffuseness of brainstem limbic tracts made observation of their relationships nearly impossible until the advent of specialized neurohistochemical techniques like the Nauta-Gygax (1954) degeneration stain, and histofluorescence (Falck et al.,

1962) which allow selective visualization of some fibers within a reticulum, while the others remain in a uniform, non-obfuscating, background.

Even before the advent of these techniques, several relatively compact hypothalamic-midbrain tracts were well known:

1. the mamillary peduncle, a predominantly ascending tract comprising fibers from midbrain dorsal and ventral tegmental nuclei, and collaterals from ascending sensory pathways terminating in the mamillary bodies (Fox, 1941; Guillery, 1957; Morest, 1961; Nauta, 1958). Some of the mamillary peduncle's fibers bypass the mamillary bodies, continuing rostrally to contribute to the medial forebrain bundle (Nauta & Haymaker, 1969)

2. the mamillo-tegmental tract, which originates in the mamillary bodies and descends to the dorsal and ventral tegmental nuclei (of Guddens)

3. the dorsal longitudinal fasciculus, both an ascending and a descending tract composed of fibers interconnecting various groups of neurons lying adjacent to the hypothalamic III ventricle, the mesencephalic cerebral aqueduct of Sylvius, and the pontine and medullary IV ventricle (Crosby & Woodburne, 1951).

Numerous investigators have observed degeneration in the midbrain periaqueductal gray area after hypothalamic lesions (e.g., Guillery, 1957; Nauta, 1958; Wolf & Sutin, 1966) and degeneration in hypothalamic nuclei after periaqueductal gray lesions (e.g., Bucher & Burgi, 1953; Chi, 1970; Hamilton & Skultety, 1970). Nauta (1958) noting that both the periaqueductal gray area and the dorsal and ventral tegmental nuclei exchange projections with the hypothalamus, suggested that such anatomical reciprocity

is indicative of functional reciprocity between the midbrain, and forebrain limbic structures, such interactions being mediated by hypothalamic nuclei. Nauta's hypothesis of functional reciprocity among midbrain and forebrain limbic structures is supported by Steiner and Ellman's (1972) discovery of functional reciprocity between REM sleep and hypothalamic self-stimulation (see Introduction), and by the present tegmental/hypothalamic interaction study.

Thus, the anatomical substrate for the observed interactions between midventral and lateral periaqueductal electrode sites, and hypothalamic electrode sites, are well established; the periaqueductal-hypothalamic interconnections of the dorsal longitudinal fasciculus can easily account for them. A possible difficulty with such an account is the fact that the dorsal longitudinal fasciculus runs to and from medial hypothalamic nuclei, whereas in the present study hypothalamic electrodes were generally more lateral, most frequently near the fornix, and, less frequently, the lateral hypothalamic area (medial forebrain bundle) (see Table 7). Is there evidence, then, that influences upon medial hypothalamic neurons are relayed to lateral hypothalamic ones? Yes, several kinds of experiments suggest that the medial and lateral hypothalamus interact physiologically. For example, Oomura et al. (1964) found that stimulation of the ventromedial hypothalamic nucleus affects the firing pattern of lateral hypothalamic neurons, and vice versa.

Hoebel and Teitelbaum (1962) and, more recently, Porrino (1975) have presented evidence indicating an inhibitory influence of the medial hypothalamus upon lateral hypothalamic self-stimulation. There is some evidence that such medial-lateral hypothalamic interactions are mediated by direct neuronal connections between them. Albert and Storlien (1969) found that knife cuts between the medial and lateral hypothalamus result in hypothalamic hyperphagia, implying, of course, that interrupting the path between the medial and lateral hypothalamus is equivalent to destroying the ventromedial nucleus; in either case the inhibitory influence of the medial hypothalamus upon the lateral hypothalamus (or other laterally placed structures) is lost. However, because interconnections among hypothalamic nuclei are thin and poorly myelinated, they are difficut to trace (Arees & Mayer, 1967; Millhouse, 1969; Wolf & Sutin, 1966). As a result, data concerning intrinsic hypothalamic interconnections have been scanty. However, Krieg (1932) reported a tract (tractus hypothalamus filiformis) between the subfornical area of the lateral hypothalamus and the paraventricular nucleus of the medial hypothalamus (see also Millhouse, 1969, Fig. 8). Moreover, Guillery (1957) and Nauta (1958) have observed degenerating axons in medial hypothalamic nuclei after lateral hypothalamic and lateral preoptic lesions; Millhouse (1969) reported short projections from lateral hypothalamic neurons to medial hypothalamic nuclei. Thus, there is no

doubt that lateral hypothalamic neurons project to medial hypothalamic as well as periaqueductal nuclear groups. Krees and Mayer (1967) presented evidence for medial hypothalamus-to-lateral hypothalamus projections; they observed degenerating terminals in the lateral hypothalamus after lesioning the ventromedial and arcuate hypothalamic nuclei. Subsequently, Millhouse (1969) observed projections from ventral ventromedial neurons to lateral hypothalamic neurons.

Finally, Millhouse (1969) has made the important observation that dendrites of both lateral and medial hypothalamic neurons extend a considerable distance from their cell bodies; in fact, dendrites of ventromedial hypothalamic neurons extend into the lateral hypothalamic area, and vice versa. Therefore, axons projecting to the ventromedial nucleus are likely to terminate upon <u>both</u> ventromedial lateral hypothalamic neurons' dendrites, and, of course, the same argument applies as well to axons projecting to the lateral hypothalamic area.

The above-described studies lead to the conclusion that hypothalamic functioning is undoubtedly the consequence of rich interaction among medial and lateral hypothalamic neurons.

Neuroanatomical relationships between the hypothalamus and the locus coeruleus are not clear, partly because the locus coeruleus is not a well-defined nucleus. The term "locus coeruleus" is Latin for "sky-like place" (Spilman,

1957); this brain area acquired its name because in humans the neurons within it are pigmented, and this pigmentation gives a sky-blue appearance to the lateral borders of the pontine IV ventricle when viewed from above (Kuntz, 1936). Thus the term locus coeruleus originally described a gross brain feature only in terms of cell pigmentation. It was subsequently discovered that these pigmented neurons are a differentiated portion of the laterodorsal tegmental nucleus, a column of neurons lying in the ventrolateral part of the periaqueductal gray at the level of the isthmus (posterior midbrain/anterior pons) (Huber & Crosby, 1943). An important anatomical detail is that the neurons composing the laterodorsal tegmental nucleus are not confined to the borders of the central gray; neurons in the ventral part of the nucleus "spill over" into the adjacent dorsal tegmentum. Another important fact is that the posterior neurons of the nucleus are consistently pigmented only in primates (Russell, 1955); therefore, in animals like dogs, cats, and rats, the original defining feature of the locus coeruleus is generally absent, and in such species the locus coeruleus had to be defined in terms of cytoarchitectonic comparisons with corresponding primate sections. This difficulty was circumvented by the discovery that in rats neurons composing the laterodorsal tegmental column can be made to fluoresce (Dahlstrom & Fuxe, 1965) because they are noradrenergic. Happily, this feature, unlike pigmentation, is ubiquitous; it is true of all species thus far

tested (cats: Chu & Bloom, 1974; Jones & Moore, 1974; monkeys: Felton et al., 1974; Garner & Sladek, 1975; Hubbard & DiCarlo, 1973; Humans: Nobin & Bjorklund, 1973).

These fluorescence studies have confirmed the important anatomical facts that: (a) the ventral borders of the nucleus are poorly delineated, and (b) the locus coeruleus, though reported to be a "pontine" nucleus, in fact protrudes anteriorly well into the posterior midbrain; therefore, it is possible to distinguish between the "principal" locus coeruleus which lies within the central gray, and the "subcoeruleus" which lies ventrolateral to the central gray in the adjacent tegmentum.

The latter fact (b) is of critical importance to this discussion because it is the rostral locus coeruleus neurons which project to midbrain and forebrain structures (Maeda & Shimizu, 1972) and because rostral locus coeruleus neurons most affect REM sleep; most of Jouvet's locus coeruleus lesions were in the isthmus region (posterior midbrain and anterior pons).

Recall that several studies have reported degenerating fibers within the periaqueductal gray as far posterior as the isthmus region following posterior lateral hypothalamic lesions (Guillery, 1957; Nauta, 1958; Wolf & Sutin, 1966) and many investigators have reported interconnections among the periventricular nuclei (see discussion of dorsal longitudinal fasciculus). In addition, Conrad et al. (1974)

have reported degeneration in the area of the locus coeruleus after periaqueductal lesions. Therefore, a careful reading of the literature would leave little doubt that there are both direct and indirect descending pathways between the posterior hypothalamus and the locus coeruleus. What doubt might remain is dispelled by a recent electron microscopic study by Mizuno and Nakamara (1970) who demonstrated degenerated pre-synaptic membranes impinging on the dendritic membranes of locus coeruleus neurons following posterior hypothalamic lesions.

The case for ascending connections between the locus coeruleus and the hypothalamus is less clear. Loizou (1969) reported that after unilateral and bilateral locus coeruleus lesions there is a loss of fluorescence in the premamillary, mamillary, posterior, arcuate, and periventricular hypothalamic nuclei, and, in some cases, the dorsomedial, paraventricular, supraoptic, preoptic and perifornical hypothalamic nuclei. Loizou remarked that loss of terminal fluorescence was more noticeable after bilateral lesions as compared to unilateral lesions, and that loss of terminal fluorescence was seen both ipsilateral and contralateral to unilateral locus coeruleus lesions; both findings were taken as indicating bilateral distribution of locus coeruleus fibers.

Ungerstedt (1971, p. 18) also reported a decrease in the number of hypothalamic noradrenergic terminals after bilateral locus coeruleus lesions. In addition, he

distinguished between a dorsal noradrenergic bundle composed mostly of locus coeruleus fibers, and a ventral noradrenergic bundle composed of fibers from noradrenergic cell groups A1, A2, A5, and A7.

Maeda & Shimizu (1972) observed fluorescence reductions following scattered brain lesions. They concluded that noradrenergic fibers emanating from the "principal" locus coeruleus and forming a dorsal (ascending) noradrenergic pathway have fine terminations on thalamic and cortical neurons, while fibers emanating from the anterior locus coeruleus, subcoeruleus, and noradrenergic group A5, form an "intermediate" ascending noradrenergic pathway between the dorsal and ventral noradrenergic bundles. These fibers have thick terminations in the hypothalamus, mainly in the "periventricular zone."

Olson and Fuxe (1972) presented evidence that fibers from the ventral part of the "principal" locus coeruleus and the subcoeruleus, as well as fibers from noradrenergic group A7, have thick terminations on periventricular and preoptic neurons, results similar to those of Maeda and Shimizu (1969); however, Olson and Fuxe maintained that these fibers join the dorsal part of the ventral noradrenergic pathway, rather than forming a distinct "intermediate" pathway as suggested by Maeda and Shimizu.

In a radioautographic study Pickel et al. (1974) failed to find labeling in any hypothalamic nucleus following injection of radioactive proline into the locus

coeruleus. The most likely reason for this failure is that the injection micropipettes were lowered only 6.3 mm into the brain, thereby preventing injection into the more ventral parts of the "principal" locus coeruleus, or into the subcoeruleus (it is the more ventral coeruleus neurons which most investigators have found to project to the hypothalamus).

Kobayashi et al. (1974) assayed norepinephrine levels of individual hypothalamic nuclei following locus coeruleus lesions; they found that destruction of locus coeruleus neurons significantly reduced norepinephrine levels in the ipsilateral periventricular and paraventricular nuclei. They did not find significant reduction in the lateral hypothalamus (medial forebrain bundle). However, their lesions spared the most anterior portions of the locus coeruleus and they inexplicably sampled the medial forebrain bundle just medial to the optic tract at the level of the posterior hypothalamic region, where it is far more likely that ventral bundle (non-locus coeruleus) noradrenergic fibers were sampled, than locus coeruleus noradrenergic terminals (see Ungerstedt, 1971).

Ross and Reis (1974) measured the decrease in dopamine-β-hydroxylase activity in various brain nuclei following locus coeruleus lesions (recall that dopamine-β-hydroxylase is the enzyme which converts dopamine to norepinephrine, and is indigenous to noradrenergic neurons). They found that locus coeruleus lesions resulted in nearly

50% decreases in dopamine-β-hydroxylase activity in both the medial and the lateral hypothalamus.

Lindvall et al. (1974) and Lindvall and Bjorklund (1974) reinvestigated the ascending catecholamine pathways with an improved fluorescence technique, and they attempted to correlate these pathways with pathways long known in the "classical" neuroanatomical literature. In addition to the previously described dorsal noradrenergic bundle (renamed the "dorsal tegmental bundle" by them), and the ventral noradrenergic bundle (which they found to be within the classical central tegmental tract), they discovered noradrenergic fibers and cell bodies within the dorsal longitudinal fasciculus (recall that the dorsal longitudinal fasciculus interconnects periaqueductal and periventricular nuclei, including the medial hypothalamic nuclei. They reported that some fibers from the locus coeruleus contribute to the dorsal longitudinal fasciculus; others project to the ventral tegmental nucleus and then continue on a path resembling that of the classical mamillary peduncles. Noradrenergic dorsal longitudinal fasciculus fibers also innervate the rostral dorsal tegmental nucleus and adjacent periaqueductal gray area. Thus, they firmly established the intimate relation between the locus coeruleus and midbrain limbic structures. Lindvall et al. (1974) and Lindvall and Bjorklund (1974) failed to find any locus coeruleus terminals in the hypothalamus. However, their criterion for distinguishing

between coeruleus and non-coeruleus terminals was size: fine terminal associated with the locus coeruleus, thick terminals associated with non-coeruleus noradrenergic neurons. But previous investigators (Maeda & Shimizu, 1972; Olson et al., 1972) reporting coeruleus terminals in the hypothalamus have described them as uncharacteristically thick. Therefore, Lindvall et al.'s use of terminal size to distinguish between coeruleus and non-coeruleus terminals in the hypothalamus is inappropriate.

To summarize, the locus coeruleus, periaqueductal gray area and medial hypothalamus certainly have reciprocal interconnections. Fibers from the posterior and lateral hypothalamus certainly descend to ventral central gray nuclei at least as far caudal as the isthmus, which comprises the anterior locus coeruleus. Fibers originating in the periaqueductal nuclei descend to the locus coeruleus and ascend to the posterior hypothalamus and possibly to the lateral hypothalamus (Chi, 1970). Finally, locus coeruleus fibers probably terminate in the medial hypothalamus and may also terminate in the lateral hypothalamus as well. Even if it were the case that no locus coeruleus fibers reached the lateral hypothalamus directly, there are sufficient indirect pathways from limbic structures which are indisputably innervated by the locus coeruleus (e.g., anterior thalamic nucleus, cingulate cortex and hippocampus) to the lateral hypothalamus to account for the interactions observed in the present study.

Poschel (1969) and Crow (1972) previously reported self-stimulation from electrodes near the interpeduncular nucleus. Interactions between the locus coeruleus and interpeduncular nucleus might be mediated by the medial flow of the catecholaminergic portion of the central tegmental radiation (Lindvall & Bjorklund, 1974), to which the locus coeruleus contributes. Interaction between the hypothalamus and the interpeduncular nucleus might be mediated by fibers descending from the medial forebrain bundle (Nauta, 1958) or by fibers ascending from dopaminergic neurons (A10) in the area of the interpeduncular nucleus. A difficulty with the latter hypothesis is that the interpeduncular nucleus had a large response rate enhancement under d-amphetamine, but not l-amphetamine, indicating noradrenergic mediation of self-stimulation. Also, although dopaminergic fibers course throughout the hypothalamus as part of the medial forebrain bundle, no dopaminergic terminals have been demonstrated in the hypothalamus outside of the arcuate nucleus-pituitary region (Bjorklund et al., 1975). The same difficulty arises in attempting to account for hypothalamus/substantia nigra interactions. To make matters worse, although extensive degeneration has frequently been reported in ventral tegmental areas following hypothalamic lesions, such fiber degeneration has not been reported within the substantia nigra. However, several forebrain nuclei (e.g., caudoputamen) have been shown to have both noradrenergic

and dopaminergic post-synaptic receptors (Forn et al., 1974), and it is conceiveable that hypothalamic and substantia nigral impulses converge there.

Experiment 2: Simultaneous Stimulation Threshold Reductions

The results of this experiment indicate that there are systematic differences in the magnitude of interaction among different electrode site combinations. Such a result has never been reported before. The possible significance of these differences in interaction magnitude will be discussed in the following sections.

It should be noted that the contralateral hypothalamic combination yielded the smallest interactions. This result is important because if the observed response enhancements were due merely to non-physiological summation of passively spreading current, the contralateral hypothalamic electrodes, being much closer to each other than any of the other combinations tested, should have evidenced the greatest behavioral enhancements rather than the smallest as was the case. There are no reported commissures or direct fiber pathways between the contralateral lateral hypothalamic areas; therefore, physiological influences between them must travel a circuitous route. A recent study by German and Holloway (1973) suggests that impulses from the opposing lateral hypothalami converge posteriorly, probably to the midbrain limbic structures.

Experiment 3: d- and l-Amphetamine

If one accepts the generalization that noradrenergic

areas respond differentially to d- and l-amphetamine, while dopaminergic sites respond near-equally to d- and l-amphetamine, then the present results can be explained. Results from the locus coeruleus (dorsal noradrenergic bundle) and the lateral periaqueductal gray area showing response enhancement under d-amphetamine, but not l-amphetamine would then converge with previous histofluorescent and pharmacological studies indicating that the locus coeruleus and its ascending projection are noradrenergic Ellman et al., 1975 a; Ritter & Stein, 1973; Ungerstedt, 1971). The results from midventral periaqueductal gray sites, showing that d- and l-amphetamine have nearly equal response enhancement, are similar to results obtained from the substantia nigra by Phillips and Fibiger (1973). Such a result, of course, implies the presence of dopamine-containing cell bodies and/or fibers in the midventral periaqueductal area. Although there is considerable evidence indicating that the substantia nigra has dopamine-containing cell bodies (Ungerstedt, 1971), until recently evidence for the presence of any catecholamine substance in the midventral periaqueductal area was scanty. However, investigators employing fluorescent histochemical techniques had repeatedly detected catecholaminergic cell bodies and terminals within the periaqueductal gray area. For example, Dahlstrom and Fuxe (1964) described a catecholaminergic area (A10), located in the mid-sagittal plane of the rat midbrain, extending dorsally to the ventral border of the

periaqueductal gray area; they also described catecholamine-containing cell bodies within the periaqueductal gray area (oculomotor and Edinger-Westphal nuclei). Area A10 was subsequently shown to be a dopaminergic area with terminals in the nucleus accombens (Anden et al., 1964,1965) where near-equal self-stimulation enhancement for both amphetamine isomers has recently been reported (Phillips et al., 1975).

Subsequent investigations have confirmed that there are distinct groups of catecholamine-containing cell bodies in the periaqueductal gray area as far anterior as the oculomotor nuclei and as far posterior as the dorsal raphe nucleus; distinct catecholamine-containing fibers are interspersed within the medial longitudinal fasciculus as far posterior as the level of the locus coeruleus (Felton et al., 1974; Lindvall et al., 1974; Lindvall & Bjorklund, 1974; Nobin & Bjorklund, 1973; Palkovitz & Jacobowitz, 1974). The present study's midventral periaqueductal gray electrodes are located within these catecholaminergic areas.

Dahlstrom and Fuxe (1965) suggested that the periaqueductal catecholaminergic cells may be a dorsal extension of the A10 (dopaminergic) cell group. Lindvall and Bjorklund (1974) also speculated that catecholaminergic cell bodies in the midventral periaqueductal gray area at the level of the rostral dorsal raphe nucleus might be a dorsal extension of A10. Most importantly, Bjorklund et al. (1974) described a group of dopaminergic neurons (A11) interspersed among fibers of the dorsal longitudinal

fasciculus from the hypothalamus to the rostral mesencephalon. Thus, a dopaminergic hypothesis accounting for midventral periaqueductal self-stimulation has a firm histochemical base. The present suggestion that midventral periaqueductal gray self-stimulation is mediated primarily by dopamine rests heavily on the d- and l-amphetamine behavioral screening procedure, and like Phillips and Fibiger's investigations is dependent on the validity of this procedure. Thus, these results (Ellman et al., 1975b; Phillips & Fibiger, 1973; Phillips et al., 1975) are vulnerable to some investigators' (Ferris et al., 1972; Harris & Baldessarini, 1973) doubts about the validity of the d- and l-amphetamine screening procedure to distinguish between noradrenergic and dopaminergic neurons. Poschel (1969) tested numerous forebrain and midbrain self-stimulation sites under a monoamine oxidase blocker, tranylcypromine. He found that the drug generally increased self-stimulation rates significantly. However, at some sites self-stimulation rates inexplicably decreased; most of these latter sites have subsequently been found to be predominantly dopaminergic (e.g., substantia nigra, nucleus accumbens, caudate nucleus). Therefore, the ability of a second drug to differentiate among noradrenergic and dopaminergic self-stimulation sites increases our confidence in corresponding results under the amphetamine isomers, controversies concerning the biochemical mechanisms of these effects notwithstanding.

At first glance, serotonin might be considered as the most likely mediator of periaqueductal self-stimulation because until recently, serotonin appeared to be the only monoamine present in large quantities within the periaqueductal area (dorsal raphe nucleus [B7]). Therefore, it might be hypothesized that the present results were mediated by augmented release of serotonin, but such an hypothesis would face several difficulties. First, it has been shown that for raphe serotonergic neurons, release of serotonin is greatest for low frequency stimulation, but minimal for higher frequencies, such as that employed in the present studies (Kostowski et al., 1969). Second, it has been repeatedly shown that serotonin release results in decreased self-stimulation rates (Poschel & Ninteman, 1971; Poschel et al., 1974; Stein & Wise, 1974), not increased rates as in the present experiments.

Third, Margules (1969) tested self-stimulation near the dorsal raphe nucleus (B7) with amphetamine, chlorpromazine, and parachlorophenylalanine (PCPA). Amphetamine is a catecholamine enhancer, chlorpromazine is a catecholamine blocker, while PCPA depletes both catecholamines and serotonin (Miller et al., 1970). Margules (1969) found that amphetamine increases dorsal raphe self-stimulation rates, while chlorpromazine decreases self-stimulation rates. He also found that PCPA decreases self-stimulation rates over the first 24 hours post-administration during

which time all monoamine levels are decreased; however, PCPA has no effect on dorsal raphe self-stimulation at 72 hours or thereafter, at which time catecholamine levels have begun to recover but serotonin levels remain depressed (Miller et al., 1970).

From these results Margules concluded that dorsal raphe self-stimulation is not mediated by serotonin-containing neurons near the electrodes, but by hypothesized noradrenergic fibers of passage, most probably within the ventral division of the dorsal longitudinal fasciculus. However, it is unlikely that Margules' results were due to stimulation of the dorsal longitudinal fasciculus since his electrode placements were ventral to most dorsal longitudinal fasciculus fibers (Konig & Klippel, 1963). Also, his results indicated only that dorsal raphe self-stimulation is mediated by a catecholaminergic transmitter; his experimental procedure did not distinguish between norepinephrine and dopamine as possible mediators. However, his results together with the results from the present study do pose insuperable problems for a serotonergic hypothesis of periaqueductal self-stimulation. It is ironic that although Margules' own electrode placements were probably too ventral to stimulate the dorsal longitudinal fasciculus, the present study's amphetamine isomer results indicate that self-stimulation elicited from dorsal longitudinal fasciculus fibers is mediated by norepinephrine, as he claimed.

Other possibilities are mediation of midventral periaqueductal self-stimulation by norepinephrine alone, or by norepinephrine in conjunction with other transmitters. If norepinephrine is the sole mediating transmitter substance, then it is difficult to explain the difference in pharmacological response between midventral periaqueductal sites and nearby lateral periaqueductal, or dorsal noradrenergic bundle sites, which are clearly noradrenergic. If all periaqueductal self-stimulation is mediated by norepinephrine, then why do adjacent periaqueductal areas (lateral, midventral) respond differentially to the same pharmacological agents? The second possibility, that norepinephrine is involved in, and perhaps necessary for, the mediation of midventral periaqueductal self-stimulation is not ruled out by the present data; however, these data lead inescapably to the inference that midventral periaqueductal self-stimulation is subserved by dopamine-mediated mechanisms. Whether or not the hypothesis that dopamine is the principal mediator of midventral periaqueductal self-stimulation is correct, it is clear from the data of both the present study and Phillips and Fibiger's (1973) and Phillips et al.'s (1974) studies that the neurohumoral substrates of midventral periaqueductal, substantia nigra, and nucleus accombens self-stimulation must differ from the neurohumoral substrates of hypothalamic, dorsal noradrenergic bundle, and dorsal longitudinal fasciculus self-stimulation.

Implications

The results of the present experiments have implications for various investigators' conceptions of brain "systems" for reward and/or punishment. The response enhancement data from Experiment 1 indicate that dorsal and ventral midbrain, and dorsal pontine, self-stimulation sites interact with the hypothalamus as do forebrain limbic structures and the hypothalamus (German & Holloway, 1973; Jackson & Gardner, 1974; Szabo et al., 1972; Ungerleider & Coons, 1970).

These results support Nauta's (1958) hypothesis that dorsal and ventral midbrain structures are interacting components of the "limbic" motivational system.

These results also support Steiner and Ellman's (1972) hypothesis that structures which mediate REM sleep (i.e., locus coeruleus) interact with forebrain limbic structures which mediate motivated behaviors.

Conversely, they do not support notions of multiple reward "systems" defined in terms of (a) general location, response threshold, and response rate (Olds et al., 1960), or (b) putative heurohumoral mediators (Stein & Wise, 1973). Recall that Olds et al. (1960) hypothesized the existence of two reward systems, one ventral, having moderate thresholds and high response rates, the other dorsal, having low thresholds and lower response rates. However, response rates elicited from many dorsal electrodes (in the midventral periaqueductal, and locus coeruleus

[dorsal noradrenergic bundle] groups) were as high as response rates elicited from ventral (hypothalamic and substantia nigra) electrodes (see Ellman et al., 1974). Even if that were not true, the fact that dorsal and ventral electrode sites interact is prima facie evidence that they do not belong to separate and distinct self-stimulation "systems."

Recall also that in this study the largest interactions were between "noradrenergic" electrode sites (e.g., lateral hypothalamus) and "dopaminergic" electrode sites (e.g., substantia nigra); such results imply that there is a single "reward system" which comprises both noradrenergic and dopaminergic elements. Data obtained by Farber et al. (1975) bear on this point. They found that locus coeruleus (noradrenergic) lesions resulted in a complete and permanent cessation of self-stimulation elicited from substantia nigra (dopaminergic) electrodes and from hypothalamic sites known to be rich in dopamine (nigrostriatal bundle and H_2 fields of Forel); the same lesions left other hypothalamic self-stimulation sites (e.g., medial forebrain bundle) unaffected. Belluzzi et al. (1975) also presented evidence suggesting that self-stimulation elicited from the substantia nigra depends on the integrity of noradrenergic pathways. These results imply that dopaminergic mechanisms cannot by themselves subserve a complete self-stimulation "system"; rather, the results imply that dopaminergic sites support self-stimulation only in conjunction with noradren-

ergic sites. As of this writing, there is no data bearing on the converse question: do lesions of dopaminergic nuclei like the substantia nigra reduce self-stimulation elicited from noradrenergic sites like the medial forebrain bundle or locus coeruleus? If the answer turns out to be "yes," then it will be obvious that neither dopamine nor norepinephrine qualifies as <u>the</u> substrate of "reward." Instead, the question will then be how and where dopamine-containing and norepinephrine-containing neurons interact to produce rewarding brain activity. The fact that in the present study interactions between dopaminergic sites and noradrenergic sites were generally larger than interactions between pairs of noradrenergic sites suggests that dopaminergic and noradrenergic structures normally potentiate one another's rewarding effects. If, instead, dopaminergic structures and noradrenergic structures belonged to distinct systems, one might expect a disruption of behavior, or at best, small potentiations, rather than the large potentiations which actually do occur. The latter point is discussed in more detail in the following paragraphs.

Although it must be conceded that the <u>post hoc</u> analysis of the present interaction and drug data which sorts interacting site combination into a small-threshold-reduction, common neurotransmitter group (Group A), or a large-threshold-reduction, mixed transmitter group (Group B) can be only tentative until verified by further investigation, it does lead to several interesting theoretical notions about

the nature of interactions among neuroanatomical nuclei (or "systems" of intimately related nuclei). When an animal is presented with two electrical stimuli simultaneously, there are several possible outcomes:

1. one of the stimuli may be "ignored" while the other is "attended to" and, as a consequence, the animal may respond as though only the "attended" stimulus had been presented (no interaction). In the context of the present study, the animal may simply respond at the response rate determined by the more rewarding stimulus. In the present study, this type of interaction occurred in the control animals in which one electrode site was rewarding and the other site neutral;

2. the stimuli may interfere with each other, and as a consequence, the subject may respond at a lower rate than if only one stimulus had been presented (negative interaction). Olds and Olds (1962) reported such an effect of dorsal tegmental stimulation on hypothalamic self-stimulation. Valenstein (1965) reported a similar result for dorsal periaqueductal stimulation on hypothalamic stimulation. Also, Hoebel and Teitelbaum (1962) and Porrino (1975) reported similar results for ventromedial hypothalamic stimulation on lateral hypothalamic self-stimulation;

3. the overall reinforcing value of the simultaneously presented stimuli may be averaged and the animal may "split the difference" between the rates elicited when the stimuli are presented alone (averaged interaction). Studies like the present study are performed at threshold self-stimulation intensities because at intensities eliciting near-peak response rates, animals tend to show this type of interaction (pilot data, this study);

4. the reinforcing value of the stimuli may be added together and as a consequence, simultaneous stimulation response rates may be a simple sum of the rates elicited by the stimuli when presented alone (summative interaction);

5. the presentation of a second rewarding stimulus may potentiate the effects of the first, and as a consequence, simultaneous stimulation response rates may be greater than the sum of rates elicited when presented alone (potentiating interaction). In the present study, this type of interaction occurred when both electrode sites were rewarding and when threshold or below-threshold intensities were employed.

How might a potentiating interaction be interpreted? Albino and Lucas (1962), borrowing a notion from Freud, suggested that the activity of reward-mediating structures is fed back upon them, causing them to increase their activity (positive feedback). If this were so, stimulating two anatomically related limbic structures would multiply the activity level in a neuronal circuit comprising both structures. The circularity of limbic interconnections certainly makes such a model plausible.

Another possibility is that interacting reward structures have potentiating behavioral effects because they converge on the dendrites of a third group of neurons. Therefore, stimulating one rewarding site would apply an excitatory bias to those dendrites, potentiating the effects of a secondary excitatory influence. If such a population of "reward" impulse-receiving dendrites received impulses from two distinct populations of reward neurons, each population coded by a characteristic neurotransmitter (e.g., norepinephrine and dopamine), then stimulating two noradrenergic reward sites would stimulate twice the same general input to the convergent dendritic field. This would result in a temporal summation at the convergent

dendritic fields and the result would be a significant but small behavioral potentiation. However, if a noradrenergic reward site and a dopaminergic reward site were stimulated, then separate inputs would summate spatially at the convergent dendritic field, and the result would be larger behavioral potentiation.

The latter model, though admittedly speculative, does account for the present data, some of which is otherwise difficult to explain. For example, Rat 68F had two hypothalamic electrodes, one of which was insensitive to l-amphetamine, the other, sensitive to l-amphetamine. If the d- and l-amphetamine drug technique does accurately acreen for noradrenergic and dopaminergic self-stimulation sites, and if the norepinephrine-dopamine interaction model of reward-mediation is correct, then we would predict that: (a) Rat 68F's l-amphetamine sensitive hypothalamic site, the anterior hypothalamic nucleus, is rich in dopamine; and (b) there should have been a large threshold reduction when Rat 68F's two hypothalamic electrodes were <u>simultaneously stimulated</u>. The latter prediction (b) was correct; Rat 68F's threshold reduction was the largest of all hypothalamus/contralateral hypothalamus electrode combinations, twice the magnitude of the next largest threshold reduction. Two studies are relevant to the former (a) prediction. First, in Poschel's (1969) study of the effects of monoamine oxidase blockade on self-stimulation, most electrode sites had increased response rates

under drug. The sites which instead showed decreased response rates are generally characterized by their richness in dopamine, and the only hypothalamic site listed in that study as showing decreased response rates under drug is the anterior hypothalamic nucleus. Second, Bjorklund et al. (1975) have recently described an intrahypothalamic dopamine system; according to them, the anterior hypothalamic nucleus is permeated with dopaminergic fibers of passage.

Additional evidence for a noradrenergic-dopaminergic reward interaction model is that despite the fact that there are no known connections between the locus coeruleus and substantia nigra, nor substantia nigral terminals in the hypothalamus (Bjorklund et al., 1975), Farber et al. (1975) abolished substantia nigra self-stimulation with locus coeruleus lesions, and, in the present study, large threshold reductions were observed upon <u>simultaneous stimulation</u> of hypothalamus and substantia nigra electrodes. These results lead to the inference that in both instances the two interacting structures interact, not upon each other, but at a third region, which receives terminals from both. One possible "third" region is the caudate nucleus which is known to have both noradrenergic and dopaminergic receptor sites (Forn et al., 1974).

Epilogue

The history of physiological psychology has been to study intensively those manipulations which result in gross, easily detected changes in behavior; manipulations resulting in small behavioral changes are frequently overlooked or dismissed as unimportant. Such an approach has limited our understanding of brain functioning in at least two significant ways.

First, despite the almost universal ridicule of Gall's phrenology by modern brain researchers, the association between a particular brain manipulation and a large behavioral effect makes the temptation to attribute the effect to the manipulated structure nearly overwhelming. Witness the postulation of lateral hypothalamic "hunger centers" and ventromedial hypothalamic "satiety centers" which were in vogue during the 1950's and 1960's as explanations for the cyclic variation in food-seeking behavior (see Albert & Storlien, 1969).

Second, the individual differences in behavior which most interest psychologists are usually quite subtle, rarely like the relatively gross differences preferred by physiological psychologists. There are many people whose behavior is considered "abnormal" or even bizarre who are nevertheless quite able to seek food, water, and mates, produce and comprehend language, and process visual and auditory information. Unlike the case in most physiological experiments, most behavioral differences of interest

appear to be under the control of many variables, and attempts to account for human behavior in terms of simple-minded models postulating control of this or that behavior by this or that nucleus or fiber tract have proven uniformly fruitless (see Valenstein, 1973).

Undoubtedly, if we are to eventually understand how brains produce behavior, we must abandon the notion that small neuronal subunits can independently regulate complex muscular and glandular activity patterns, free of influence from surrounding structures. The activity of each neuron is regulated by projections from many different nuclei, and, in turn, projects to, and influences, many nuclei. Therefore, to understand the role of any particular nucleus in the production or inhibition of a particular behavior, we must understand how the nuclei which project to it interact on its dendrites and how the output of that nucleus interacts with the output of other nuclei on common dendritic fields. Multiple site stimulation and ablation studies such as those described in this thesis are primitive first attempts to gain knowledge of how the activity of all the brain's nuclei are combined to produce singular and coordinated responses appropriate to the constellation of stimuli impinging on an organism at any given moment.

Table 1

Protocol for Simultaneous Stimulation Showing Data for Rat 87F, an Animal Which Self-Stimulates at Each of Its Two Electrode Sites (Double Presser)

Site	Stimulation Intensity (uA)	Mean Responses/min. First 2 min.	Mean Responses/min. Last 5 min.
Extinction	0.0	1.5	0.6
Hypothalamus	28.2	42.5	0.0
Extinction	0.0	1.0	1.8
Substantia Nigra	21.2	13.0	0.2
Extinction	0.0	0.0	0.4
Hypothalamus/ Substantia Nigra	28.2/21.2	89.5	42.0
Extinction	0.0	2.0	0.0
Substantia Nigra	21.2	5.0	0.0
Substantia Nigra	63.6	92.5	62.8
Hypothalamus	35.4	104.5	115.2

Table 2

Protocol for Simultaneous Stimulation Showing Data for Rat 79F, a Control Animal Which Self-Stimulates at One of Its Electrode Sites (Hypothalamus) but not at Its Other Electrode Site (Medial Lemniscus)

Site	Stimulation Intensity (uA)	Mean Responses/min.	
		First 2 min.	Last 5 min.
Extinction	0.0	8.2	0.5
Hypothalamus	28.2	32.0	24.6
Extinction	0.0	18.5	3.2
Hypothalamus/ Medial Lemniscus	28.2/21.2	34.0	19.2
Extinction	0.0	8.0	0.0
Hypothalamus	28.2	33.5	16.6
Medial Lemniscus	21.2	8.0	4.0
Hypothalamus	127.3	45.5	56.0

Table 3

Effect of Subthreshold Simultaneous Stimulation on Response Rates for Double Presser and Control Subjects

Animal	Threshold Intensity (uA)		Mean Responses/min.		
	Site 1	Site 2	Site 1	Site 2	Simultaneous Stimulation
I. Double Pressers					
Site 1: Locus Coeruleus (Dorsal Noradrenergic Bundle) / Site 2: Hypothalamus					
3E	10.6	53.0	9.2	3.8	142.6
7E	141.4	24.7	3.1	4.7	85.4
9E	21.2	63.6	3.6	8.5	45.1
25E	42.4	14.1	6.8	3.7	16.9
37E	21.2	14.1	7.0	0.0	23.0
74E	60.8	28.2	8.8	4.1	25.9
Site 1: Periaqueductal Midbrain Central Gray Area / Site 2: Hypothalamus					
40E	21.2	46.0	7.2	1.1	11.5
43E	81.3	21.2	6.1	0.2	20.4
19F	63.6	14.1	7.1	4.0	33.0
44F	89.1	33.9	5.0	7.9	20.2
51F	46.7	21.2	1.5	1.4	52.2

Table 3, cont'd.

Site 1: Substantia Nigra / Site 2: Hypothalamus					
81E	106.1	50.9	7.5	2.0	71.2
58F	38.2	28.2	6.3	9.0	105.5
80F	36.8	18.4	9.8	4.5	58.7
82F	87.7	28.2	4.4	4.8	28.2
87F	28.2	21.2	5.4	2.0	30.5
Site 1: Hypothalamus / Site 2: Contralateral Hypothalamus					
50E	12.7	22.6	0.3	5.5	12.6
74E	14.1	21.2	8.7	0.9	40.7
66F	17.0	18.4	3.8	9.7	40.1
68F	32.5	24.7	8.7	7.6	23.8
Site 1: Interpeduncular Nucleus / Site 2: Hypothalamus					
41E	28.2	30.4	4.5	7.0	81.1
Site 1: Interpeduncular Nucleus / Site 2: Locus Coeruleus					
41E	21.2	84.8	6.4	7.7	54.6
Site 1: Locus Coeruleus / Site 2: Substantia Nigra					
89F	63.6	127.3	6.0	0.0	9.6
90F	36.8	67.9	6.2	8.8	37.1

Table 3, cont'd.

II. Controls					
Site 1: Self-Stimulation Site / Site 2: Control Site					
22E	14.1	177.0 35.0	3.4	1.4 2.4	6.0 8.0
23E	14.1	141.0 35.0	2.5	0.0 0.0	8.8 0.2
8F	106.1	177.0 35.0	10.7	0.3 0.1	2.2 1.5
43F	46.0	177.0 49.5	6.5	0.2 0.3	9.3 6.3
79F	28.2	177.0 21.2	14.2	0.5 2.4	5.1 21.9

Table 4

Comparison of Single Site Peak Response Rates with Simultaneous Stimulation Response Rates

Animal	Peak Response Rate (resp./min.) Site 1	Site 2	Simultaneous Stimulation Response Rate (resp./min.)
	I. Double Pressers		
Site 1: Locus Coeruleus (Dorsal Noradrenergic Bundle) / Site 2: Hypothalamus			
3E	400.0	230.0	142.6
7E	10.0	165.0	85.4
9E	30.0	170.0	45.1
25E	40.8	141.0	16.9
37E	39.6	24.8	23.0
74E	78.2	76.4	25.9
Site 1: Periaqueductal Midbrain Central Gray Area / Site 2: Hypothalamus			
40E	54.2	28.6	11.5
43E	22.2	172.0	20.4
19F	48.6	79.6	33.0
44F	44.8	43.4	20.2
51F	58.8	169.2	52.2

Table 4, cont'd.

Site 1: Substantia Nigra / Site 2: Hypothalamus			
81E	108.8	71.6	71.2
58F	120.4	59.0	105.5
80F	49.8	141.4	58.7
82F	57.6	19.6	28.2
87F	75.6	143.2	30.5
Site 1: Hypothalamus / Site 2: Contralateral Hypothalamus			
50E	79.0	11.8	12.6
74E	134.0	76.4	40.7
66F	44.2	148.6	40.1
68F	25.0	74.4	23.8
Site 1: Interpeduncular Nucleus / Site 2: Hypothalamus			
41E	159.0	149.6	81.1
Site 1: Interpeduncular Nucleus / Site 2: Locus Coeruleus			
41E	159.0	27.8	54.6
Site 1: Locus Coeruleus / Site 2: Substantia Nigra			
89F	25.6	intermittent presser-positive	9.6
90F	86.6	107.8	37.1

Table 4, cont'd.

II. Controls			
Site 1: Self-Stimulation Site / Site 2: Control Site			
22E	219.0	-	6.0 8.0
23E	127.0	-	8.8 0.2
8F	103.2	-	2.2 1.5
43F	56.1	-	9.3 6.3
79F	65.8	-	5.1 21.9

Table 5

Threshold Reductions Between the Varied Site Threshold Intensity and the Simultaneous Stimulation Threshold Intensity

Animal	Threshold Intensity (uA)			Difference Between Varied Site and Simultaneous Stimulation Thresholds (uA)	Interaction Category
	Single Site Stimulation		Simultaneous Stimulation		
	Constant Site	Varied Site			
Varied Site: Locus Coeruleus (Dorsal Noradrenergic Bundle)/Constant Site: Hypothalamus					
3E	53.0	11.3	9.2	2.1	A
7E[a]	141.4	25.1	22.6	2.5	A
9E	63.6	22.6	14.1	8.5	A
25E	14.1	42.8	40.7	2.1	A
37E	14.1	20.5	18.9	1.6	A
74E	28.2	59.0	53.7	5.3	A
Varied Site: Periaqueductal Midbrain Central Gray Area / Constant Site: Hypothalamus					
43E	21.2	82.0	72.1	9.9	B
19F	14.1	63.5	49.1	14.4	B
51F	21.2	51.3	40.3	11.0	B
40E	46.0	21.9	18.5	3.4	A

[a]Varied Site: Hypothalamus

Table 5, cont'd.

Varied Site: Substantia Nigra / Constant Site: Hypothalamus					
81E	50.9	94.0	77.5	16.5	B
82F	28.2	99.7	83.4	16.3	B
87F	21.2	29.0	19.9	9.1	B
80F	18.4	35.4	32.2	3.2	C
Varied Site: Hypothalamus / Constant Site: Contralateral Hypothalamus					
50E	12.7	24.0	21.7	2.3	A
74E	21.2	13.8	12.2	1.6	A
66F	17.0	17.7	11.3	6.4	A
68F	24.7	30.4	18.0	12.4	B
Varied Site: Interpeduncular Nucleus / Constant Site: Hypothalamus					
41E	28.2	28.3	19.1	9.2	C
Varied Site: Locus Coeruleus / Constant Site: Interpeduncular Nucleus					
41E	21.2	86.2	43.8	42.4	C

Table 6

Self-Stimulation Response Rates Under Saline and Under d- or l-Amphetamine Sulfate

Animal	Mean Responses/min.				Response Rate Difference Score (d-amphetamine minus l-amphetamine)
	Peak Intensity	Threshold Intensity			
	Saline	Saline	d-amphetamine	l-amphetamine	
Drug Category I Electrode Sites					
Locus Coeruleus (Dorsal Noradrenergic Bundle) Electrode Sites					
9E	14.7	6.5	36.9	15.9	+21.0
13E	13.8	3.0	15.3	4.4	+10.9
14E	22.1	1.7	3.6	0.9	+ 2.7
18E	110.4	0.7	0.7	0.0	+ 0.7
33E	58.9	8.4	27.6	7.1	+20.5
54E	26.5	3.4	83.9	10.5	+73.4
74E	42.4	3.9	52.9	3.4	+49.5
23F	26.8	2.8	23.8	0.0	+23.8
26F (left)	19.0	1.0	13.3	1.0	+12.3
26F (right)	34.3	1.6	61.1	4.6	+56.5
Mean:	36.9	3.3	31.9	4.8	+27.1

Table 6, cont'd

Lateral (Periventricular System) Electrode Sites					
97C	11.2	9.4	24.7	16.7	+ 8.0
1E	14.0	5.2	31.0	4.7	+26.3
15E	14.4	2.9	2.3	0.7	+ 1.6
40E	54.2	0.7	19.3	1.4	+17.9
44F	45.1	1.0	32.2	0.2	+32.0
Mean:	27.8	3.8	21.9	4.7	+17.2
Interpeduncular Nucleus Electrode Site					
41E	157.6	0.2	20.9	0.1	+20.8
Drug Category II Electrode Sites					
Mid-Ventral Periaqueductal Gray Electrode Sites					
98C	56.0	5.0	18.4	6.5	+11.9
30E	98.2	16.4	39.9	26.7	+13.2
31E	86.3	15.7	46.6	8.1	+38.5
32E	195.6	27.0	18.0	31.8	-13.8
43E	22.2	5.8	39.3	21.6	+17.7
93E	29.7	5.3	65.2	52.8	+12.4
3F	35.0	6.4	17.5	21.7	- 4.2

Table 6, cont'd.

19F	45.2	4.8	1.0	14.2	-13.2
43F	55.5	1.9	5.2	39.0	-33.8
51F	43.5	9.5	14.9	20.7	- 5.8
Mean:	66.7	9.8	26.6	24.3	+ 2.3
Rat 68F Left Hypothalamus (Drug Category II) and Right Hypothalamus (Drug Category I)					
68F left	27.7	5.9	22.2	15.0	+ 7.2
68F right	36.6	8.4	32.0	11.4	+20.6

Table 7

Histological Determination of Electrode Placements

Animal	Area Aimed For	Actual Electrode Locus
97C	Tegmentum	Dorsal longitudinal fasciculus
	Hypothalamus	Globus pallidus/putamen
98C	Tegmentum	Oculomotor nucleus
	Hypothalamus	H_2 fields of Forel
1E	Tegmentum	Dorsal longitudinal fasciculus
	Hypothalamus	Fornix
3E	Tegmentum	Dorsal noradrenergic bundle
	Hypothalamus	Lateral hypothalamus/crus cerebri
7E	Tegmentum	Medial locus coeruleus
	Hypothalamus	Perifornical nucleus
9E	Tegmentum	Ventral tegmental nucleus
	Hypothalamus	Medial forebrain bundle/fornix
13E	Tegmentum	Dorsal noradrenergic bundle
	Hypothalamus	Lateral part of dorsomedial nucleus
14E	Tegmentum	Dorsal noradrenergic bundle/anterior locus coeruleus
	Hypothalamus	Medial forebrain bundle
15E	Tegmentum	Periaqueductal gray behind nucleus Darkschewitsch
	Hypothalamus	Between fornix and dorsomedial nucleus
18E	Tegmentum	Dorsal noradrenergic bundle/anterior locus coeruleus
	Hypothalamus	Medial part of medial forebrain bundle/fornix

Table 7, cont'd.

22E	Tegmentum	Pontine reticular formation*
	Hypothalamus	Medial forebrain bundle/lateral hypothalamus
23E	Tegmentum	Pontine raphe*
	Hypothalamus	Fornix
25E	Tegmentum	Ventral tegmental nucleus
	Hypothalamus	Ventral medial forebrain bundle/lateral hypothalamus
30E	Tegmentum	Oculomotor nucleus
31E	Tegmentum	Dorsal tegmental decussation/nucleus linearis pars caudalis
32E	Tegmentum	Dorsal raphe nucleus
33E	Tegmentum	Dorsal noradrenergic bundle/anterior locus coeruleus
37E	Tegmentum	Dorsal noradrenergic bundle/locus coeruleus
	Hypothalamus	Posterior hypothalamic nucleus
40E	Tegmentum	Dorsal longitudinal fasciculus, pars tegmentalis
	Hypothalamus	Zona incerta
41E	Tegmentum	Locus coeruleus/dorsal tegmental nucleus
	Tegmentum	Interpeduncular nucleus
	Hypothalamus	Lateral hypothalamus/crus cerebri
43E	Tegmentum	Oculomotor nucleus
	Hypothalamus	Lateral hypothalamus/dorsomedial nucleus

*electrode did not support self-stimulation

Table 7, cont'd.

50E	Hypothalamus	Left medial forebrain bundle/optic tract
	Hypothalamus	Right lateral hypothalamus
54E	Tegmentum	Locus coeruleus/dorsal noradrenergic bundle
74E	Tegmentum	Locus coeruleus
	Hypothalamus	Left dorsomedial nucleus/lateral hypothalamus
	Hypothalamus	Right fornix
81E	Tegmentum	Substantia nigra, pars compacta
	Hypothalamus	Zona incerta
93E	Tegmentum	Pontine medial longitudinal fasciculus
3F	Tegmentum	Interstitial nucleus (mesencephalon)
8F	Tegmentum	Oculomotor nucleus (electrode malfunction)
	Hypothalamus	Fornix
19F	Tegmentum	Midline gray between dorsal tegmental nuclei
	Hypothalamus	Lateral hypothalamus/dorsomedial nucleus
23F	Tegmentum	Dorsal noradrenergic bundle
26F	Tegmentum	Left anterior locus coeruleus
	Tegmentum	Right anterior locus coeruleus/dorsal noradrenergic bundle
43F	Tegmentum	Dorsal raphe nucleus
	Hypothalamus	Nucleus reuniens (subthalamus)*

*electrode did not support self-stimulation

Table 7, cont'd.

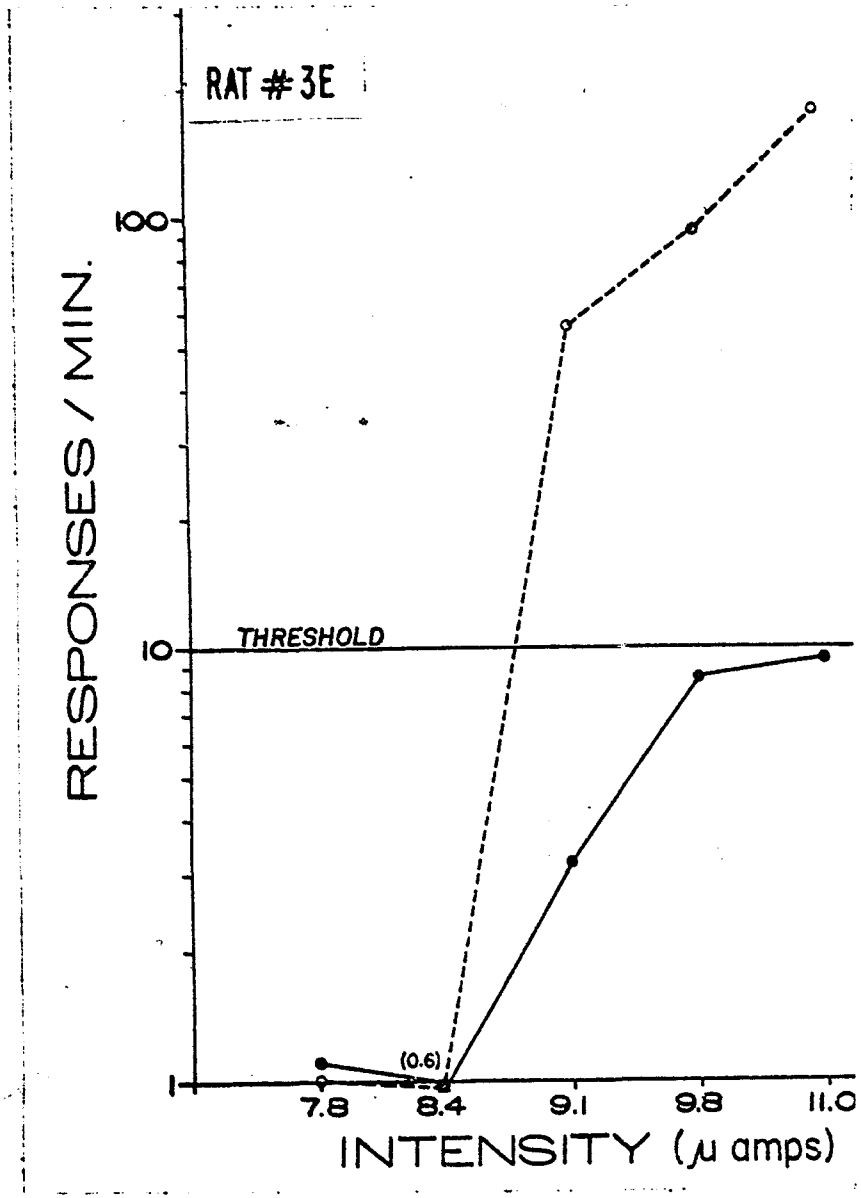
44F	Tegmentum	Lateral to periaqueductal gray
	Hypothalamus	H_1 fields of Forel/dorsomedial nucleus
51F	Tegmentum	Oculomotor nucleus
	Hypothalamus	Stria terminalis
58F	Tegmentum	Substantia nigra, pars compacta
	Hypothalamus	Lateral hypothalamus/fornix
66F	Hypothalamus	Left lateral hypothalamus
	Hypothalamus	Right fornix
68F	Hypothalamus	Left anterior nucleus of hypothalamus
	Hypothalamus	Right lateral hypothalamus
79F	Tegmentum	Lateral to medial lemniscus*
	Hypothalamus	Fornix/dorsomedial nucleus
80F	Tegmentum	Substantia nigra, pars reticulata
	Hypothalamus	H_2 fields of Forel/lateral hypothalamus
82F	Tegmentum	Substantia nigra, pars lateralis
	Hypothalamus	Dorsomedial nucleus
87F	Tegmentum	Substantia nigra, pars compacta
	Hypothalamus	Lateral hypothalamus/H_2 fields of Forel

* electrode did not support self-stimulation

Table 7, cont'd.

89F	Tegmentum	Locus coeruleus/pontine dorsal noradrenergic bundle
	Tegmentum	Substantia nigra, pars reticulata
90F	Tegmentum	Mesencephalic IV nucleus
	Tegmentum	Substantia nigra, pars compacta

Figure 1. Comparison of simultaneous stimulation interaction between dorsal noradrenergic bundle and hypothalamic self-stimulation sites (3E), and simultaneous stimulation interaction between a hypothalamic self-stimulation site and a tegmental non-self-stimulation site (22E).


RAT # 3E
RESPONSES / MIN.
100
10
1
THRESHOLD
(0.6)
7.8
8.4
9.1
9.8
11.0
INTENSITY (μ amps)

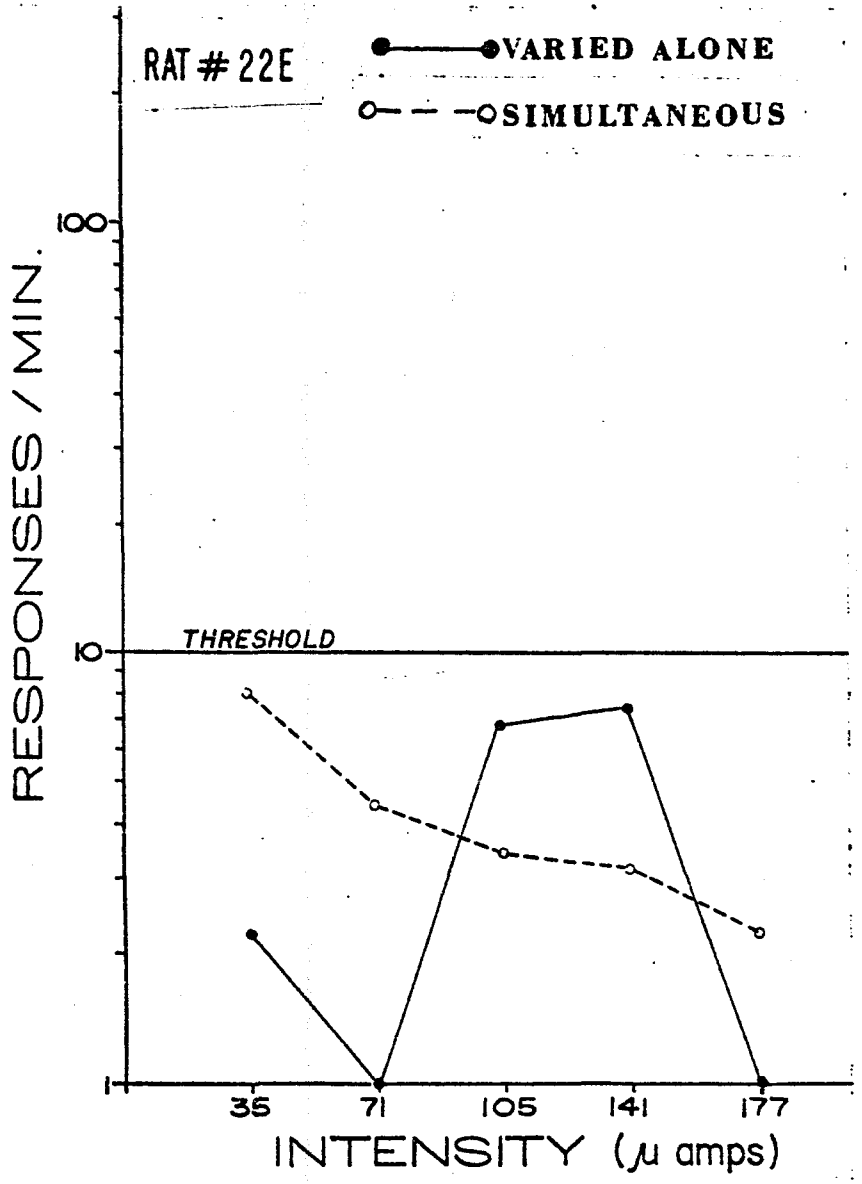

RAT # 22E
VARIED ALONE
SIMULTANEOUS
RESPONSES / MIN.
100
10
1
THRESHOLD
35
71
105
141
177
INTENSITY (μ amps)

Figure 2. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for three locus coeruleus (dorsal noradrenergic bundle)/hypothalamus electrode site combinations (74E, 37E, 25E).

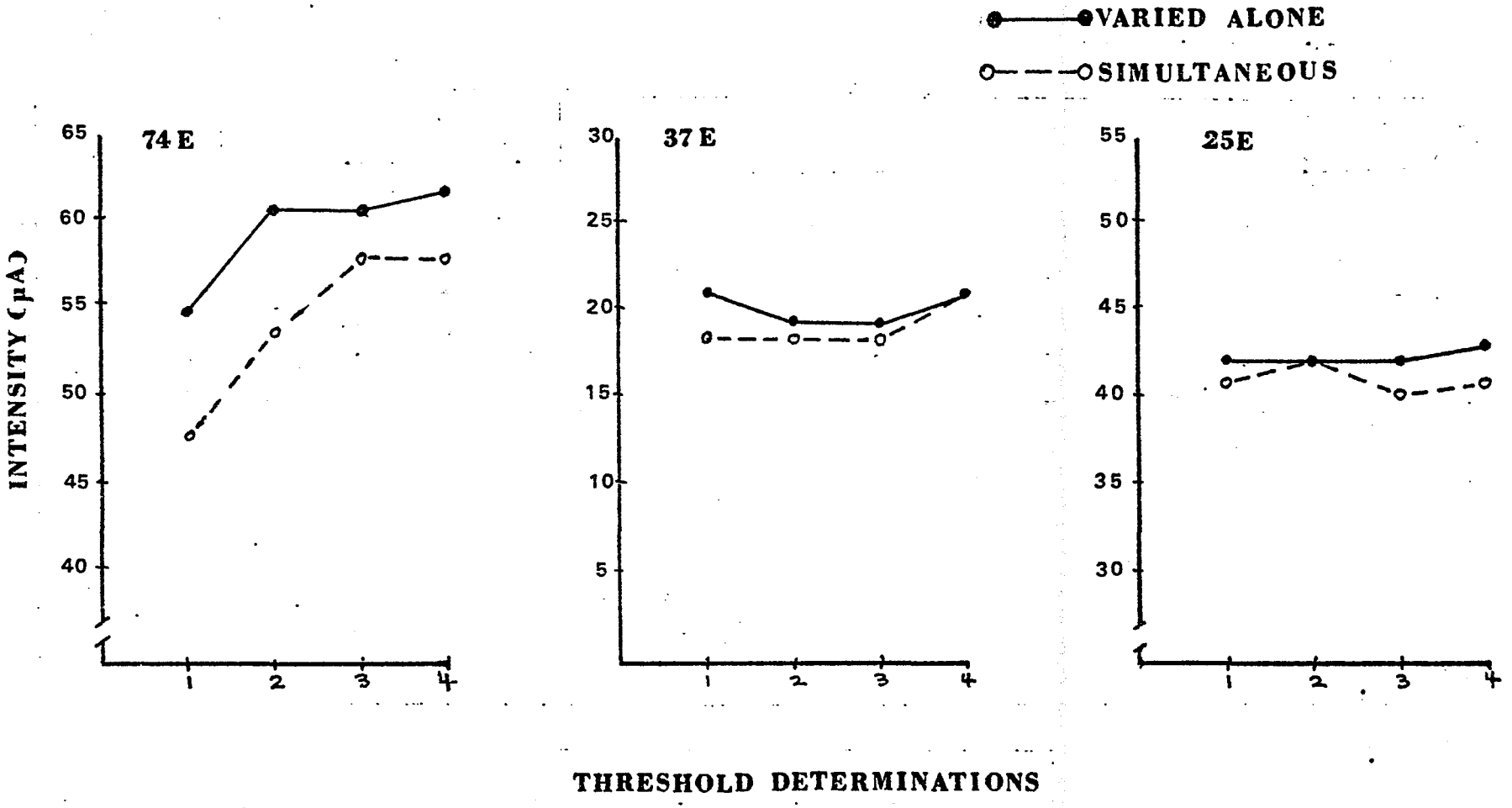

VARIED ALONE
SIMULTANEOUS
74 E
37 E
25E
INTENSITY (μA)
65
60
55
50
45
40
30
25
20
15
10
5
55
50
45
40
35
30
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 3. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for three locus coeruleus (dorsal noradrenergic bundle)/hypothalamus electrode site combinations (9E, 3E, 7E).

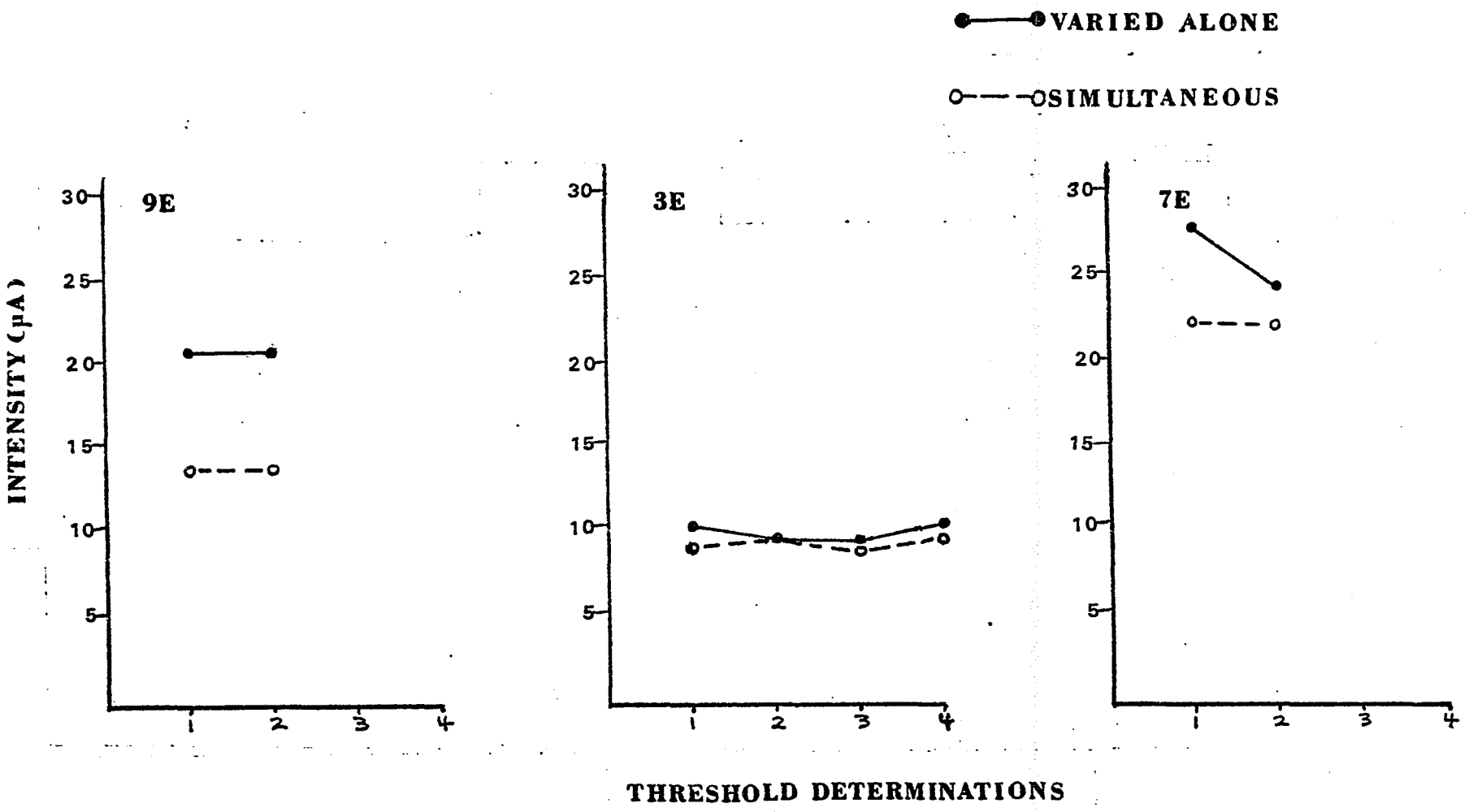

VARIED ALONE
SIMULTANEOUS
9E
3E
7E
INTENSITY (μA)
30
25
20
15
10
5
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 4. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for two midbrain periaqueductal gray area/hypothalamus electrode site combinations (51F, 19F).

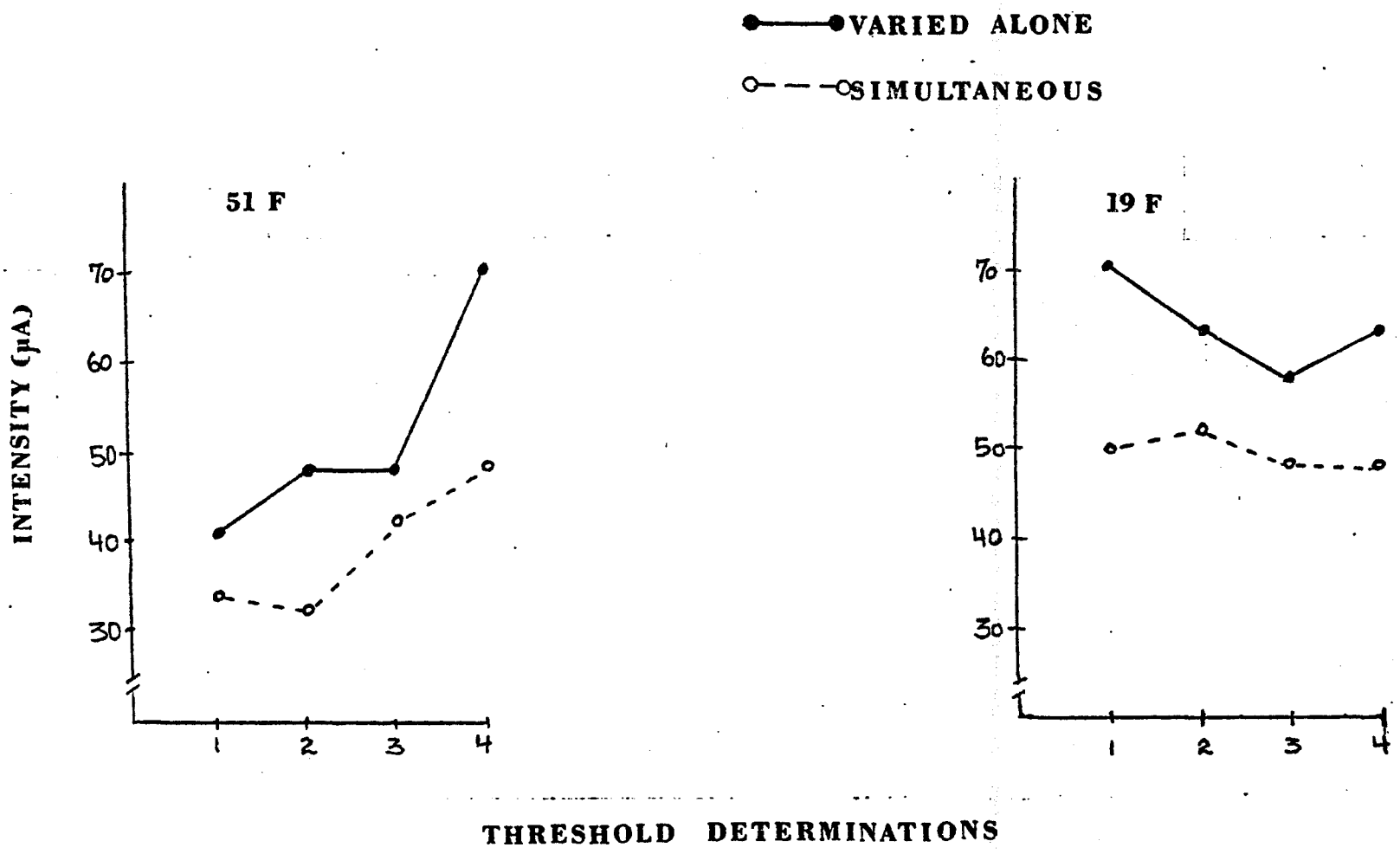

VARIED ALONE
SIMULTANEOUS
51 F
19 F
INTENSITY (μA)
70
60
50
40
30
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 5. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for two midbrain periaqueductal gray area/hypothalamus electrode site combinations (43E, 40E).

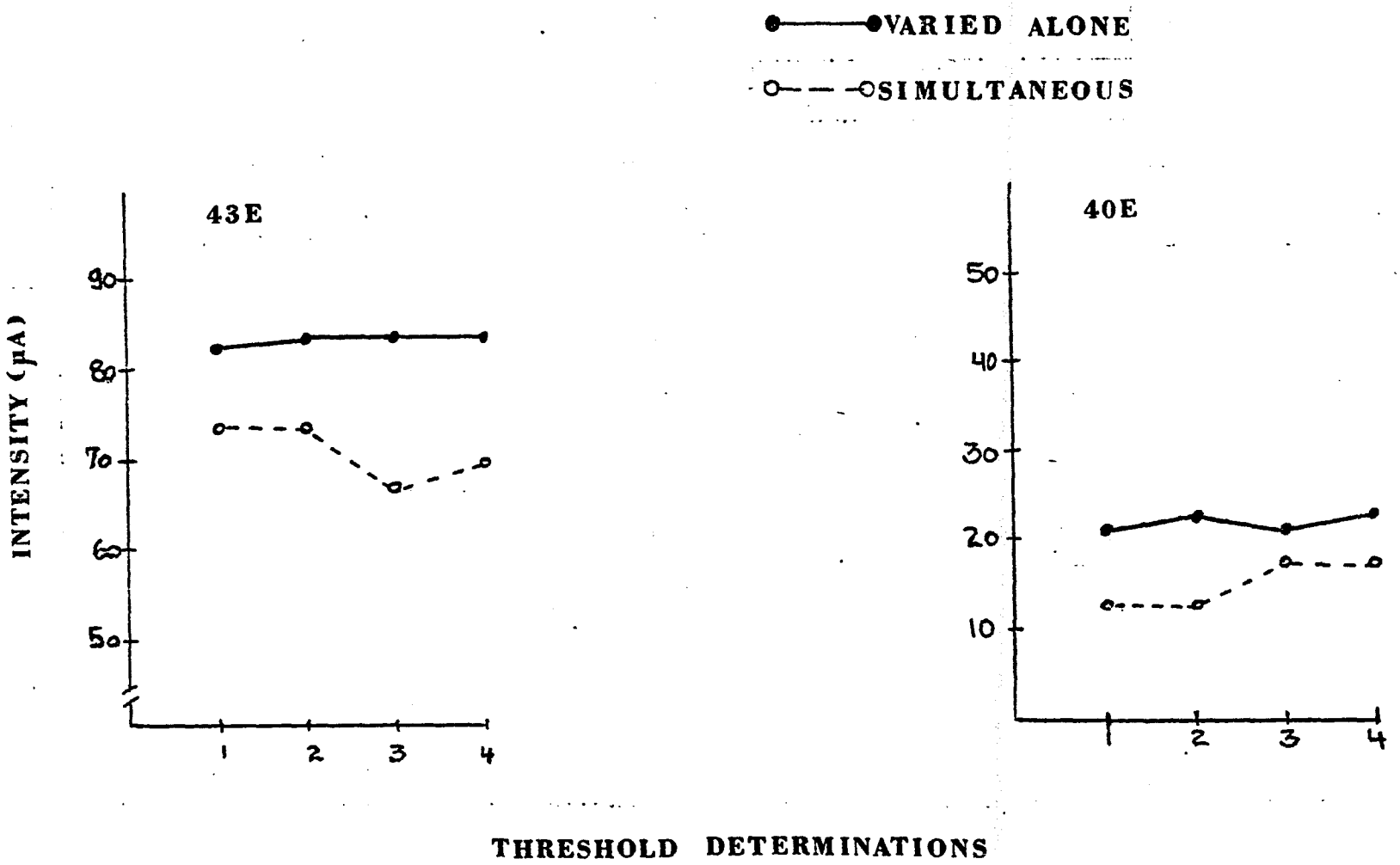

VARIED ALONE
SIMULTANEOUS
43E
40E
INTENSITY (μA)
90
80
70
60
50
40
30
20
10
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 6. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for two substantia nigra/hypothalamus electrode site combinations (82F, 81E).

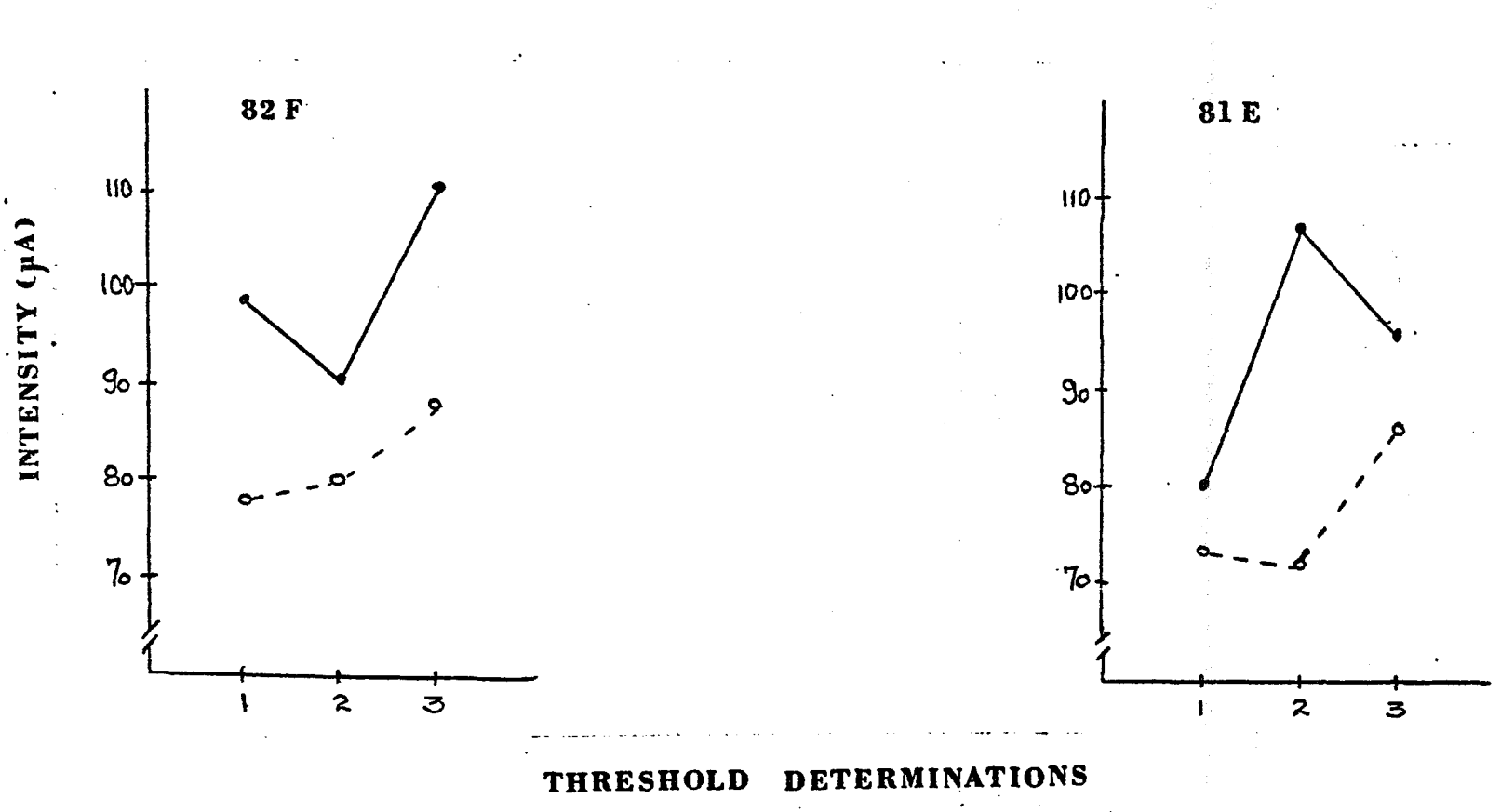

VARIED ALONE
SIMULTANEOUS
82 F
81 E
INTENSITY (μA)
110
100
90
80
70
1
2
3
THRESHOLD DETERMINATIONS

Figure 7. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for two substantia nigra/hypothalamus electrode site combinations (87F, 80F).

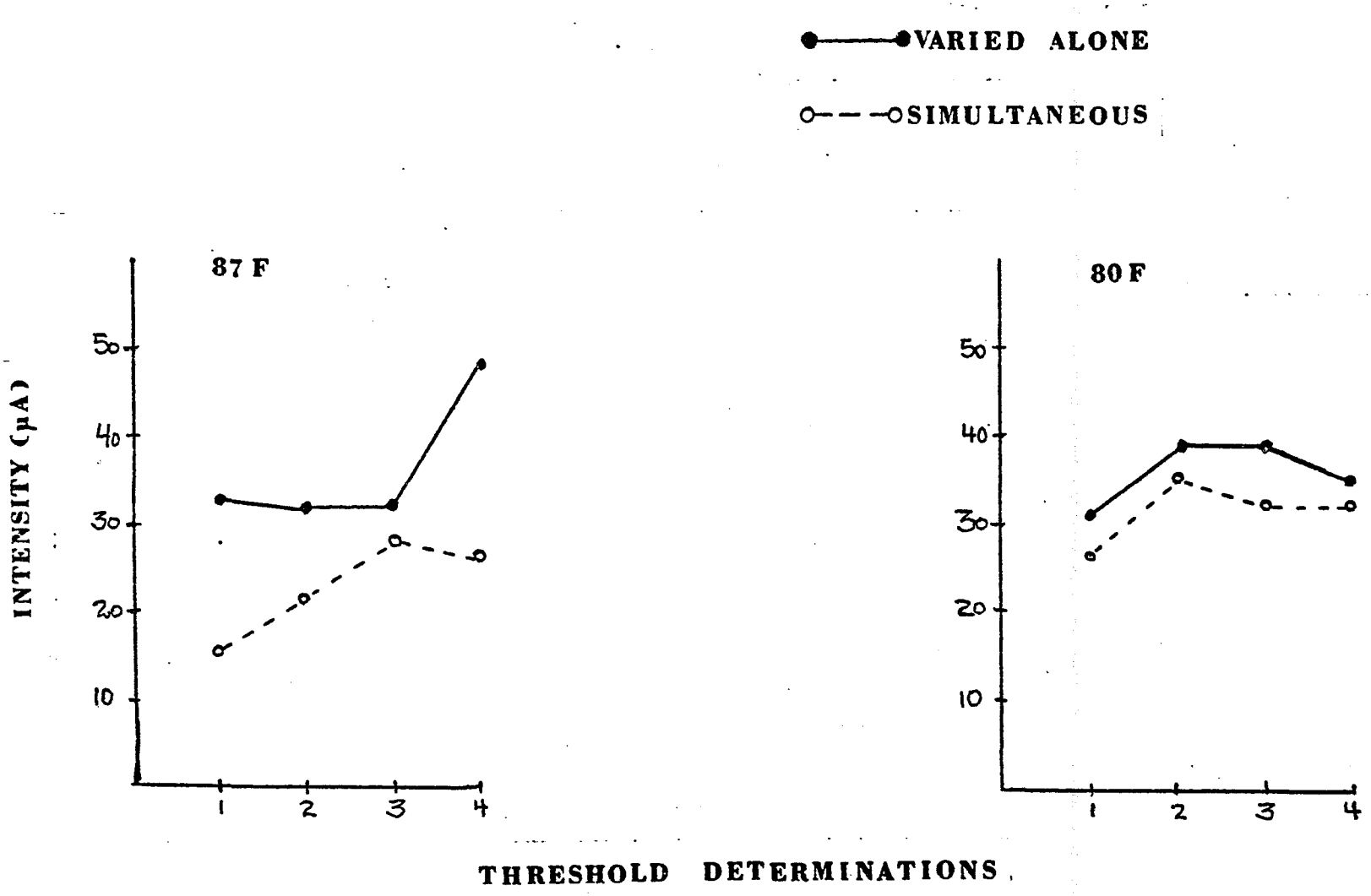

VARIED ALONE
SIMULTANEOUS
87 F
80 F
INTENSITY (μA)
50
40
30
20
10
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 8. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for two hypothalamus/contralateral hypothalamus electrode site combinations (74E, 50E).

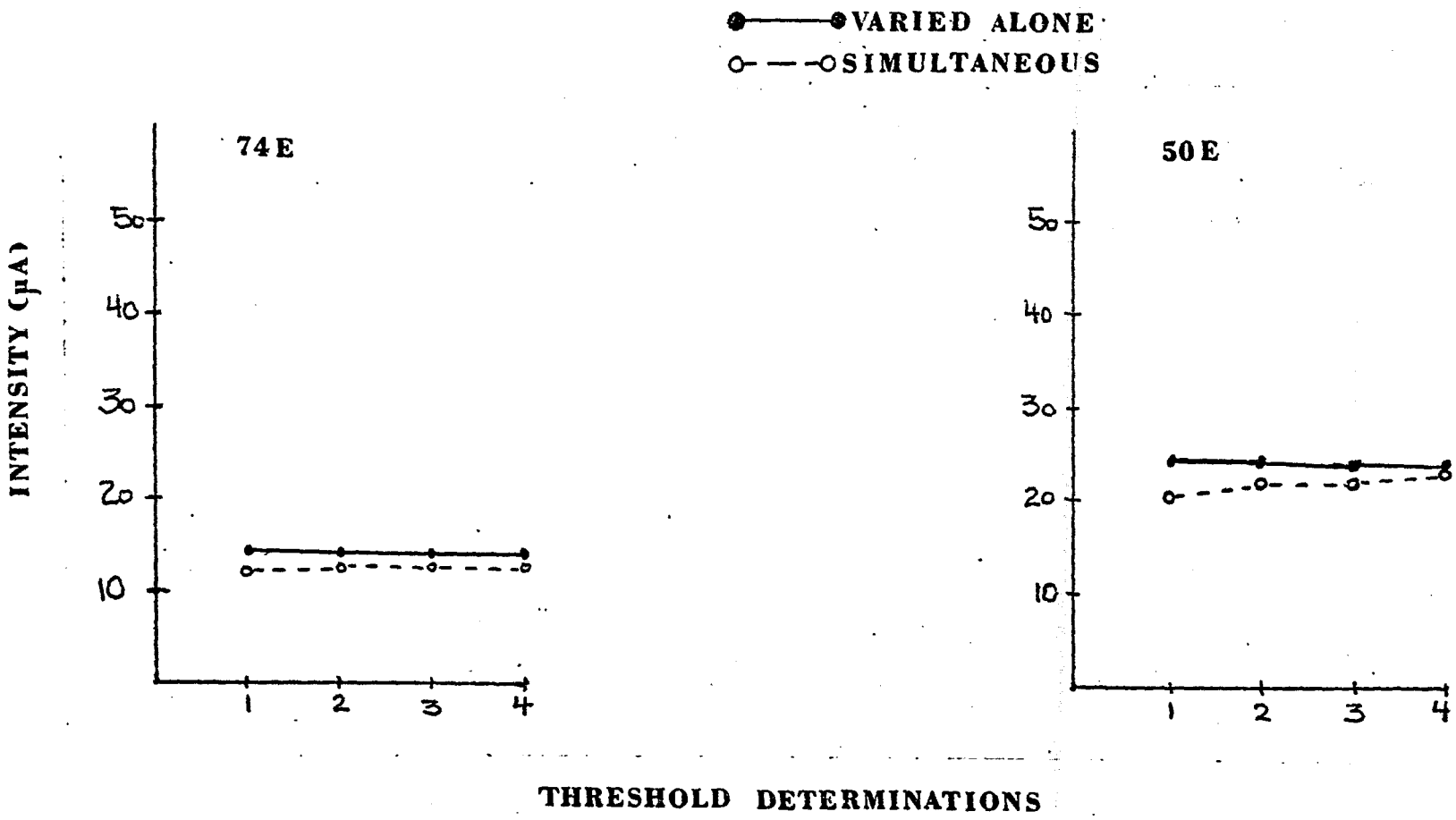

VARIED ALONE
SIMULTANEOUS
74E
50E
INTENSITY (μA)
50
40
30
20
10
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 9. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for two hypothalamus/contralateral hypothalamus electrode site combinations (66F, 68F).

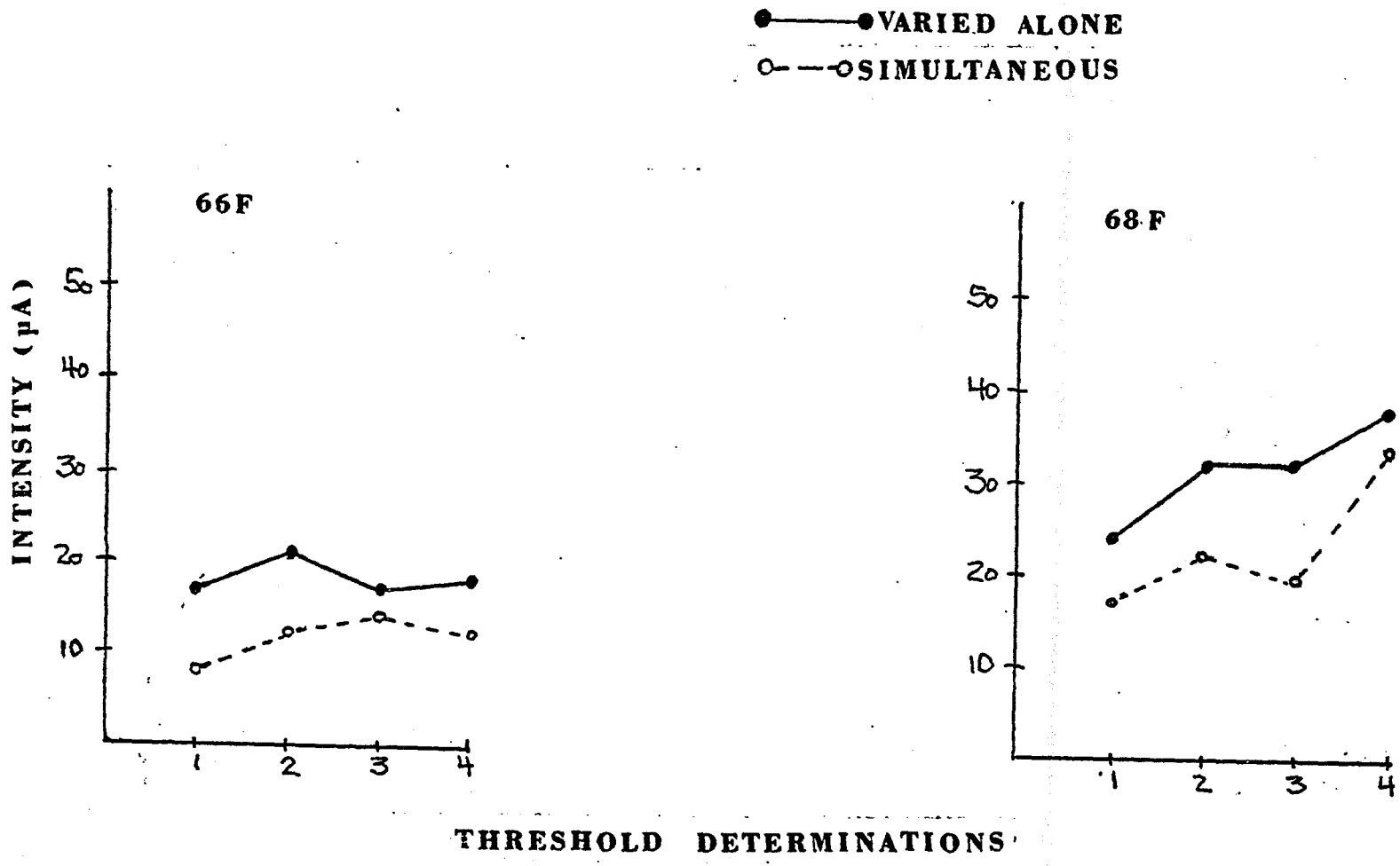

VARIED ALONE
SIMULTANEOUS
66F
68F
INTENSITY (μA)
10
20
30
40
50
1
2
3
4
THRESHOLD DETERMINATIONS

Figure 10. Difference between simultaneous stimulation response threshold and varied-site-alone response threshold obtained for an interpeduncular nucleus/locus coeruleus electrode site combination (left) and for an interpeduncular nucleus/hypothalamus electrode site combination (right).

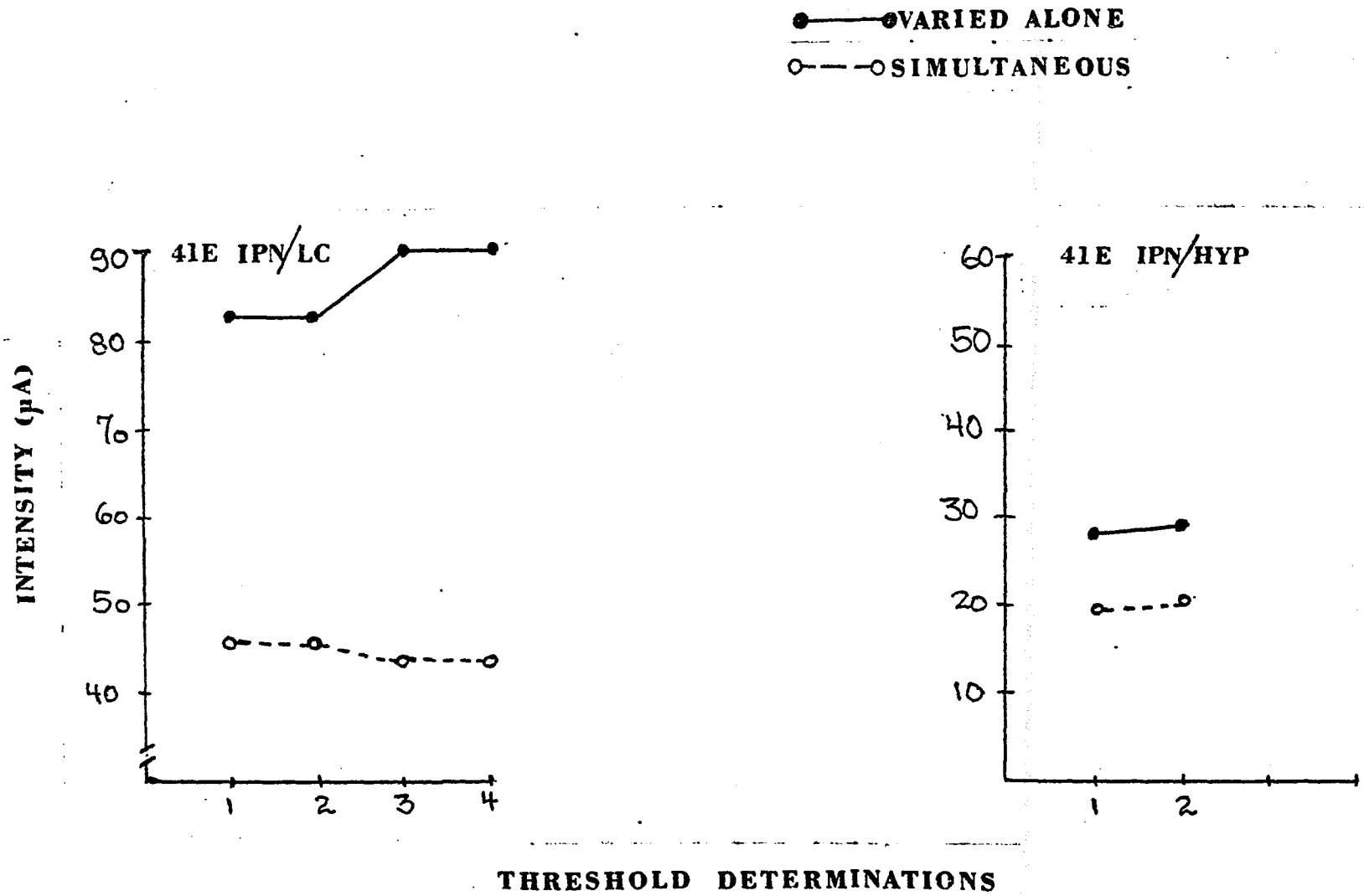

VARIED ALONE
SIMULTANEOUS
41E IPN/LC
41E IPN/HYP
INTENSITY (μA)
90
80
70
60
50
40
60
50
40
30
20
10
1
2
3
4
1
2
THRESHOLD DETERMINATIONS

Figure 11. Reduction in simultaneous stimulation threshold compared to varied-site-alone threshold for Rat 3E, a locus coeruleus (dorsal noradrenergic bundle)/hypothalamus electrode site combination.

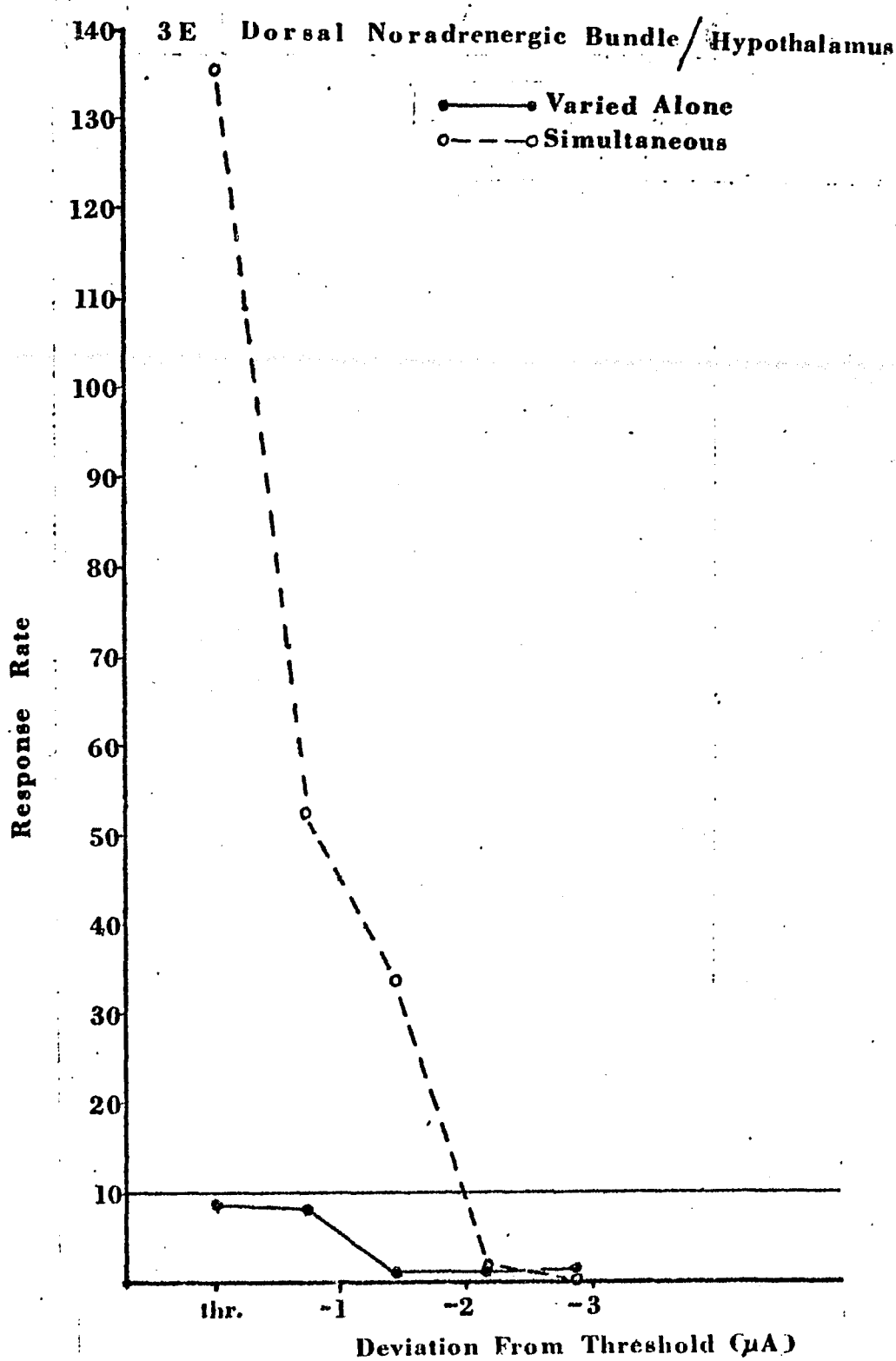

3E Dorsal Noradrenergic Bundle / Hypothalamus
Varied Alone
Simultaneous
Response Rate
140
130
120
110
100
90
80
70
60
50
40
30
20
10
thr.
-1
-2
-3
Deviation From Threshold (μA)

Figure 12. Reduction in simultaneous stimulation threshold compared to varied-site-alone threshold for Rat 43E, a mid-ventral periaqueductal gray/hypothalamus electrode site combination.

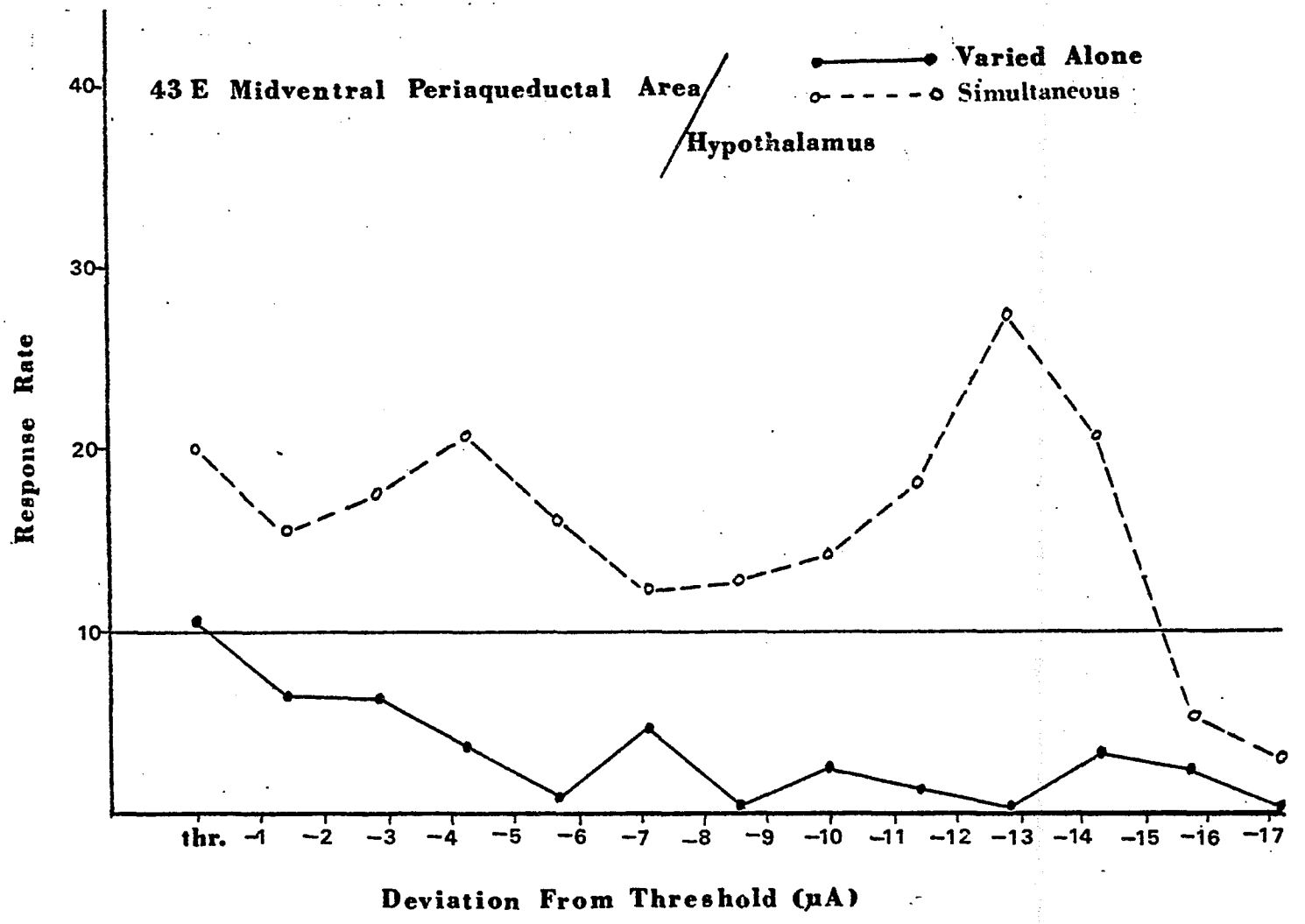

43 E Midventral Periaqueductal Area / Hypothalamus
Varied Alone
Simultaneous
Response Rate
40
30
20
10
thr.
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
-16
-17
Deviation From Threshold (μA)

Figure 13. Reduction in simultaneous stimulation threshold compared to varied-site-alone threshold for Rat 40E, a dorsal longitudinal fasciculus/hypothalamus electrode site combination.

40 E Dorsal Longitudinal Fasciculus/ Hypothalamus

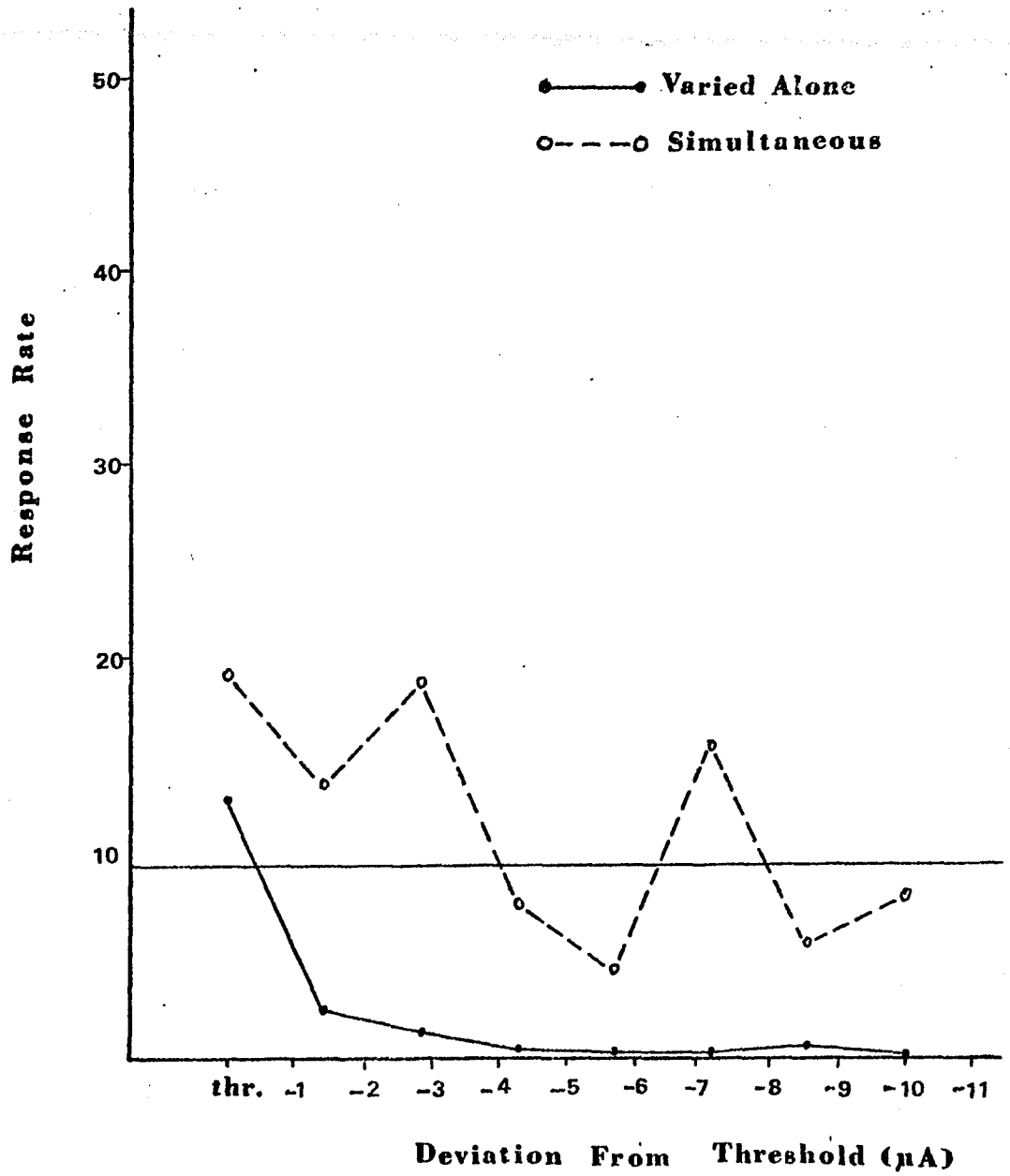


Figure 14. Reduction in simultaneous stimulation threshold compared to varied-site-alone threshold for Rat 81E, a substantia nigra/hypothalamus electrode site combination.

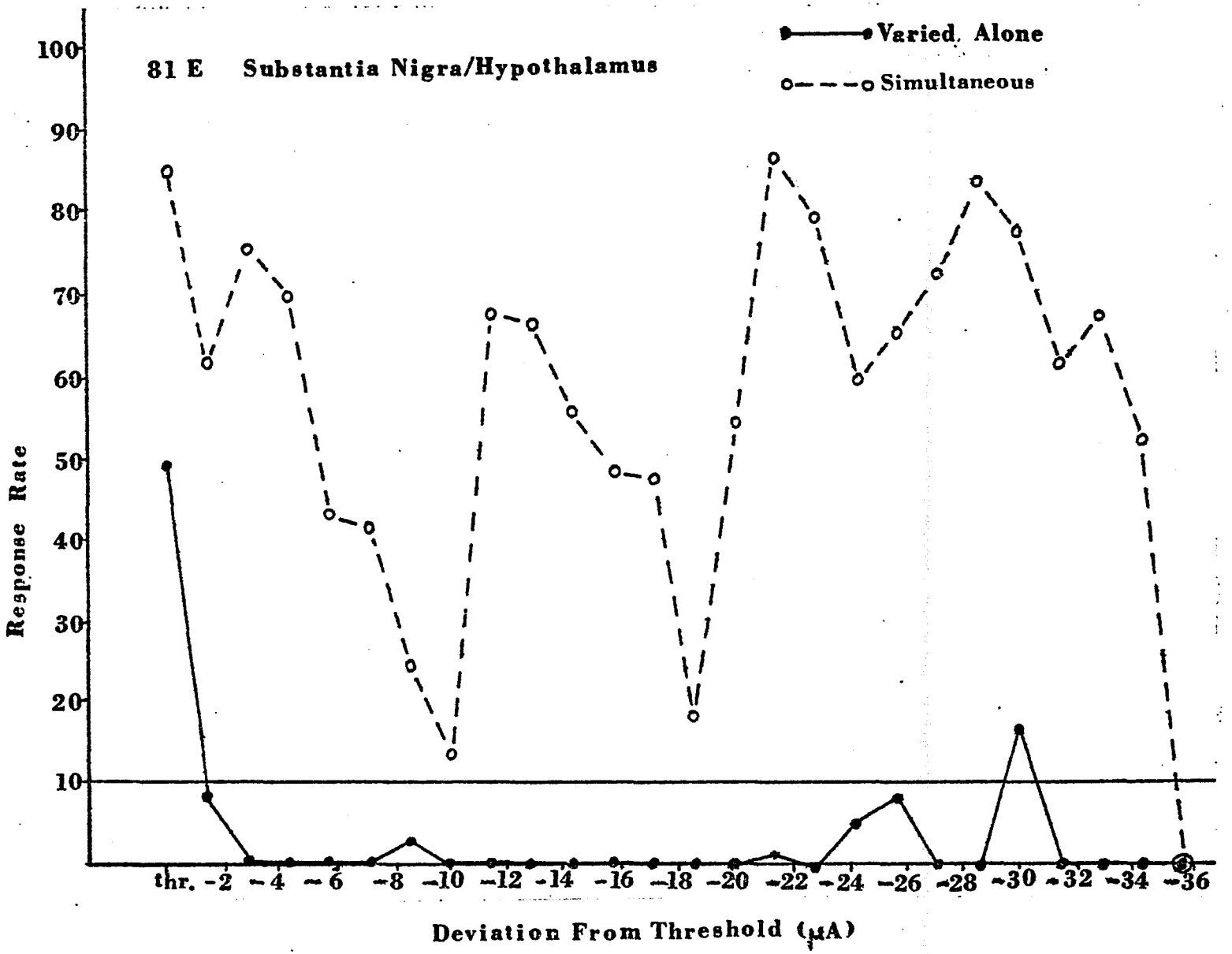

81 E Substantia Nigra/Hypothalamus
Varied, Alone
Simultaneous
Response Rate
100
90
80
70
60
50
40
30
20
10
thr. -2 -4 -6 -8 -10 -12 -14 -16 -18 -20 -22 -24 -26 -28 -30 -32 -34 -36
Deviation From Threshold (μA)

Figure 15. Reduction in simultaneous stimulation threshold compared to varied-site-alone threshold for Rat 74E, a hypothalamus/contralateral hypothalamus electrode site combination.

74 E Hypothalamus/Hypothalamus

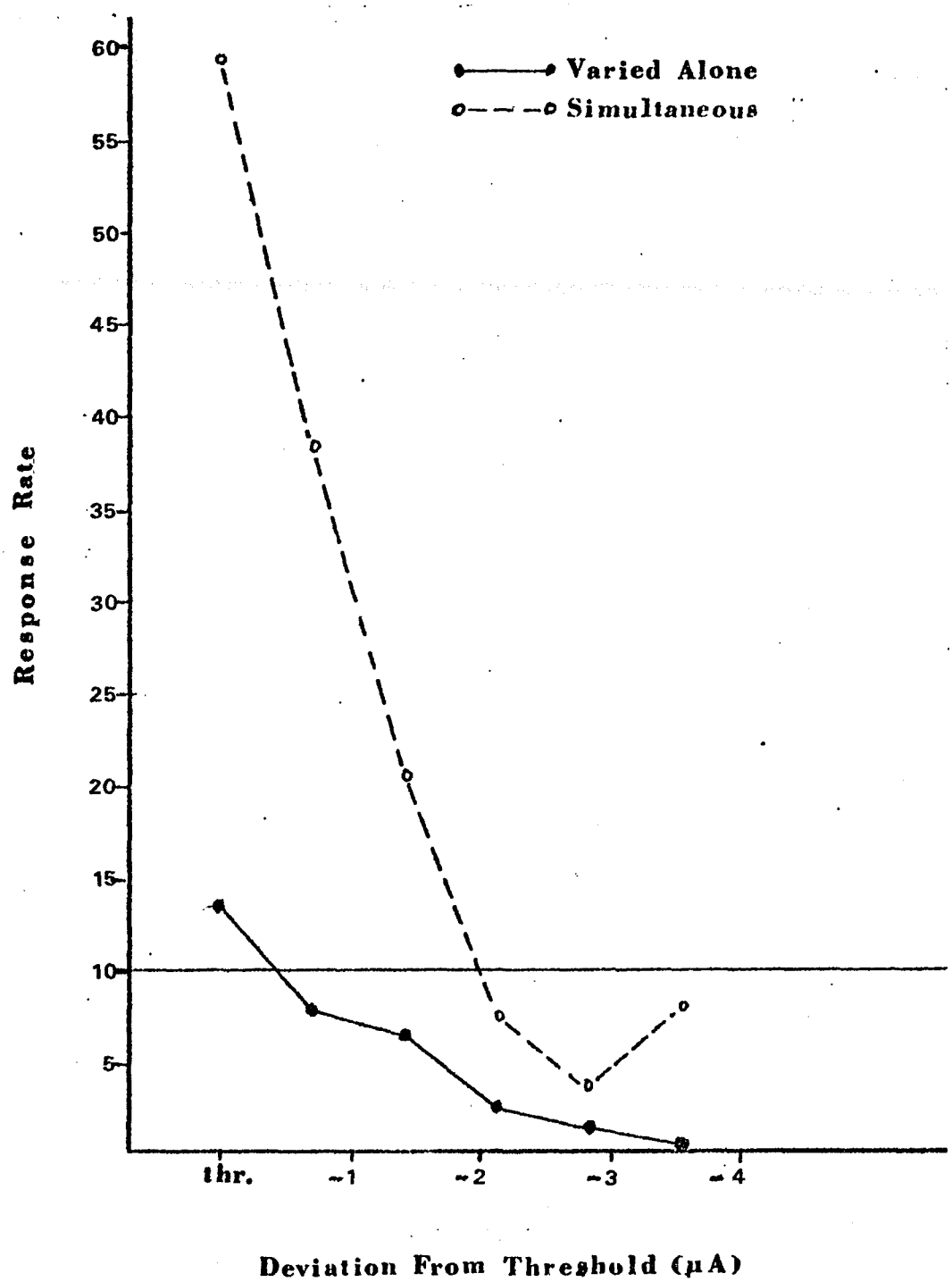


Figure 16. The effect of d-amphetamine and l-amphetamine on seven Drug Category I hypothalamic self-stimulation sites (97C, 1E, 9E, 13E, 14E, 15E, 18E) over five ascending current intensities. Response rate under drug conditions is expressed as a multiple of the saline rate which has been assigned a value of 1.

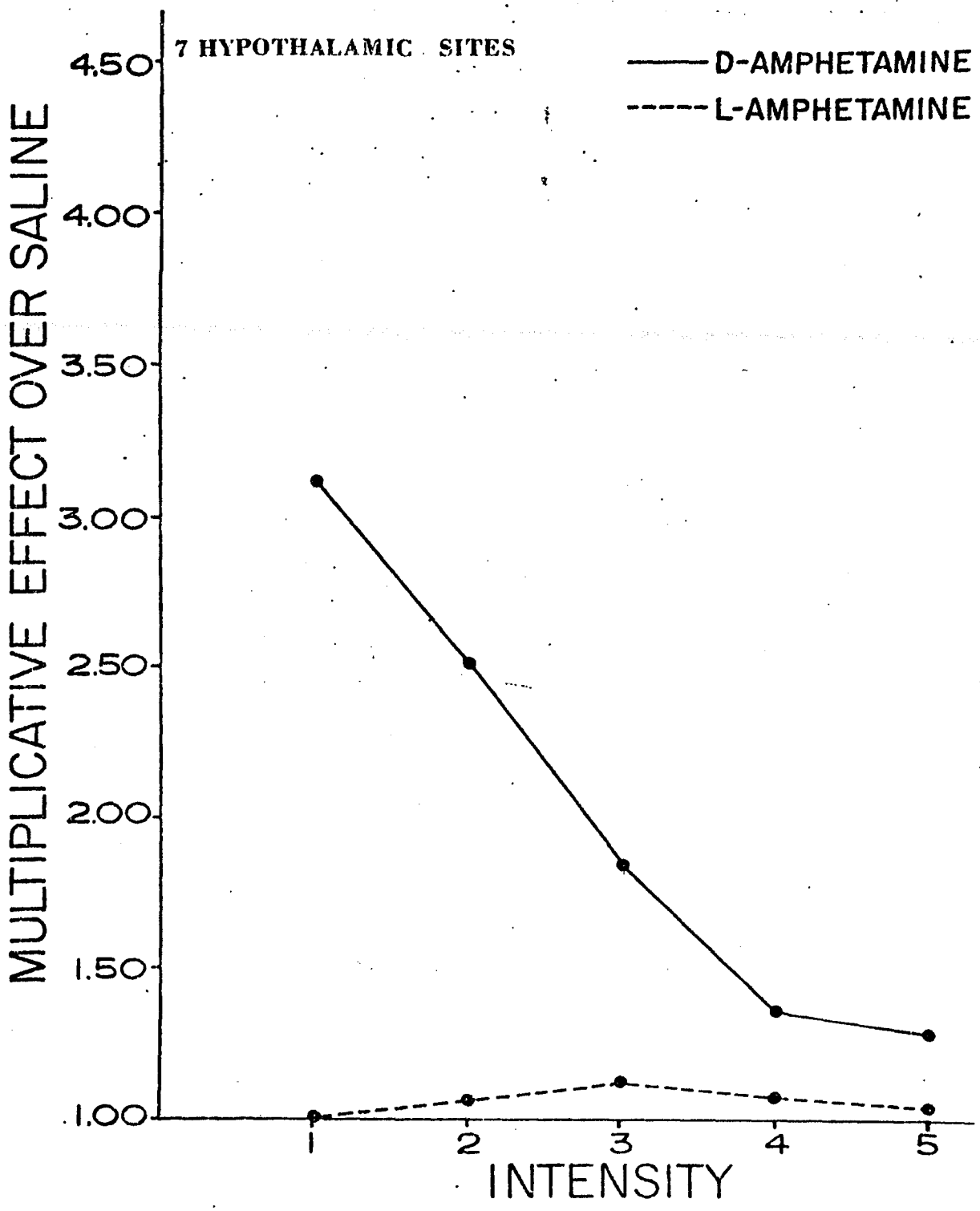

7 HYPOTHALAMIC SITES
D-AMPHETAMINE
L-AMPHETAMINE
MULTIPLICATIVE EFFECT OVER SALINE
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
1
2
3
4
5
INTENSITY

Figure 17. The effect of d-amphetamine and l-amphetamine on midventral periaqueductal, and dorsal noradrenergic bundle self-stimulation over five ascending current intensities. Response rate under drug conditions is expressed as a multiple of the saline rate which has been assigned a value of 1.

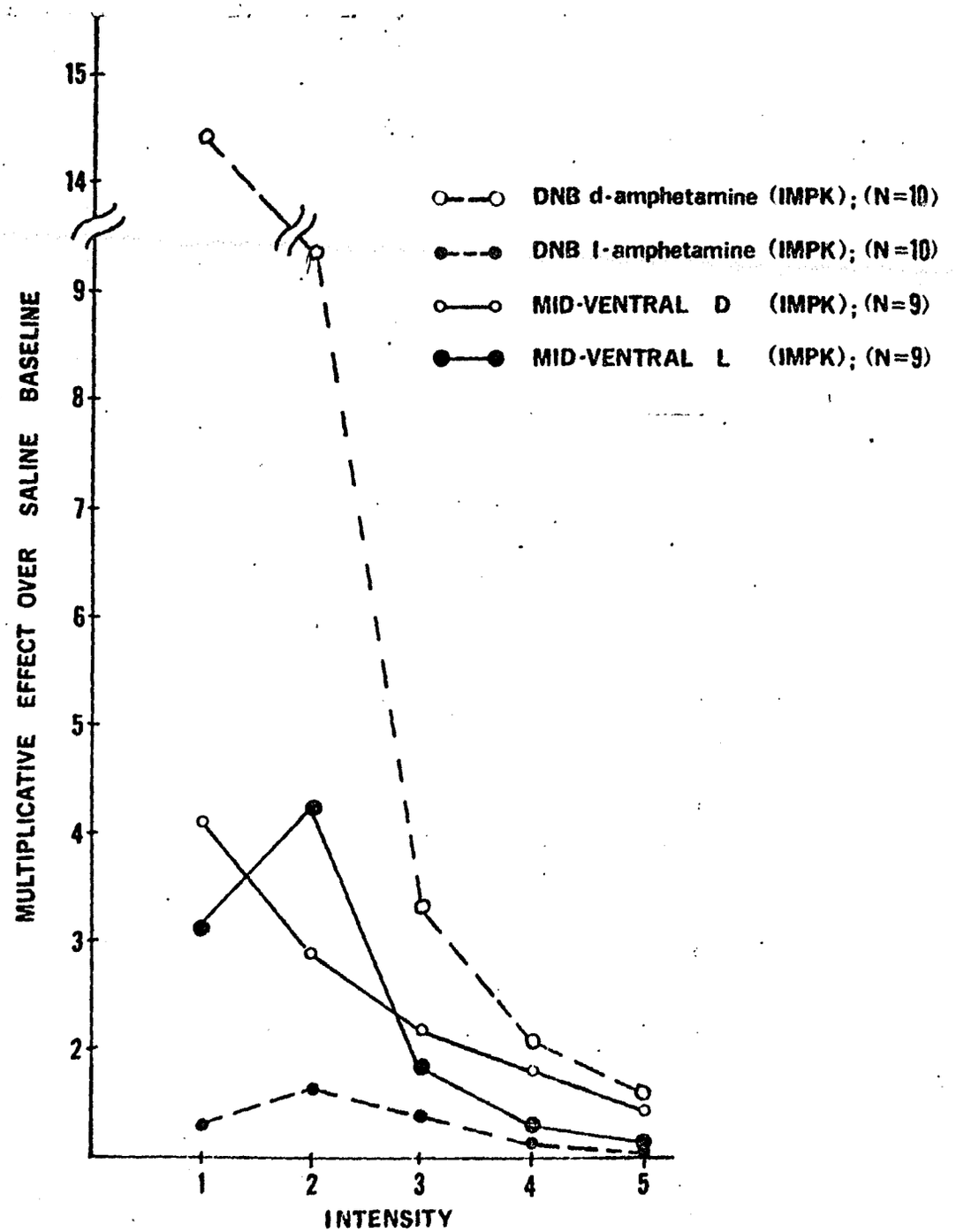

MULTIPLICATIVE EFFECT OVER SALINE BASELINE
15
14
9
8
7
6
5
4
3
2
1
2
3
4
5
INTENSITY
DNB d-amphetamine (IMPK); (N=10)
DNB l-amphetamine (IMPK); (N=10)
MID-VENTRAL D (IMPK); (N=9)
MID-VENTRAL L (IMPK); (N=9)

Figure 18. The effect of d-amphetamine and l-amphetamine on lateral periaqueductal self-stimulation over five ascending current intensities. Response rate under drug conditions is expressed as a multiple of the saline rate which has been assigned a value of 1.

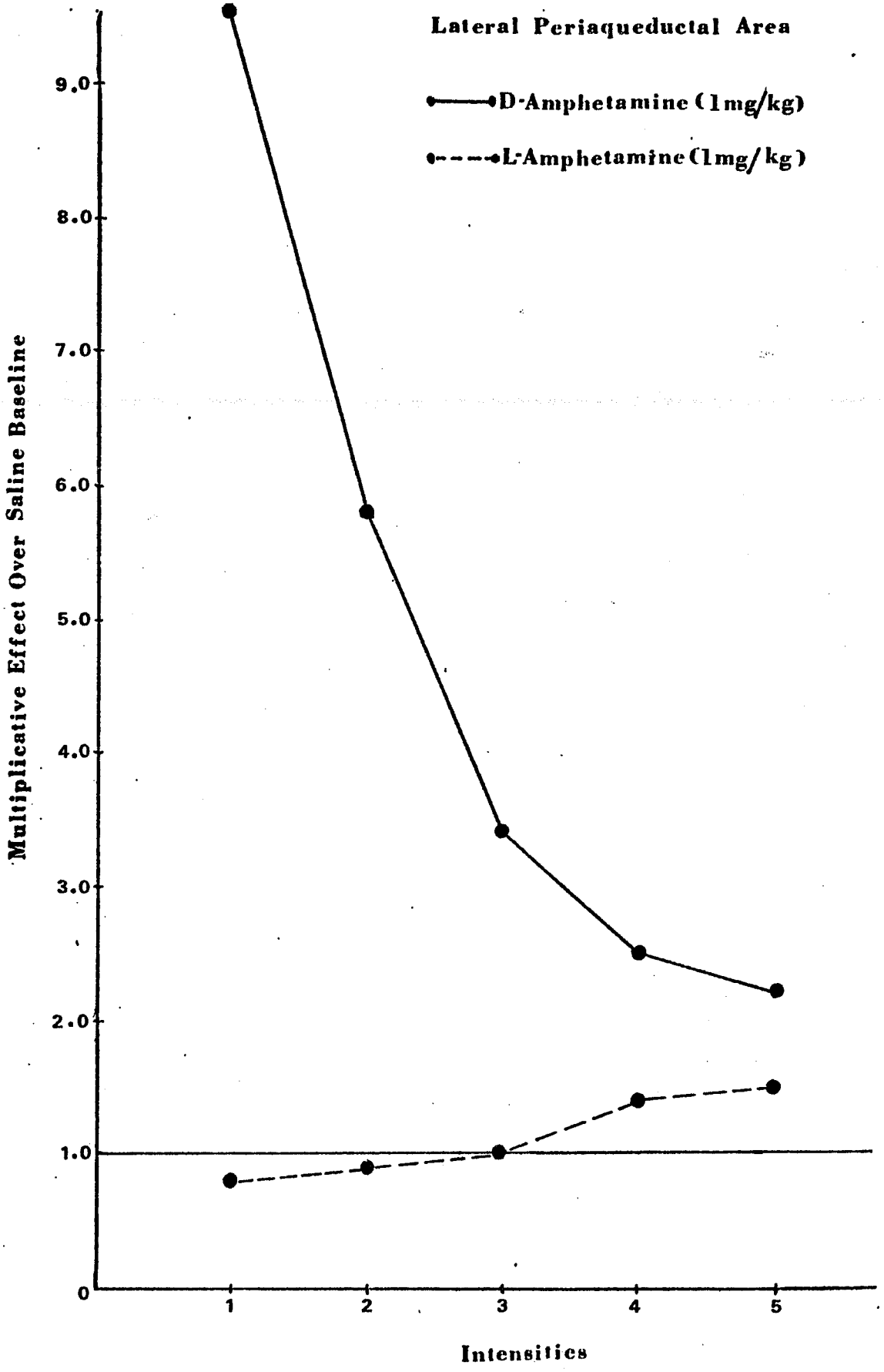

Lateral Periaqueductal Area
D-Amphetamine (1mg/kg)
L-Amphetamine (1mg/kg)
Multiplicative Effect Over Saline Baseline
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
2
3
4
5
Intensities

Figure 19. Effect of d-amphetamine and l-amphetamine on midventral periaqueductal, and dorsal noradrenergic bundle self-stimulation over four dosages. Response rate under drug conditions is expressed as a multiple of the saline rate which has been assigned a value of 1.

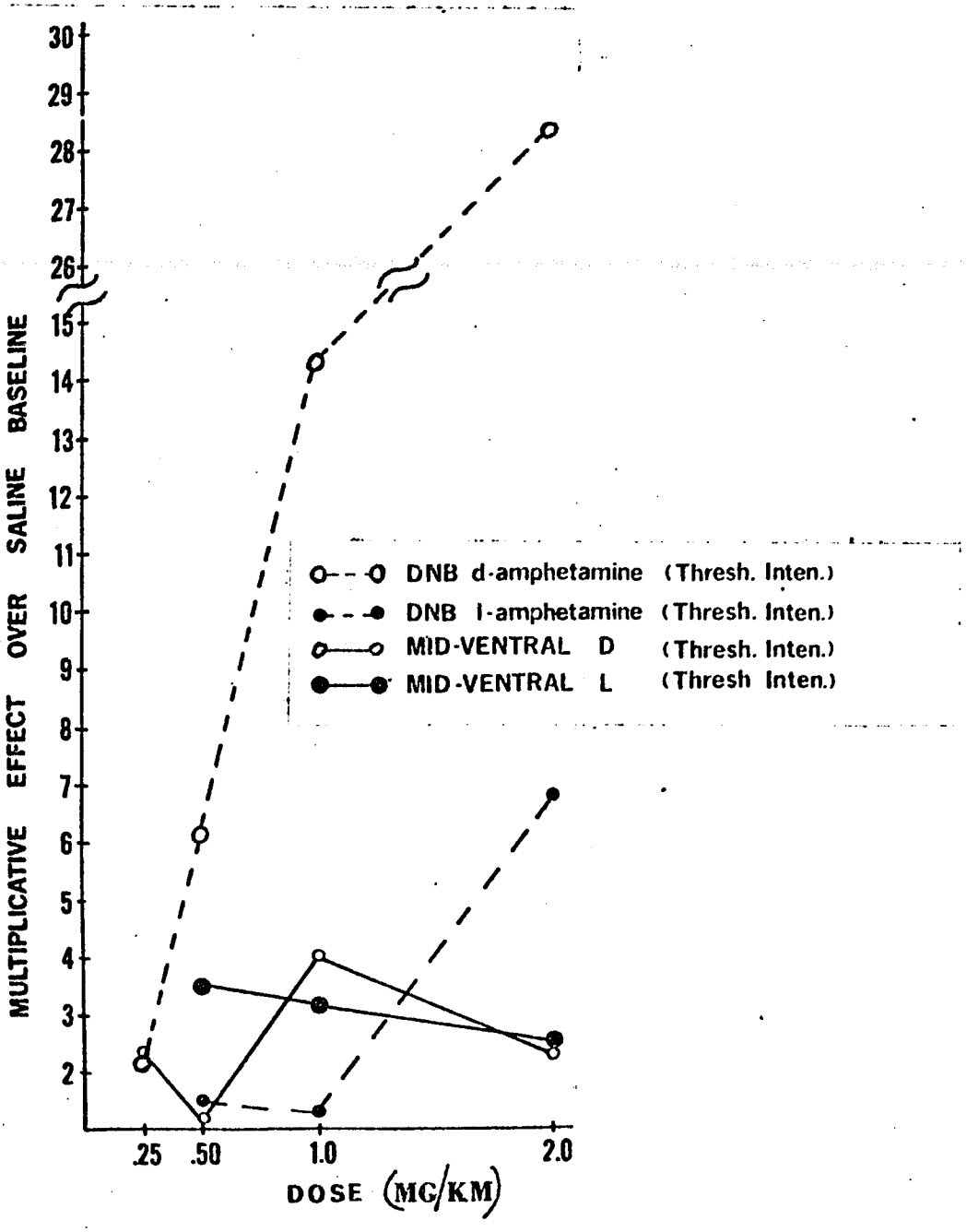

MULTIPLICATIVE EFFECT OVER SALINE BASELINE
30
29
28
27
26
15
14
13
12
11
10
9
8
7
6
5
4
3
2
.25
.50
1.0
2.0
DOSE (MG/KM)
DNB d-amphetamine (Thresh. Inten.)
DNB l-amphetamine (Thresh. Inten.)
MID-VENTRAL D (Thresh. Inten.)
MID-VENTRAL L (Thresh Inten.)

Figure 20. Location of locus coeruleus (dorsal noradrenergic bundle) Drug Category I electrodes (circles) and midventral periaqueductal Drug Category II electrodes (triangles). The upper left section is the most anterior; the lower right section is the most posterior.

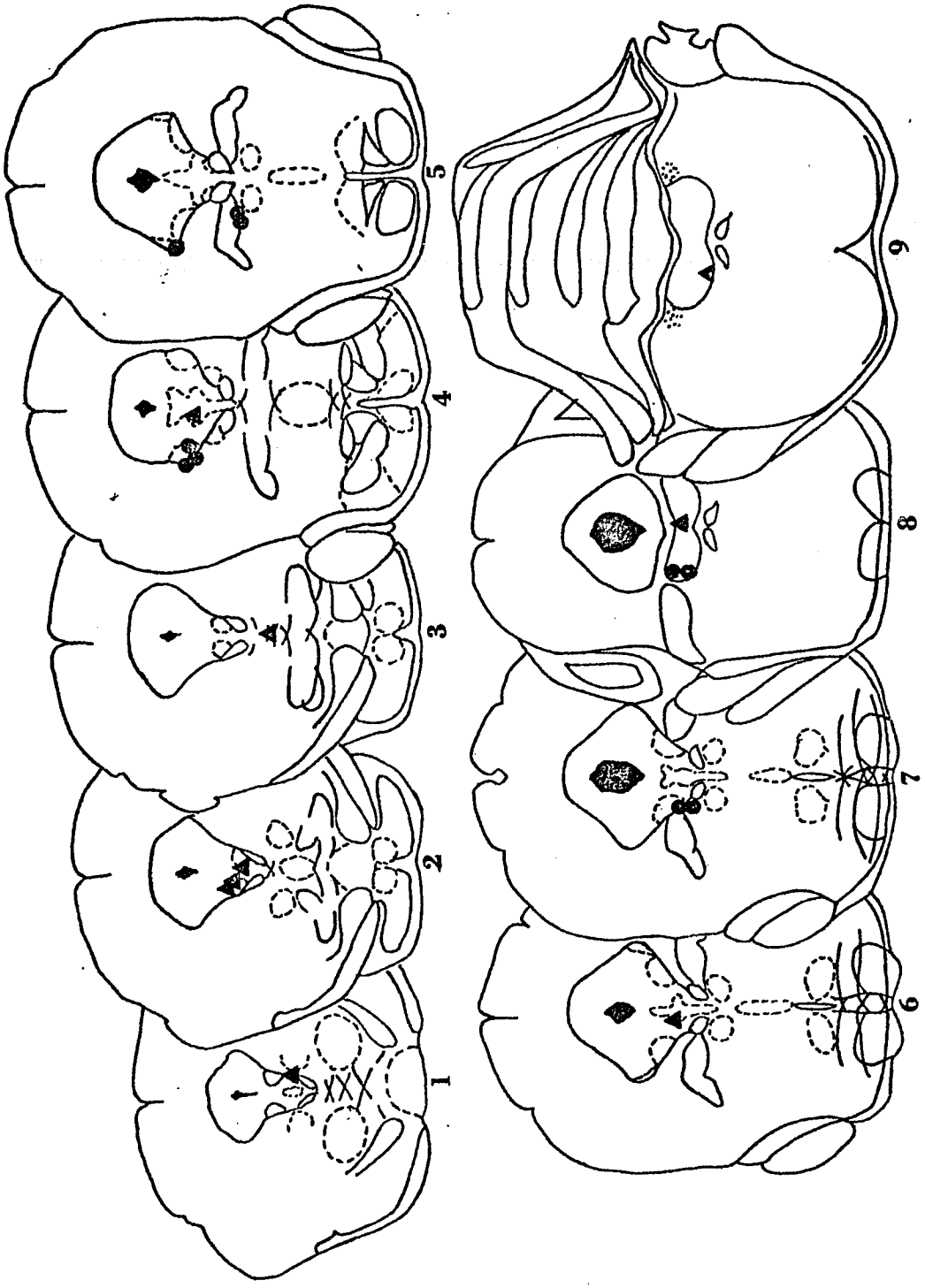

1
2
3
4
5
6
7
8
9

Figure 21. Location of midbrain lateral periaqueductal Drug Category I electrodes (circles). The most anterior midventral periaqueductal Drug Category II electrodes (triangles) are indluded for comparison. A substantia nigra Drug Category II electrode and interpeduncular nucleus Drug Category I electrode are also included (8). The upper left section is the most anterior; the lower right section is the most posterior.

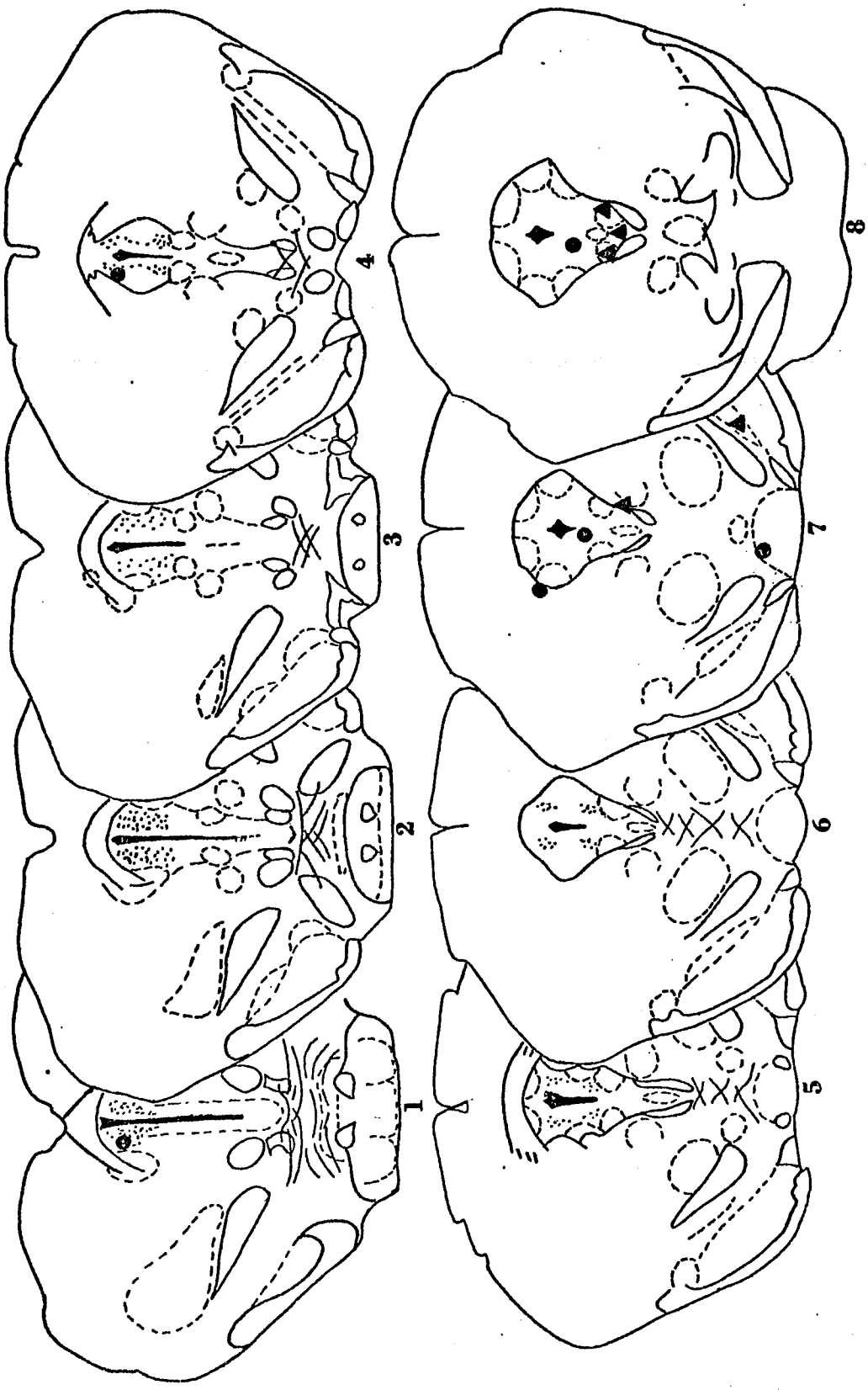
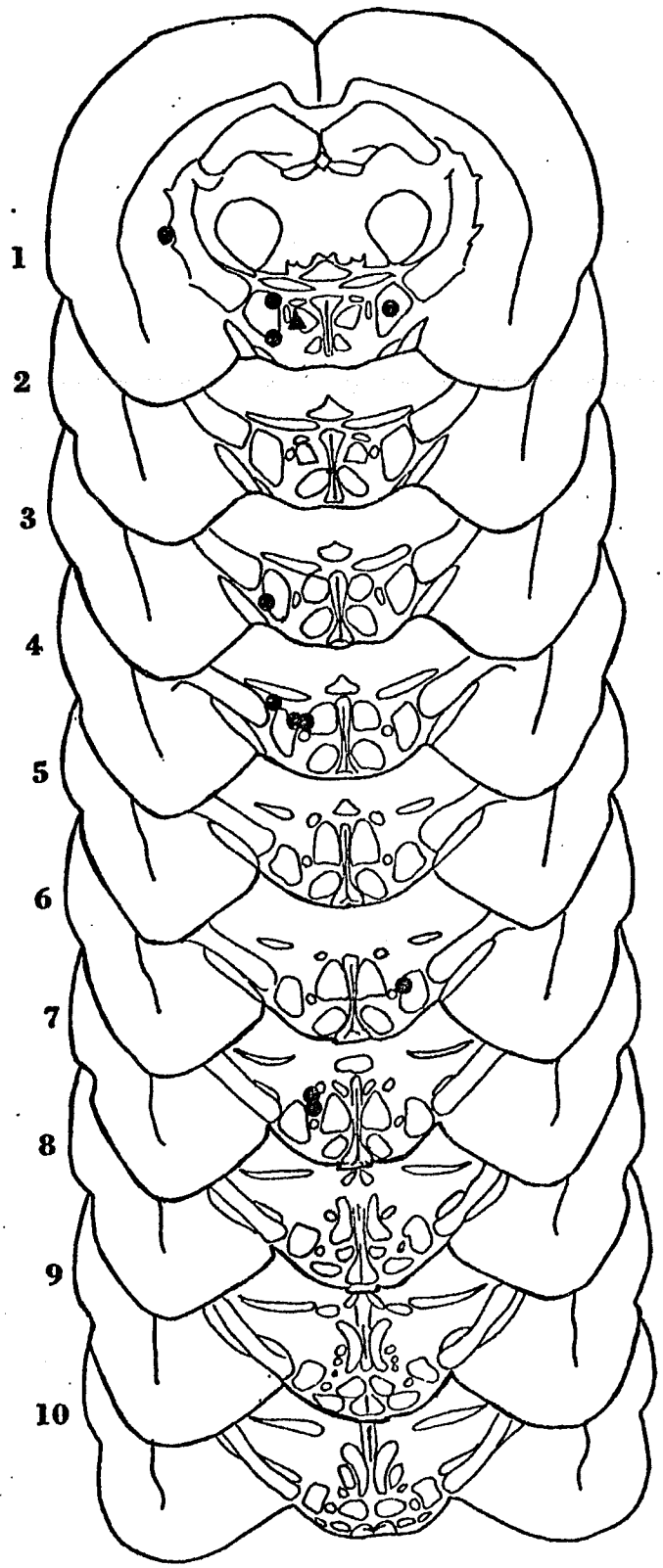

1
2
3
4
5
6
7
8

Figure 22. Location of hypothalamic Drug Category I electrodes (circles) and one Drug Category II electrode (triangle) (1). The top section is the most anterior.


1
2
3
4
5
6
7
8
9
10

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