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LOCALIZATION OF SITES OF ACTION OF HIGH-ABUSE-LIABILITY DRUGS I--ETC(U)
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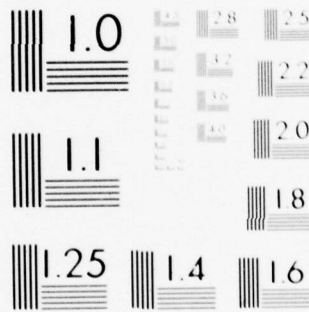
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on self-stimulation are obtained upon repeated morphine administration. These effects are site and dose-dependent. Synergistic effects are obtained with cholinergic agents. Effects similar to those of morphine were found with pentobarbital. These diverse agents facilitated self-stimulation, though the time course, amount of facilitation, and site of effect differed across pharmacological agents. We investigated specificity of response rate and refractoriness within the central reward system using the monophasic cathodal/anodal technique to differentiate between heretofore homogeneous self-stimulation areas from which differential drug effects are obtained by superimposing the isomers of amphetamine on this technique. We are able to determine directionality of fiber pathways in the central reward system(s). Destruction of one part of the reward system was found to abolish or drastically reduce self-stimulation behavior in another part, but d-amphetamine reverses this process while l-amphetamine does not.

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REPORT NUMBER 1

LOCALIZATION OF SITES OF ACTION OF HIGH-ABUSE-
LIABILITY DRUGS IN THE CENTRAL NERVOUS SYSTEM

Final Report

Solomon S. Steiner, Ph.D.

30 September 1975

Supported by

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The findings in this report are not to be construed as
an official Department of the Army position unless so
designated by other authorized documents.

SUMMARY

We have investigated the hypothesis that high-abuse pharmacological agents with ostensibly different biochemical and behavioral effects have a common influence on the central reward system as measured by intracranial self-stimulation. We have investigated the effects of the optical isomers of amphetamine on different central reward sites and have found that differential behavioral effects can be obtained within the reward system. Biphasic effects on self-stimulation are obtained upon repeated morphine administration. These effects are site and dose-dependent. Synergistic effects are obtained with cholinergic agents. Effects similar to those of morphine were found with pentobarbital. These diverse agents facilitated self-stimulation, though the time course, amount of facilitation, and site of effect differed across pharmacological agents.

We investigated specificity of response rate and refractoriness within the central reward system using the monophasic cathodal/anodal technique to differentiate between heretofore homogeneous self-stimulation areas from which differential drug effects are obtained by superimposing the isomers of amphetamine on this technique. We are able to determine directionality of fiber pathways in the central reward system(s). Destruction of one part of the reward system was found to abolish or drastically reduce self-stimulation behavior in another part, but d-amphetamine reverses this process while l-amphetamine does not.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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ATTACHMENTS:

1. D- and L-Amphetamine Differentially Mediates Self-Stimulation in Rat Dorsal Midbrain Area, by S. J. Ellman, R. F. Ackermann, R. J. Bodnar, F. Jackler and S. S. Steiner. Reprint from Physiology & Behavior, 1976, 16, 1-7.
2. Differential Effects of Unilateral Dorsal Hindbrain Lesions on Hypothalamic Self-Stimulation in the Rat, by J. Farber, S. J. Ellman, L. A. Mattiace, A. Holzman, P. Ippolito, R. Halperin and S. S. Steiner. Reprint from Brain Research, 1976, 112, 148-155.
3. Alteration of Escape from Rewarding Electrical Brain Stimulation by D-Amphetamine, by S. S. Steiner, R. F. Ackermann, R. J. Bodnar, F. Jackler, J. M. Healey and S. J. Ellman. Reprint from International Journal of Neuroscience, 1976, 6, 273-278.
4. Comparison of Behaviors Elicited by Electrical Brain Stimulation in Dorsal Brain Stem and Hypothalamus of Rats, by S. J. Ellman, R. F. Ackermann, R. J. Bodnar, F. Jackler and S. S. Steiner. Reprint from Journal of Comparative and Physiological Psychology, 1975, 88(2), 816-828.

ATTACHMENTS (continued)

5. Behavioral Interactions among Rat Brainstem and Hypothalamic Self-Stimulation Sites, by R. F. Ackermann, S. J. Ellman, R. J. Bodnar, F. Jackler and S. S. Steiner. Submitted to Physiology and Behavior.

6. Directionality of Neurophysiological Interactions between Brainstem and Hypothalamic Self-Stimulation Loci in Rats, by R. J. Bodnar, S. J. Ellman, R. F. Ackermann, E. R. Greenblatt, S. S. Steiner and E. E. Coons. Reprint from Neuroscience Abstracts, Society for Neuroscience, 5th Annual Meeting, New York City, 1975.

7. Intracranial Self-Stimulation Site Specificity: Monopolar Activation of Bipolar Electrodes, by S. S. Steiner, R. J. Bodnar, R. F. Ackermann, W. T. Nelson and S. J. Ellman. Paper presented at the 84th Annual Convention of the American Psychological Association, Washington, D. C., September, 1976.

GENERAL INTRODUCTION

Throughout this contract, we have investigated the hypothesis that high-abuse pharmacological agents with ostensibly different biochemical and behavioral effects have a common influence on the central reward system as measured by intracranial self-stimulation. We contend that pharmacological agents with high-abuse liability alter the central reward system in addition to exerting their specific behavioral effects; in contrast, non-abuse agents do not facilitate the central reward system. Hence, such diverse agents of high abuse as stimulants, narcotics and barbiturates should alter self-stimulation in a facilitatory manner because their common basis of abuse is the activation of central reward mechanisms.

Our studies thus far have comprised two major approaches: (1) investigation of localization of sites of action within the central reward systems of stimulants, narcotics and barbiturates; and (2) investigation of those sites altered by high abuse potential drugs to elucidate the nature of central reward mechanisms, to characterize their physiological interactions, and to systematically manipulate one part of the reward mechanism in order to affect another part. Specifically, the first approach has investigated the effects of the optical isomers of amphetamine on different central reward sites and has found that differential

behavioral effects can be obtained within the reward system. The first approach also included investigation of the effects of repeated morphine administration across doses. These studies demonstrated biphasic (depressive followed by facilitatory) dose-dependent effects on self-stimulation from distinct neuroanatomically distinct loci. We report the synergistic effects of morphine and cholinergic agonists/antagonists. Finally, the first approach investigated the effects of sodium pentobarbital (Nembutal) on two neuroanatomical loci over repeated administrations. In all three cases, these diverse agents facilitated self-stimulation, though the time-course, amount of facilitation, and activated loci differed across pharmacological agents.

The second approach concerned three categories of experiments; the first investigated specificity of response rate and refractoriness within the central reward system of discrete and distinct neuroanatomical loci that are closely adjacent. We used the monophasic cathodal/anodal technique to differentiate between heretofore homogeneous self-stimulation areas where differential drug effects could be obtained. We have established that these distinct neuroanatomical loci which sustain self-stimulation behavior are indeed a physiologically interrelated system. This was done by using behavioral measures to estimate the magnitude of neurophysiological interactions between intracranial self-stimulation (ICSS) sites; moreover, we present preliminary data determining directionality (ascending or descending) of the system. It was determined

that this system is pharmacologically differentiable, that is, when a part of the system which equipotently reacts to both isomers of amphetamine interacts with a part of the system which reacts only to d-amphetamine, a large behavioral interaction occurs. When both interacting parts are only d-amphetamine reactive, a small interaction occurs. Thus, discrete localization of sites which are differentially affected by drugs behave differently with respect to one another.

Finally, but certainly not least important, it was found that destruction of one part of the reward system will abolish or drastically reduce self-stimulation behavior in another part, but that d-amphetamine, a high-abuse agent, reverses this process while l-amphetamine, a non-abuse agent, does not.

During the contract period, we tested over 300 rats, filling 212 conditions, in various phases of these experiments; rats were introduced to these conditions after they had achieved stable baseline responding (a minimum of three days to a maximum of three months). The following list enumerates ten series of experiments which were run during the contract period.

- I. Differential effects of morphine on self-stimulation over hours, dosage and days.
- II. Effects of cholinergic agents on self-stimulation over hours and days.

- III. Interactive effects of cholinergic agents and morphine on self-stimulation over hours, dosage and days.
- IV. Effects of morphine on rate-intensity functions.
- V. Neuroanatomic differentiation of the d- and l-isomers of amphetamine.
- VI. Differential effects of d- and l-amphetamine on the reinstatement of ICSS following locus coeruleus lesions.
- VII. Effects of pentobarbital on self-stimulation over hours and days.
- VIII. Behavioral evidence for neurophysiological interactions between intracranial "reward" loci.
- IX. Neuroanatomical differentiation of self-stimulation sites: specificity, refractoriness, and directional interactions of self-stimulation sites.
- X. Preliminary testing of the effects of amphetamine as determined by a double-pulse (C-T) technique.

SPECIFIC EXPERIMENTS

The following seven specific experiments supported in part by U. S. Army Contract DADA 17-73-C-3072 are attached:

1. D- and L-Amphetamine Differentially Mediates Self-Stimulation in Rat Dorsal Midbrain Area
2. Differential Effects of Unilateral Dorsal Hindbrain Lesions on Hypothalamic Self-Stimulation in the Rat
3. Alteration of Escape from Rewarding Electrical Brain Stimulation by D-Amphetamine
4. Comparison of Behaviors Elicited by Electrical Brain Stimulation in Dorsal Brain Stem and Hypothalamus of Rats
5. Behavioral Interactions among Rat Brainstem and Hypothalamic Self-Stimulation Sites
6. Directionality of Neurophysiological Interactions between Brainstem and Hypothalamic Self-Stimulation Loci in Rats
7. Intracranial Self-Stimulation Site Specificity: Monopolar Activation of Bipolar Electrodes.

The following publications and presentations have been supported in part by U. S. Army Contract DADA 17-73-C-3072 (copies were previously submitted with Report Number 2 dated 30 April 1975).

Publications

Farber, J., Ellman, S. J., Mattiace, L. A., Holtzman, A., Ippolito, P. A., Halperin, R. D., & Steiner, S. W. Selective abolition of hypothalamic self-stimulation by locus coeruleus lesions. Submitted to Science.

Jackler, F., Ackermann, R. F., Bodnar, R. J., Steiner, S. S., & Ellman, S. J. I. Differential effects of morphine on self-stimulation over hours, dosage and days. In preparation.

Jackler, F., Ackermann, R. F., Bodnar, R. J., Steiner, S. S., & Ellman, S. J. II. Effects of cholinergic agents on self-stimulation over hours and days. In preparation.

Jackler, F., Ackermann, R. F., Bodnar, R. J., Steiner, S. S., & Ellman, S. J. III. Interactive effects of cholinergic agents and morphine on self-stimulation over hours, dosage and days. In preparation.

Presentations

Jackler, F., Bodnar, R. J., Ackermann, R. F., Slavik, S., Steiner, S. S., & Ellman, S. J. Dose-dependent biphasic effects of morphine

on intracranial self-stimulation in rats. Presented at the Eastern Psychological Association, New York, 1975.

Steiner, S. S. Symposium: The role of catecholamines in central mechanisms of reward. Participants: A. Phillips, S. J. Ellman, B. Cooper, & J. Belluzzi. Discussant: L. Stein. Presented at the Eastern Psychological Association, New York, 1975.

Steiner, S. S. Neuroanatomical differentiation of the effects of psychopharmacological agents within the reward system. Presented at the Annual Meeting of the Behavioral Pharmacology Society, Princeton, New Jersey, May, 1975.

ATTACHMENT 1

D- and L-Amphetamine Differentially Mediates Self-Stimulation in Rat Dorsal Midbrain Area¹

STEVEN J. ELLMAN,² ROBERT F. ACKERMANN, RICHARD J. BODNAR
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(Received 16 September 1974)

ELLMAN, S. J., R. F. ACKERMANN, R. J. BODNAR, F. JACKLER AND S. S. STEINER. *d- and l-Amphetamine differentially mediates self-stimulation in rat dorsal midbrain area*. *PHYSIOL. BEHAV.* 16(1) 1-7, 1976. — Intracranial self-stimulation (ICSS) response rates elicited from the dorsal noradrenergic bundle are enhanced by d-amphetamine but not by l-amphetamine, suggesting noradrenergic mediation. Both isomers equally enhance ICSS response rates elicited from the mid-ventral periaqueductal area (the oculomotor nuclei, the interstitial nuclei, the dorsal raphe nuclei, and the medial longitudinal fasciculus), suggesting dopaminergic mediation.

Intracranial self-stimulation	Dorsal noradrenergic bundle	Periaqueductal midbrain	Norepinephrine
Dopamine	d- and l-Amphetamine		

BASED on pharmacological data obtained from hypothalamic and locus coeruleus (LC) intracranial self-stimulation (ICSS) sites, some investigators [2, 31, 32, 36] have concluded that ICSS is mediated primarily by norepinephrine. However, Phillips and Fibiger [27] have posited that at least one ICSS site, the substantia nigra (A-9), is mediated by dopamine. The rationale for their experiment was based on findings [5, 6, 35] that d-amphetamine is 8 to 10 times as potent as l-amphetamine in releasing catecholamines in noradrenergic terminals, while equipotent in dopaminergic terminals. Phillips and Fibiger compared the effects of d- and l-amphetamine on hypothalamic and substantia nigra ICSS response rates and found that hypothalamic ICSS rates are significantly enhanced by d-amphetamine, while substantia nigra response rates are equally enhanced by both isomers.

Several investigators have found that midbrain and pontine dorsal brainstem electrode sites support ICSS. For example, periaqueductal midbrain and adjacent tegmental areas support ICSS [4, 7, 10, 19, 23, 25, 34], as does the LC (a noradrenergic pontine periventricular nucleus) and its ascending projection, the dorsal noradrenergic bundle (DNB) [8, 11, 31, 29]. Stein, in several studies [31, 32, 36] using pharmacological agents such as d-amphetamine, diethylthiocarbamate and pimozide, concluded that ICSS

elicited from the hypothalamus and LC is mediated by norepinephrine. Indeed, Stein concluded that all ICSS is mediated by norepinephrine. We [11] found that LC ICSS resembles hypothalamic ICSS in that d-amphetamine enhances LC ICSS rates, but l-amphetamine does not.

This report investigates the effect of d- and l-amphetamine upon the anterior portions of the LC, or its ascending projection, the DNB, and the mid-ventral portions of the periaqueductal and pontine periventricular gray area and adjacent tegmentum, specifically such areas as the oculomotor nuclei, the interstitial nuclei, the dorsal raphe nuclei, and the medial longitudinal fasciculus.

METHOD

Animals and Procedure

Twenty-one male albino Sprague-Dawley rats (375-500 g) were anesthetized with Equithesin (Jensen, 1 ml/kg), placed in a Kopf stereotaxic instrument, and implanted with a stainless steel bipolar electrode which was 0.5 mm at its widest extent and completely insulated except at the tip. In each animal, the electrode was aimed at 1 of 2 areas: (a) the periaqueductal midbrain in the area of the medial longitudinal fasciculus, designated mid-ventral (MV), and (b) the DNB. With the incisor bar at -5 mm, coordinates

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were 0.6 mm anterior to lambda, 1.8 mm lateral to the sagittal suture, and 6.8 mm from the top of the skull and angled 12° toward the mid-sagittal plane for MV sites; 1.0 to 1.2 mm posterior to lambda, 1.0 mm lateral to the sagittal suture, and 7.0 mm from the top of the skull for DNB sites.

Ten days after recovery from surgery, each animal was shaped to barpress in an operant conditioning chamber (see [11] for dimensions) on a continuous reinforcement schedule. Reinforcements consisted of 250 msec trains of sinusoidal 60 Hz waves passed between the bipolar electrodes. Current was continuously monitored on a differential input cathode ray oscilloscope by observing the voltage drop across a 1000 ohm 1% resistor in series with the animal. Animals were shaped for a minimum of 15 successive daily sessions at a variety of current intensities. Rats that did not self-stimulate were discarded. All remaining animals self-stimulated daily throughout a 40 min session which was divided into five 7 min periods separated by 1 min timeouts. Data from the first 2 min of each 7 min period were disregarded to reduce carry over effects. The mean response rates over the last 5 min of each 7 min period were recorded and constituted the dependent variable in all conditions. Criteria for determining a rate-intensity function were the following; during the first 7 min period, the stimulation intensity was sufficiently low so that the animal's mean response rate over the last 5 min of the period was below an arbitrarily defined response threshold of about 10 responses per min. The fifth intensity sustained self-stimulation behavior at rates which approached or reached the animal's highest response rates. The second, third, and fourth intensities yielded response rates that were between threshold and peak intensity response rates. Rate-intensity functions, averaged over 5 days, were determined for each animal.

After stable rate-intensity functions were established, each animal entered the following drug paradigm which consisted of a series of six 3 day sequences. Days 1 and 3 of each 3 day sequence served as pre- and postdrug saline controls respectively. On these days, only saline solution (1 ml/kg, i.p.) was injected 30 min before self-stimulation sessions. On the second day of each 3 day sequence, animals were injected 30 min before self-stimulation sessions with either d- or l-amphetamine sulfate (Smith, Kline & French; 1 mg/ml/kg in saline, IP). Each animal received both d- and l-isomers in successive 3 day sequences in an a-b-b-a-a-b order which was counterbalanced across animals. Thus, each animal received 18 injections: 3 of the d-isomer, 3 of the l-isomer and 12 of saline. All comparisons were made between drug days (Day 2) and predrug saline days (Day 1). Data from postdrug saline days (Days 3) were disregarded to exclude drug carryover effects.

Eight of the animals (3 MV, 5 DNB) were tested in the above procedure under a variety of drug doses for d-amphetamine (0.25, 0.5, 1.0, 2.0 mg/ml/kg in saline, IP) and l-amphetamine (0.5, 1.0, 2.0 mg/ml/kg in saline, IP). These animals received 18 injections per dose in random order as described above.

After completion of the experiment, animals were overdosed with Equithesin and perfused with normal saline followed by 10% Formalin. Serial frozen sections were stained with luxol fast blue and cresyl violet [15] and electrode loci determined by microscopic examination of the sections.

Response rates under both isomers were compared for

each electrode site. 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. Each electrode focus was determined without knowledge of its drug category.

RESULTS

In every instance Drug Category I electrodes ($n = 10$) were localized within the ventro-lateral portions of the periaqueductal gray (anterior LC) and adjacent tegmentum (DNB). In every instance Drug Category II electrodes ($n = 9$) were localized in the mid-ventral portions of the periaqueductal and pontine periventricular areas, and in the adjacent tegmentum (MV). The latter included placements as far rostral as the interstitial nucleus and as far caudal as the pontine medial longitudinal fasciculus at the level of the dorsal tegmental nucleus (Fig. 1).

Table 1 shows each animal's (a) response rates for peak intensities under saline, (b) threshold intensities under saline and drug conditions, and (c) difference score between d- and l-amphetamine (1 mg/kg). Figure 2 shows the multiplicative effects on rate-intensity curves obtained from both sites under d- or l-amphetamine (1 mg/kg) as compared to the saline condition. This ratio was obtained by assigning saline response rates a value of one and drug response rates a value proportionate to the ratio between drug response rate and saline response rate (i.e., multiplicative effects). Both Fig. 2 and Table 1 show that in DNB sites, response enhancement under d-amphetamine was significantly greater than response enhancement under l-amphetamine (correlated difference score t test, $p < 0.005$), while in MV sites, there was no significant difference between response enhancement under d- as compared to l-amphetamine (correlated difference score t test, $p > 0.05$). Furthermore, response enhancement under d-amphetamine was significantly greater for DNB compared to MV sites (Mann-Whitney U Test, $p < 0.05$). On the other hand, response enhancement under l-amphetamine was significantly greater for MV sites compared to DNB sites (Mann-Whitney U Test, $p < 0.05$).

Figure 3 depicts the multiplicative drug effect plotted against dosage. Note that at 1 mg/kg doses, the data are comparable to those shown in Fig. 2, that is, DNB sites show differential responding under d- and l-amphetamine while MV sites show virtually equal responding across doses.

DISCUSSION

If one accepts the generalization that noradrenergic areas respond differentially to d- and l-amphetamine, while dopaminergic sites respond equally to d- and l-amphetamine, then the present results can be easily explained. The results obtained from stimulation of DNB sites would be compatible with previous histofluorescent and pharmacological studies indicating that the LC and its ascending projections are noradrenergic [11, 20, 22, 31, 33, 39]. The results from our MV sites, showing that d- and l-amphetamine have virtually equal response enhancement on ICSS, are similar to results obtained from the substantia nigra [18, 27]. While there is considerable evidence that the substantia nigra contains dopaminergic cell bodies [39], the evidence for catecholamine neuronal elements in MV sites

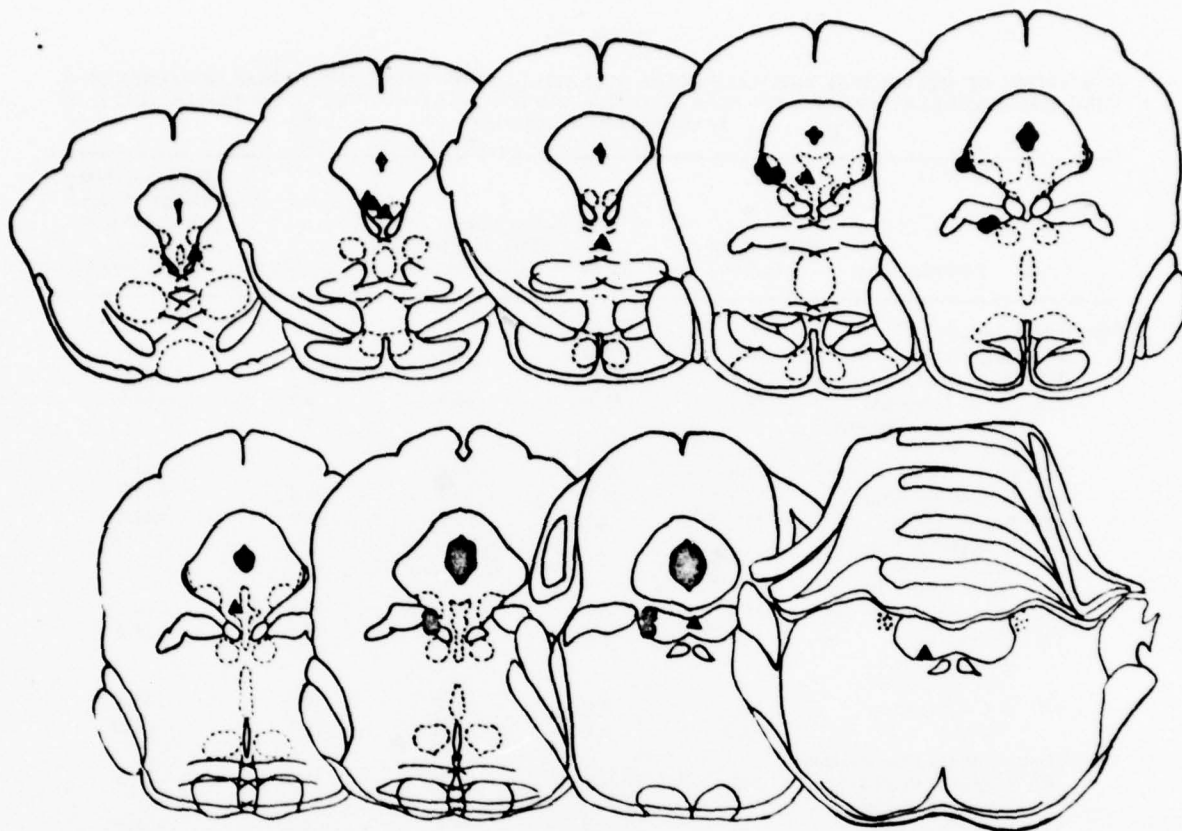


FIG. 1. Location of electrode tips for dorsal noradrenergic bundle sites (circles) and for mid-ventral periaqueductal/periventricular sites (triangles). These sections progress posteriorly from the anterior midbrain at the level of the oculomotor nuclei (upper left) to the anterior pons at the level of the locus coeruleus (lower right). All sections except the two rightmost in the bottom row are adapted from König and Klippel [15].

has only recently become clear. Investigators employing fluorescent histochemical techniques have repeatedly detected catecholaminergic cell bodies and terminals within the periaqueductal gray area. Early reports [9] described a catecholaminergic area (A-10) located in the mid-sagittal plane of rat midbrain which extends dorsally to the ventral border of the periaqueductal gray area. Catecholamine cell bodies have also been reported within the periaqueductal gray area (oculomotor and Edinger-Westphal nuclei). Area A-10 was subsequently shown to be dopaminergic with terminals in the nucleus accumbens [1,39] where equal ICSS enhancement for both amphetamine isomers has been recently reported [28]. Subsequent investigation has confirmed that the MV area contains (a) 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, and (b) distinct catecholamine containing fibers interspersed within the medial longitudinal fasciculus as far posterior as the level of the locus coeruleus [12, 20, 21, 24, 26]. Our animals' MV electrodes were located within these areas.

On the basis of the above data, a catecholaminergic hypothesis accounting for MV periaqueductal self-stimulation has a firm anatomical base. However, it has also been suggested that these cells may be a dorsal extension of the

A-10 (dopaminergic) cell group [9,20]. Moreover, recent reports [21] indicate that group A-11, composed of dopaminergic neurons (3), extends along the dorsal longitudinal fasciculus from the caudal hypothalamus to the periaqueductal gray area as far caudal as the dorsal raphe nucleus. The present suggestion that MV ICSS is mediated by dopaminergic neurons, possibly an extension of the A-10 dopaminergic group, or the periaqueductal portion of the A-11 dopaminergic group 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. These results [11, 27, 28, the present study] are therefore vulnerable to some criticism [13, 14, 38] of the validity of *d*- and *l*-amphetamine screening to distinguish between noradrenergic and dopaminergic neurons.

If we must be tentative in concluding that ICSS in the MV area is mediated by dopamine, it then becomes important to explore alternative explanations. Until recently, serotonin was the only known monoamine found in large quantities within the periaqueductal area [9]. Thus, it might be hypothesized that our results were mediated by an augmented release of serotonin. However, such a hypothesis would face several difficulties. First, it has been shown that release of serotonin is greatest after low frequency

TABLE I

COMPARISON OF MID-VENTRAL PERIAQUEDUCTAL SELF-STIMULATION RATES AND DORSAL NORADRENERGIC BUNDLE SELF-STIMULATION RATES FOR PEAK INTENSITY AND THRESHOLD INTENSITY UNDER SALINE AND D- OR L-AMPHETAMINE SULPHATE

Animal	Electrode Site	Peak Intensity Saline	Response Rate		Response Rate Difference Score D-amphetamine minus l-amphetamine)	
			Saline	Threshold Intensity D-amphetamine L-amphetamine		
Mid-Ventral Animals						
98C	Oculomotor Nuclei	56.0	5.0	18.4	6.5	+11.9
30E	Oculomotor Nuclei	98.2	16.4	39.9	26.7	+13.2
31E	Dorsal Tegmental Decussation-Linearis Pars Caudalis	86.3	15.7	46.6	8.1	+38.5
32E	Dorsal Raphe	195.6	27.0	18.0	31.8	-13.8
43E	Oculomotor Nuclei	22.2	5.8	39.3	21.6	+17.7
93E	Pontine Medial Longitudinal Fas- ciculus-Ventral Tegmental Nucleus	29.7	5.3	65.2	52.8	+12.4
3F	Interstitial Nuclei	35.0	6.4	17.5	21.7	-4.2
19F	Midline Gray Between Dorsal Tegmental Nuclei	45.2	4.8	1.0	14.2	-13.2
43F	Dorsal Raphe	55.5	1.9	5.2	39.0	-33.8
	Mean:	69.3	9.8	27.9	24.7	+3.2*
Dorsal Noradrenergic Bundle Animals						
9E	Lateral to Ventral Tegmental Nucleus	14.7	6.5	36.9	15.9	+21.0
13E	Dorsal Noradrener- gic Bundle-Medial to Mesencephalic V	13.8	3.0	15.3	4.4	+10.9
14E	Dorsal Noradrener- gic Bundle-Anterior Locus Coeruleus	22.1	1.7	3.6	0.9	+2.7
18E	Dorsal Noradrener- gic Bundle-Anterior Locus Coeruleus	110.4	0.7	0.7	0.0	+0.7
33E	Dorsal Noradrener- gic Bundle-Anterior Locus Coeruleus	58.9	8.4	27.6	7.1	+20.5
54E	Ventral Locus Coeruleus-Dorsal Noradrenergic Bundle	26.5	3.4	83.9	10.5	+73.4
74E	Dorsal Locus Coeruleus	42.4	3.9	52.9	3.4	+49.5
23F	Dorsal Noradrener- gic Bundle	26.8	2.8	23.8	0.0	+23.8
26F	Anterior Locus - (left) Coeruleus	19.0	1.0	13.3	1.0	+12.3
26F	Anterior Locus (right) Coeruleus-Dorsal Noradrenergic Bundle	34.3	1.6	61.1	4.6	+56.5
	Mean:	36.9	3.3	31.9	4.8	+27.1†

*t act. = 0.5 ($p > 0.05$).†t act. = 3.5 ($p \leq 0.05$).

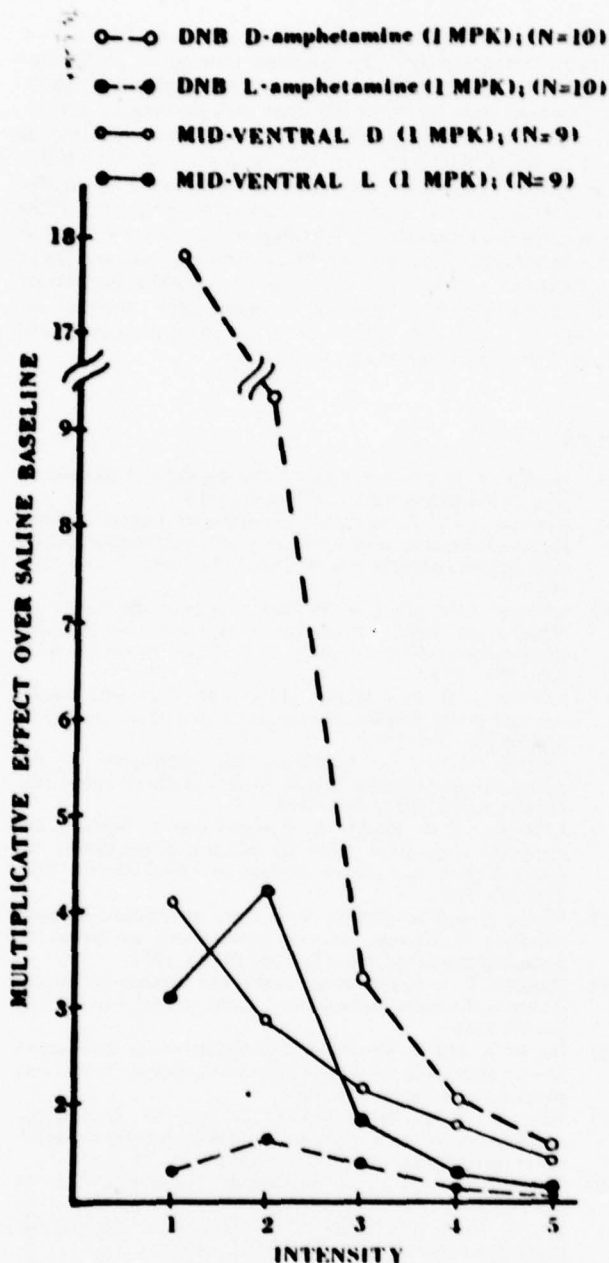


FIG. 2. Proportional comparison of 1 mg of d- or l-amphetamine/kg of body weight upon rate-intensity functions in the dorsal noradrenergic bundle (DNB), and mid-ventral periaqueductal/periventricular midbrain area (MV).

raphe stimulation, but minimal after high frequency stimulation such as that employed in the present experiments [17]. Second, it has been repeatedly suggested that serotonin release produces decreased self-stimulation [29, 30, 37], not increased rates as in the present experiments. Third, Margules [23] found that amphetamine increases dorsal raphe self-stimulation rates, while chlorpromazine decreases self-stimulation rates. He found that PCPA decreases self-stimulation rates over the first 24 hours post

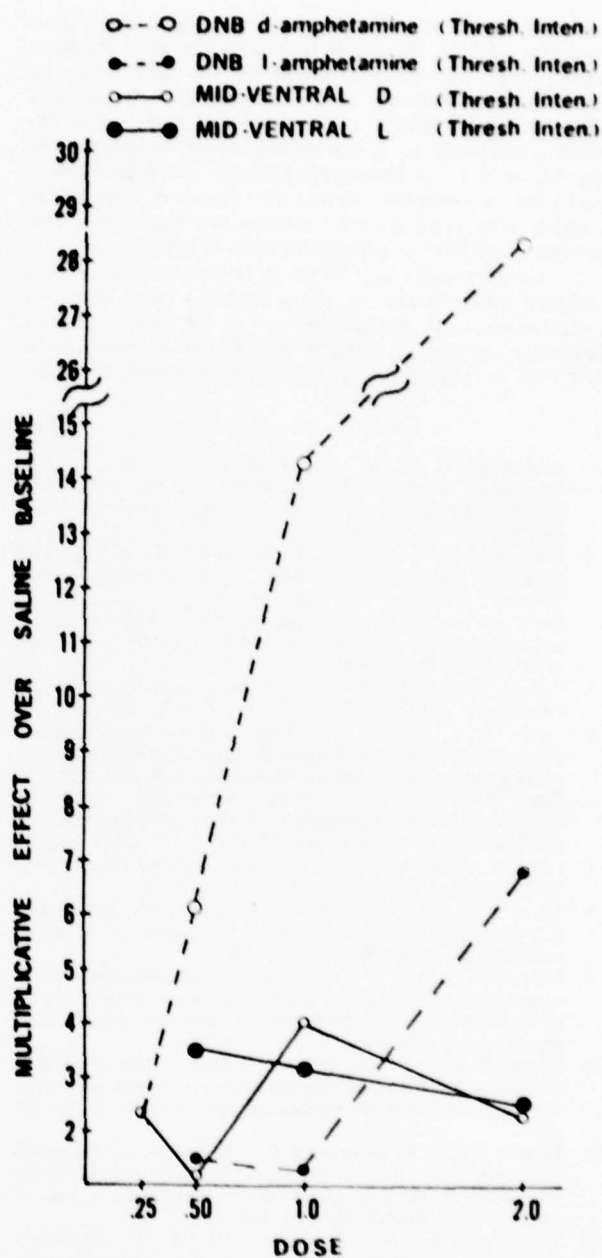


FIG. 3. Proportional comparison of dose responding d- (0.25, 0.5, 1.0, 2.0 mg/kg) and l- (0.5, 1.0, 2.0 mg/kg) amphetamine upon the threshold intensity in the dorsal noradrenergic bundle (DNB) and mid-ventral periaqueductal/periventricular midbrain area (MV).

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 recovered but serotonin levels remain depressed.

From these results, Margules concluded that dorsal raphe self-stimulation is not mediated by serotonin-containing neurons in the area of the electrodes, but rather by hypothesized noradrenergic fibers of passage, most pro-

bably within the ventral division of the dorsal longitudinal fasciculus (DLF). We think it is unlikely that Margules' results were due to stimulation of the DLF since his electrode placements are ventral to most DLF fibers [16]. Also, his results indicate only that dorsal raphe self-stimulation is mediated by a catecholaminergic transmitter; his experiment did not distinguish between norepinephrine or dopamine as possible mediators. However, his results together with ours do pose serious problems for a serotonergic hypothesis of periaqueductal self-stimulation.

It is also possible that ICSS in the mid-ventral area is mediated either solely by norepinephrine or partially by norepinephrine. If norepinephrine is the sole mediating transmitter substance, then it is difficult to explain the difference in pharmacological response between DNB and

MV sites. This is particularly striking since the DNB seems clearly noradrenergic. The question then arises as to why two sites, both mediated solely by norepinephrine, show differential responding to the same pharmacological agents. The second possibility, that norepinephrine is involved in and perhaps necessary for the mediation of MV ICSS, cannot be ruled out by the present data. In our opinion, the present data is best explained by postulating that MV ICSS is mediated by dopamine. Whether or not we are correct in this conclusion, it seems clear from both our data and those of Phillips and Fibiger that the neurohumoral substrates of MV periaqueductal, substantia nigra, and nucleus accumbens ICSS may differ from the neurohumoral substrates of hypothalamic and LC ICSS.

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ATTACHMENT 2

Differential effects of unilateral dorsal hindbrain lesions on hypothalamic self-stimulation in the rat

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In an effort to localize the cell bodies and pathways in the brain involved in the mediation of intracranial self-stimulation (ICSS) behavior, several studies have investigated the effects of electrolytic brain lesions on ICSS response rates^{2-4,11,15,17,24}. These studies were prompted by the general hypothesis that, if there is in fact, a central nervous system (CNS) 'reward center' essential to all positive reinforcement, then the destruction of such a center should reduce or abolish ICSS^{15,17,24}. Some experimenters have tested the effects of electrolytic lesions placed at the medial fore-brain bundle (MFB) on ICSS elicited from other sites throughout the limbic system^{11,24}. Others have investigated the effects of lesions placed at different ICSS sites (i.e., MFB, hippocampus, dorsal and ventral tegmentum, thalamus and septum) on MFB-lateral hypothalamic^{4,15,17} and septal^{2,3} ICSS. Although these studies have not been in complete agreement as to the effects of the lesions on ICSS and the specific loci involved, by and large most investigators have stated that lesions distant from an ICSS site have little or no long-term effect on ICSS behavior^{2,3,15,24}. Those lesions that were successful in attenuating ICSS were for the most part bilateral and the magnitude of the effect on ICSS depended on the size of the lesion and its proximity to the ICSS site¹⁷. In addition, no specific site was found which when lesioned would reliably produce complete abolition^{3,4,15}. A frequent conclusion has been that the ICSS system is highly redundant, diffuse, and capable of recovery^{3,4,15,23} and therefore, small discrete lesions should be ineffective in reducing ICSS behavior from distant sites.

We and others have reported that a number of hindbrain sites support ICSS behavior^{6-9,19,21}. Among these, the locus coeruleus (LC) and the sub-coeruleus have been described as nuclei containing noradrenergic (NE) neurons that project ascending fibers throughout the brain^{14,16,18,20,22}, including areas associated with ICSS^{5,10}. If, in addition, these hindbrain structures were actively involved in the

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mediation of ICSS at the anterior sites that receive their ascending projections, then the destruction of the hindbrain nuclei should detrimentally affect ICSS at these anterior sites. To test the above hypothesis, we investigated the effects of unilateral dorsal pons lesions on hypothalamic ICSS. Our anatomical-behavioral approach was geared to evaluate the role of dorsal tegmental pontine structures, i.e. LC, in hypothalamic ICSS behavior.

Male albino Sprague-Dawley rats (350–500 g) were anesthetized with a sodium pentobarbital and chloral hydrate combination and implanted, with the use of a stereotaxic instrument, with bipolar stainless steel electrodes (0.24 mm width between the tips) that were completely insulated except at the cross section of their tips. This procedure stimulates more discrete areas than do conventional monopolar techniques²³. In all rats, the electrodes were aimed ipsilaterally at the LC and hypothalamic areas. Ten days after surgery, the rats were trained to press a bar for electrical stimulation at both the hypothalamic and LC sites. Stimulation consisted of trains of 0.25 sec duration, 60 Hz sine waves administered on a continuous reinforcement schedule. Intensity was manipulated to optimize training. The rats' electrodes were isolated from ground and stimulation was continuously monitored on a differential input cathode ray oscilloscope. Following this, hypothalamic rate-intensity functions were obtained, as described by Ellman et al.⁸.

Subjects were tested from their hypothalamic site at a minimum of 6 different intensities, chosen to encompass the range of intensities over which the subjects would reliably self-stimulate. This procedure was employed before and after lesioning in order to evaluate the effects, if any, of the lesion at any point of the rate-intensity functions. Initially, daily trials were conducted until subjects stabilized. Rate-intensity functions collected over the next 4 days were considered as pre-lesion baseline. Following this, subjects were anesthetized and unilateral lesions were placed under the pontine electrode. The lesions were produced by passing 2–8 mA anodal radio-frequency (RF) current through the electrode tip to an indifferent cathode for a duration of 30 sec. The fact that ICSS could not be elicited again from the LC electrodes following the lesion served as a preliminary confirmation that at least the area directly under the LC electrode had been destroyed.

Following the LC lesion, the Ss were tested for 14 days (post-lesion baseline). If the Ss showed hypothalamic ICSS decrements or facilitations they were at this time 'shelved' for approximately one month and then run again for a 4-day recovery baseline (recovery-check baseline). This procedure was repeated at least one more time. If the lesion did not affect hypothalamic ICSS within the 14-day post-lesion baseline, the Ss were relesioned at the pontine site at a higher voltage and retested for 14 days. If no effects resulted from the lesion, the animal was then sacrificed. In any event, all Ss not affected by the lesion were tested for at least 14 days after the lesion. This decision was based on the fact that the time course of possible fiber degeneration or neurohumoral transmitter depletion is a maximum of 12 days^{20,22}. Those animals with ICSS alterations following the lesion were repeatedly tested 2–3 months after the lesion, thus allowing ample time for recovery to occur.

After completion of the ICSS paradigm, the animals were injected with an

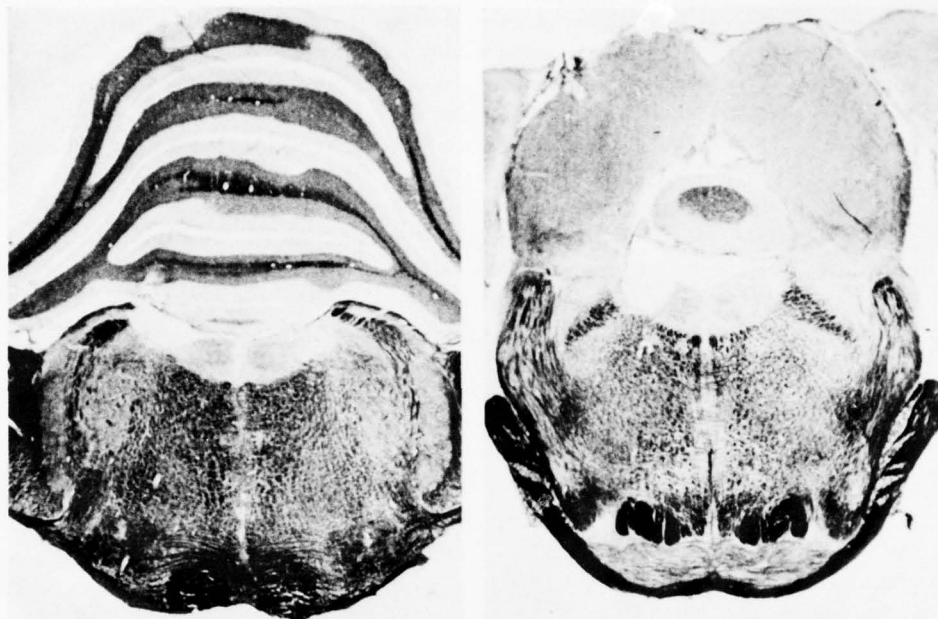


Fig. 1. Photomicrographs of pontine coronal sections showing the extent of lesion in one rat. Locus coeruleus has been unilaterally destroyed by RF coagulation. ICSS from Forel field H_2 in this rat was drastically reduced after lesion.

overdose of sodium pentobarbital and perfused via an intracardiac needle with 0.9% normal saline solution followed by 10% formalin solution. The brains were then removed from the crania and kept in 10% formalin for at least 3 days. Frozen coronal sections, 40 μ m thick were stained with Luxol fast blue and cresyl violet, according to the method of Klüver and Barrera¹². The stained brain sections were examined under the microscope. The electrode locus and size of lesions were evaluated by two judges who were not aware of the ICSS results corresponding to the brain sections. ICSS placements were classified in terms of the neuroanatomical sites under the tip of the ICSS electrode as described in Craigie's²⁵, and König and Klippel's¹³ atlases, and compared to Lindvall and Björklund's¹⁴, and Ungerstedt's²² description of the monoaminergic nuclei and pathways. The extent of the lesions under the pontine electrodes were assessed in terms of the percentage destruction of the involved structures as described in neuroanatomical maps^{13,25} and on the basis of descriptions and observations made in histofluorescence studies^{14,16,18,22}.

As evaluated by the judges, the lesions unilaterally destroyed 90–100% of the LC in all 14 Ss included in the results (Fig. 1). In addition, 30–100% of the area containing the ascending fibers stemming from the LC, as described by the histofluorescence studies^{14,16,18,22}, was destroyed by the lesion, as well as 20–100% of the nuclei of the mesencephalic V located at the level of the LC. In those Ss (N = 3) with more extensive destruction, 40–100% of the dorsal tegmental nucleus was affected by the lesion.

The hypothalamic ICSS electrodes were located in 4 distinct hypothalamic areas: 5 of the electrodes terminated along the MFB at various levels of the hypothalamus (Fig. 2A); two other ICSS electrodes were located in the perifornical area; 3 other electrode tips impinged upon the crus cerebri-internal capsule (Fig. 2B), and 4 were in the Forel field H₂, dorsal to the hypothalamus (Fig. 2C).

ICSS behavior in these 4 groups was differentially affected by the unilateral lesion. In those Ss with electrodes in the crus cerebri-internal capsule area (N = 3) or the Forel field H₂ (N = 4), the lesion resulted in either complete abolition of ICSS or a significant reduction of response rates (*t*-test, *P* < 0.05), accompanied by an increase of the ICSS threshold (Fig. 3A). The average ICSS reduction of the crus cerebri-internal capsule group, across all intensities during the 14-day post-lesion baseline, was 69–91% of pre-lesion rates. The Forel field H₂ group as a whole averaged a 29–80% reduction in ICSS response rates following the lesion. These ICSS decrements were observed up to 3 months after the lesion. In addition, the increase of current during the post-lesion runs did not elevate response rates to pre-lesion levels (Fig. 3A). Subsequent to pontine lesions, rats with electrodes in the perifornical area (N = 2) and 3 of the 5 Ss with ICSS electrodes impinging along the MFB did not show any drastic or sustained long-term changes in either ICSS response rates or ICSS thresholds. These alterations did not approach statistical significance. In fact, the ICSS response rates of the remaining 2 Ss with MFB localized electrodes were facilitated following the lesion. This elevation of response rates (up to 297% of pre-lesion levels) remained over a period of up to 2 months after the lesion (Fig. 3B).

The unilateral lesions did not result in weight losses in any of the Ss nor were there any behavioral deficits observed. Thus, the obtained results did not appear to be artifactual nor due to non-specific side effects.

As previously described, all lesions destroyed 80–100% of the LC. However, the extent of adventitious damage incurred on adjacent areas varied across Ss. Yet, the extent of the lesions of structures outside the LC does not significantly correlate with the magnitude of the effect on hypothalamic ICSS (Pearson Product-Moment correlation). Small pontine lesions that were limited primarily to the LC, with minimal impingement on adjacent sites, differentially affected hypothalamic ICSS. The same pattern of differential effects was observed in those cases where the damage caused by the lesion suffused into adjacent areas. Thus, the effect of the lesion on behavior, be it a drastic reduction or facilitation of ICSS (i.e., reduction in crus cerebri-internal capsule and Forel field H₂ ICSS; facilitation or no effect on MFB ICSS), seemed to be determined by the placement of the ICSS electrode in the hypothalamus and not by the spread of the lesion outside the LC.

Though the interpretation is partially limited by the fact that all lesions are not exactly the same, it seems to us that a likely candidate for the role of ICSS mediator in some hypothalamic areas is the nucleus LC. We cannot discount the possibility that other adjacent areas are involved in this mediation or that, more likely, areas within the LC complex that send distinct ascending pathways, as described by a number of histofluorescence studies, may assume different roles in the mediation of ICSS. We are however, impressed by the finding that similar lesions result in effects



Fig. 2. Photomicrographs of hypothalamic areas that were differentially affected by pontine lesions. A: tip of electrode impinges on MEB. ICSS from this site was facilitated following lesion (see Fig. 3B). B: tip of electrode is located in Forel field H_2 . ICSS was strongly reduced following the lesion (see Fig. 3A). C: tip of electrode is in the internal capsule. ICSS from this site was abolished by pontine lesion.

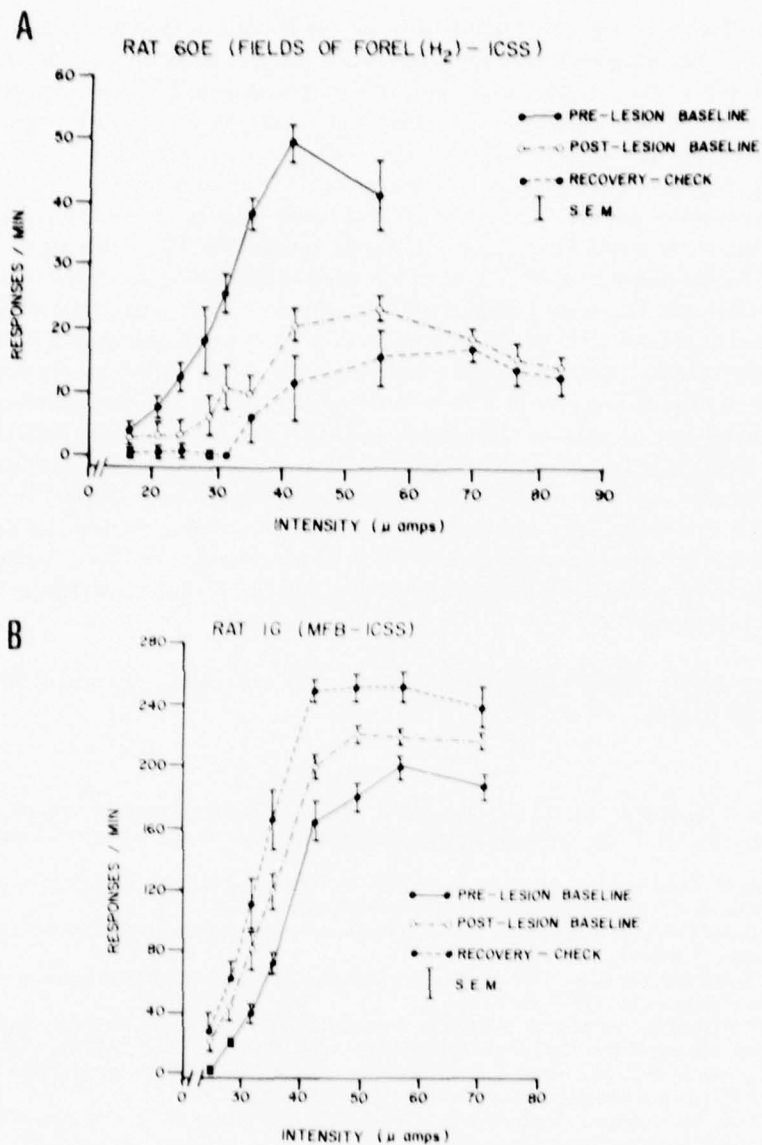


Fig. 3. ICSS rate-intensity functions of two rats before and after pontine lesions. The functions represent 4 days of pre-lesion baseline, 14 days of post-lesion baseline and 4 days recovery-check baseline 6 weeks after lesion. A: ICSS from Forel field H₂ (see Fig. 2B); B: ICSS from MFB (see Fig. 2A).

that can be differentiated in terms of distinct hypothalamic ICSS sites (Mann-Whitney U test, $P < 0.001$).

Despite the failure of other investigators to show long-term decrements in ICSS behavior as a result of a distant lesion^{2-4,13,24}, this study has shown that it is,

indeed, possible to produce long-term reductions or abolitions of ICSS behavior. Furthermore, when compared to all previous studies, the present lesions are anatomically more distant from tested ICSS sites, smaller and unilateral. Therefore, our results provide evidence contrary to the earlier conclusions that all central reward is redundant, diffuse and capable of recovery^{3,4,15,24}. This point is unequivocal in view of the long-term ICSS reductions observed up to 3 months after the lesion.

The reductions and abolitions in ICSS that result from the lesions implicate structures within the dorsal tegmentum of the pons, possibly the LC, as the area necessary for the maintenance of ICSS behavior in some hypothalamic sites Forel (field H₂, *crus cerebri-internal capsule*). Conversely, the differential effects also indicate that bilateral integrity of this same structure is not necessary for the maintenance of ICSS from all hypothalamic sites from which the behavior can be elicited (MFB, perifornical area) and that this site may even play an inhibitory role, given the facilitatory effects on two Ss. Interestingly, those areas from which ICSS was reduced, i.e., Forel field H₂ and *crus cerebri-internal capsule*, have been described as receiving ascending fibers from dopaminergic nuclei in the substantia nigra via the nigro-striatal bundle^{1,11,22}. It is possible to entertain the notion that some ICSS sites within the area of the hypothalamus, that receive dopaminergic fibers in addition to the NE fibers, depend on the integrity of a noradrenergic pontine nucleus, the LC, for the maintenance of their ICSS behavior.

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ATTACHMENT 3

**ALTERATION OF ESCAPE FROM REWARDING ELECTRICAL
BRAIN STIMULATION BY D-AMPHETAMINE†**SOLOMON S. STEINER††, ROBERT F. ACKERMANN, RICHARD J. BODNAR,
FRANCES JACKLER, JANE M. HEALEY and STEVEN J. ELLMAN*City College of New York**(Received March 2, 1976)*

Rats were trained both to barpress for and escape from locus coeruleus, midbrain periaqueductal gray and hypothalamic stimulation. Rate-intensity functions for intracranial self-stimulation (ICSS) and behavior and latency-intensity functions for escape behavior were obtained for each electrode site in each animal. Following baseline, d-amphetamine was administered and responding was compared with the saline condition for both rate-intensity and latency-intensity functions. ICSS response rates were enhanced by d-amphetamine at all loci, particularly at threshold intensities, while escape responding was biphasically affected by d-amphetamine at all loci. D-amphetamine increased escape latencies at intensities which, under saline, elicited short escape latencies, while decreasing escape latencies at intensities which, under saline, elicited long escape latencies. A significant correspondence was noted between intensities which, under the influence of d-amphetamine, both elicited longer escape latencies and higher ICSS response rates, suggesting that in both ICSS and escape paradigms, animals were titrating the duration of the stimulus train. No site-specific effects of d-amphetamine upon escape behavior were noted.

Numerous investigators have reported that animals will work both to receive electrical brain stimulation (ESB) and to escape from ESB delivered at similar parameters in the same animal to the same neuro-anatomical site (Bowie and Miller, 1958; Cooper and Taylor, 1967; Liebman, Mayer and Liebeskind, 1973; Steiner, Beer and Schaeffer, 1969; Steiner, Bodnar, Ackermann and Ellman, 1973; Ellman, Ackermann, Bodnar, Jackler and Steiner, 1975; Beer and Steiner, 1965; Steiner and D'Amato, 1964). D-amphetamine, a pharmacological agent with potent influences on catecholaminergic transmission (Stein and Wise, 1969) has been shown to lower intracranial self-stimulation (ICSS) thresholds and to increase ICSS response rates (Miller, 1957; Stein, 1964; Steiner and Stokely, 1973). However, there have been few studies (Kornblith and Hoebel, 1974) investigating the effects of d-amphetamine upon escape behavior elicited by passive electrical stimulation of ICSS sites. The present study systematically investigated the effects of d-amphetamine upon both ICSS behavior and escape behavior elicited from the same site. The sites tested,

which elicited both ICSS and escape behavior, were the locus coeruleus (LC), midbrain periaqueductal grey (MPG) and a variety of hypothalamic sites.

METHOD

Eight male albino Holtzman Sprague-Dawley rats, (375-500 gm) were anesthetized with Equithesin (Jensen; 1 ml/kg), placed in a Kopf stereotaxic instrument and implanted with two pairs of stainless steel bipolar electrodes (Plastic Products) which were 0.25 mm at their widest extent and completely insulated except at their tips. In four animals, one pair of bipolar electrodes was aimed at the hypothalamus, while the other was aimed at the MPG. The remaining four animals had electrodes aimed at the hypothalamus and the LC. With the incisor bar at -5 mm, coordinates were 4.2-4.4 mm posterior to bregma, 1.5 mm lateral to the sagittal suture, and 8.7 mm from the top of the skull for hypothalamic sites, 1.5-2.0 mm posterior to lambda, 1.0 mm lateral to the sagittal suture, and 7.0 mm from the top of the skull for the LC; and 0.6 mm anterior to lambda, 1.3 mm lateral to the sagittal suture, and 6.8 mm from the top of the skull and angled 12° toward the midsagittal plane for the MPG.

Ten days after recovery from surgery, each animal was shaped to barpress in an operant con-

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ditioning chamber (see Ellman *et al.*, 1975 for dimensions) on a continuous reinforcement schedule. Reinforcements consisted of 250 msec trains of sinusoidal 60 Hz waves passed between the bipolar electrodes. Current was continuously monitored by observing the voltage drop across a 1000-ohm 1% resistor in series with the animal on a differential input cathode ray oscilloscope (Hewlett-Packard). Animals were shaped for a minimum of 15 successive daily sessions at a variety of current intensities in each site; animals which self-stimulated in both sites continued in testing.

Rate intensity (RI) functions were derived in the following manner: each day, each animal was allowed to self-stimulate throughout a 42-min session which was divided into six 7-min periods separated by one-min timeouts. Data collected during the first 2 min of each 7-min period were disregarded; the mean response rate over the last 5 min was recorded and constituted the dependent variable. During the first 7-min period, stimulation intensity was sufficiently low so that the animal's mean response rate over the last 5 min of the period was below an arbitrarily defined threshold of about ten responses per min. The fifth intensity sustained ICSS behavior at rates which approached or reached the animal's highest response rates. The second, third, and fourth intensities yielded ICSS response rates. During the last 7-min period of each session, responding yielded no stimulation (extinction). Baseline RI functions over five days were taken for both electrode sites in each animal. The RI functions of each animal's two electrode sites were then interdigitated; i.e., delivery of the current was alternated between the two sites in an ABBAABBAABBA sequence, beginning with the posterior (LC or MPG) electrode site. The purpose of this procedure was to insure that both electrode sites would be tested equally at approximately the same time and under similar conditions.

Animals were then trained to escape from passive electrical brain stimulation in an operant chamber similar in size to the RI chamber with the following exception: in place of a retractable lever, a larger treadle was permanently inserted on the opposite wall. The onset of a stream of electrical trains (60 cycles/sec sine, 500 msec duration, 500 msec inter-train interval) delivered through one of the animal's two electrode sites marked the beginning of the first trial. Stimulation continued until either the rat depressed the treadle or 101 trains had been delivered. Either contingency constituted a single trial. After a 15-sec intertrial interval, the stimula-

tion was automatically reinitiated and the procedure repeated successively until a block of ten trials was run. The animals were trained to make a treadle-press response to terminate stimulation; all other responses were actively inhibited by the experimenter. All eight animals learned the appropriate treadle-press response for both sites in each animal.

The animals were then tested at relatively high and low intensities (5-210 μ) to find ranges of escape latencies. For each site, six intensities presented in descending order were chosen to determine a latency intensity (LI) function. At high intensities, animals often had to overcome stimulus-induced motor involvement in order to make the appropriate response; this constituted the first stimulus intensity for the paradigm. This intensity also had to sustain at least threshold responding in the self-stimulation paradigm. The next three lower intensities chosen were those from which the animal could escape reliably, that is, intensities which consistently elicited escape behavior but did not evoke any obvious involuntary movement. The fifth intensity was one which evoked variable response-latencies across days, whereas the sixth intensity was deliberately chosen to be below the threshold for eliciting escape responses.

The within-day escape procedure consisted of 80 trials; the first 10 and the last 10 trials were control trials run at zero intensity. Between the first and last blocks of trials the six chosen intensities were tested, 10 trials per intensity. For each site in each animal, baseline LI functions were taken over 5 days. Half of the animals had the posterior electrode placement tested first, followed by the hypothalamic site, while the other half had the hypothalamic site tested first.

After stable RI and LI functions were established for both its sites, each animal entered two drug paradigms: the first tested the effects of d-amphetamine upon RI functions, and the second tested the drug's effect upon LI functions. Drug effects were tested in 3-day sequences. Days 1 and 3 of each 3-day sequence served as pre- and postdrug saline controls respectively. On those days, only saline solution (1 ml/kg, IP) was injected 30 min before the testing sessions. On the second day of each 3 day sequence, animals were injected 30 min before the testing session with d-amphetamine sulfate (Smith, Kline and French; 1 mg/ml/kg in saline, IP). RI functions were tested in three 3-day sequences and LI functions were tested in five 3-day sequences. All comparisons were made between drug days (Day 2) and predrug saline days (Day 1).

Data from postdrug saline days (Day 3) were disregarded to exclude any drug carryover effects.

After completion of the experiment, each animal was injected with an overdose of Equithesin and perfused with 0.9% normal saline solution, followed by a 10% formalin solution. Frozen serial sections were then cut, mounted, and stained with luxol fast blue and cresyl violet. Electrode locus was determined by microscopic examination of the sections.

RESULTS

All 8 animals both escaped and self-stimulated from both electrode sites. All hypothalamic placements were located at or near either the fornix or medial forebrain bundle, with the exception of one animal whose electrode placement was in the globus pallidus. All LC implants were located in the dorsal noradrenergic bundle. MPG placements were located at the dorsal longitudinal fasciculus, the oculomotor nucleus, or the nucleus Darkschewisch.

RI functions

In all 16 electrode sites of the 8 animals in this experiment, there was a significant increase in ICSS response rate under d-amphetamine as compared to saline baseline at all current intensities (t -tests, $p \leq 0.05$). All sites were equally sensitive to the facilitatory effects of d-amphetamine. In order graphically to represent the data across sites, across subjects, saline ICSS response rates were grouped into 6 categories: 0-10 responses/min; 10-20 responses/min; 20-30 responses/min; 30-40 responses/min; 40-50 responses/min and 50 or more responses/min. For each category, the mean response rates of d-amphetamine were calculated and expressed as a multiple of the mean response rates under saline at the same site and at the same intensity. Figure 1 shows that d-amphetamine enhances responding at all groups, most notably at the threshold (0-10 responses/min) group.

LI functions

The effects of d-amphetamine on escape responding were studied in a similar manner since there were no differences across sites in the effects of d-amphetamine on LI functions. Mean escape latencies under saline were also grouped into 6 categories: 0-10 sec; 10-20 sec; 20-30 sec; 30-40 sec; 40-50 sec and 50 or more sec. Figure 2 displays the multi-

pliative effect of d-amphetamine over saline baseline for each category. At intensities which supported escape latencies of 0-10 sec under saline, latencies are significantly increased under d-amphetamine (t -test, $p \leq 0.05$). At intensities which supported escape latencies of 10-20 sec or 20-30 sec under saline, latencies are neither increased nor decreased under d-amphetamine (t -test, $p > 0.05$). At intensities which supported escape latencies of 30-40 sec or 40-50 sec or above 50 sec under saline, latencies are significantly decreased under d-amphetamine (t -test, $p \leq 0.05$). The latter intensities were below ICSS threshold intensities. At zero intensity, there was no significant difference (t -test, $p > 0.05$) in escape behavior between the saline and d-amphetamine conditions; therefore, the effects

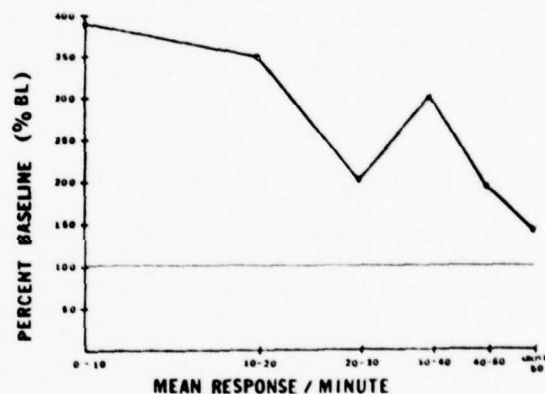


FIGURE 1 Proportional comparison of 1 mg of d-amphetamine/kg of body weight upon rate-intensity functions in LC, MPG and hypothalamic sites. Saline baseline is at 100%.

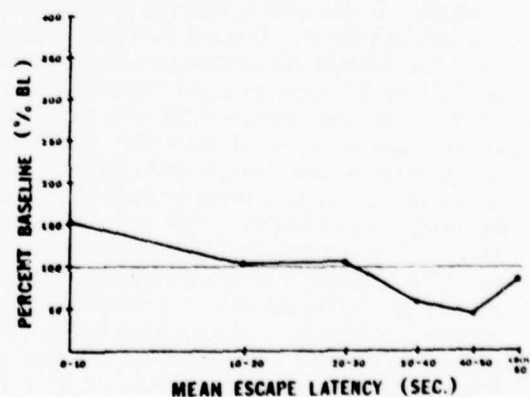


FIGURE 2 Proportional comparison of 1 mg of d-amphetamine/kg of body weight upon latency-intensity functions in LC, MPG and hypothalamic sites. Saline baseline is at 100%.

seen in escape latency categories of 30 sec or more cannot be attributed to non-specific increases in locomotor activity under d-amphetamine.

To summarize, d-amphetamine increased escape latencies at intensities which, under saline, elicited short (0-10 sec) escape latencies. In contrast, d-amphetamine decreases escape latencies at intensities which, under saline, elicited long (30-50+ sec) latencies. On the other hand, for ICSS behavior, d-amphetamine had its greatest facilitatory effect at threshold (0-10 responses/min). In all sites, there was an overlap in the intensities which, under saline, elicited both short escape responding and low ICSS response rates. At these overlapping intensities, d-amphetamine increased both escape latency and ICSS response rates (Fisher Exact Test, $p \leq 0.001$). In other words, the very same intensities which under the influence of d-amphetamine elicited longer escape latencies, supported ICSS response rates which were significantly enhanced under d-amphetamine.

DISCUSSION

All sites in all animals demonstrated differential, yet consistent ICSS and escape behaviors under baseline and drug conditions; this consistency in responding within a given condition has been reported previously (Bower and Miller, 1958; Steiner *et al.*, 1969; Steiner and D'Amato, 1964; Steiner *et al.*, 1973; Ellman *et al.*, 1975). Thus, any effect seen can be attributed to the drug and not to any non-specific artifact such as a disruption in behavior due to the initial novelty of the drug condition. As described previously, there seems to be a lack of specificity between sites in both escape and ICSS behavior. All sites in this study responded similarly for RI functions under d-amphetamine as compared to saline, that is, ICSS rates were higher under d-amphetamine at intensities which supported threshold or just above threshold rates under saline. This agrees with previous findings for these loci (Steiner and D'Amato, 1964; Ritter and Stein, 1973; Steiner and Stokely, 1973; Phillips and Fibiger, 1973; Phillips, Brooke and Fibiger, 1975; Ellman *et al.*, 1975; Ellman, Ackermann, Bodnar, Jackler and Steiner, 1976). All sites in this study produced similar LI functions in the baseline condition as described previously (Ellman *et al.*, 1975). This study failed to find any systematic differences in escape latencies under d-amphetamine that could be attributed to neuroanatomical locus.

Two striking results were seen in the escape functions under d-amphetamine as compared to saline baseline. First, there was a significant correspondence between intensities which under the influence of d-amphetamine both elicited longer escape latencies and higher ICSS response rates. At first glance, it may seem that ICSS and escape are divergent classes of responses, but as this study demonstrates, both responses act in a similar manner under d-amphetamine. In short, the animal takes more stimulation at common intensities. This result could be explained by titration studies (Steiner, *et al.*, 1969; Keeseey, 1962, 1964; Steiner and D'Amato, 1964) in which selection of preferred parameters of stimulation was demonstrated by systematic variation of parameters and preference determined by various response contingencies including operant barpressing and escape behavior. The consistency of the results between these two classes of responses suggests that the animal is not escaping from the stimulation per se, but merely "titrating" the amount of stimulation by the only means possible in the escape response contingency, that is, terminating the stimulation by an appropriate response.

The second significant result was the shorter latency to escape under d-amphetamine for intensities that were well below ICSS thresholds. These shorter escape latencies under d-amphetamine might be explained by data (Fibiger, Fibiger and Zis, 1973; Taylor and Snyder, 1970) indicating that d-amphetamine increases nonspecific locomotor activity. However, the fact that at zero intensity, there was no difference in escape latencies between the d-amphetamine and saline conditions shows that d-amphetamine did not cause a nonspecific increase in the operant (treadle-press) rate. Steiner and D'Amato (1964) reported similar results for non-pharmacological manipulation of amygdaloid stimulation-induced escape; animals escaped faster for low intensities which did not sustain ICSS, than at higher intensities which did sustain ICSS behavior. Possible explanations of these results are that d-amphetamine increases the sensitivity of an aversive and/or an inhibitory system. The elucidation of these possible mechanisms is beyond the scope of this study, but merits further examination.

Summary

LC, MPG and hypothalamic sites reacted similarly in baseline and drug conditions in both ICSS and escape paradigms; no distinctions in functions

could be attributed to electrode locus. A significant correspondence was noted between intensities which, under the influence of d-amphetamine, both elicited longer escape latencies and higher ICSS response rates, suggesting that in both ICSS and escape paradigms, animals were titrating the duration of the stimulus train. At low intensities, escape latencies under d-amphetamine were shorter than under saline; these results can not be attributed to nonspecific drug effects.

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ATTACHMENT 4

COMPARISON OF BEHAVIORS ELICITED BY ELECTRICAL
BRAIN STIMULATION IN DORSAL BRAIN STEM
AND HYPOTHALAMUS OF RATS¹STEVEN J. ELLMAN,² ROBERT F. ACKERMANN, RICHARD J. BODNAR,
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Four brain-stimulation phenomena elicited from both dorsal brain stem and hypothalamic sites were investigated with the following results: (a) intracranial self-stimulation rate-intensity functions for dorsal brain stem and hypothalamic sites yielded very high (over 1,000 responses/15 min.) to moderate (201-500 responses/15 min.) response rates; (b) *d*-amphetamine produced higher response rates than either *l*-amphetamine or saline at both dorsal brain stem and hypothalamic sites, indicating that noradrenergic dorsal brain stem fibers (or cell bodies) support intracranial self-stimulation; (c) dorsal brain stem and hypothalamic self-stimulation sites reliably produced escape behavior; (d) simultaneous stimulation of dorsal brain stem and hypothalamic sites at subthreshold intensities interacted to produce suprathreshold response rates.

In recent years, several experiments (Crow, Spear, & Arbuthnott, 1972; Ellman, Ackermann, Farber, Mattiace, & Steiner, 1973; Farber, Steiner, & Ellman, 1971; Margules, 1969; Ritter & Stein, 1973; Routtenberg & Malsbury, 1969) were performed to determine if intracranial self-stimulation behavior could be elicited from areas in the posterior midbrain and pons (previously called dorsal brain stem by Ellman et al., 1973). Although there is agreement that areas in the dorsal brain stem will yield self-stimulation, there is disagreement about the rates of self-stimulation obtained from dorsal brain stem sites. Such disagreement may be due to the fact that few studies parametrically varied intensity values to determine the range of intracranial self-stimulation response rates obtained from dorsal brain stem areas. There is also disagreement about which structures mediate dorsal brain stem self-stimulation. This is not surprising, since the cross-sectional area of electrodes used in self-stimulation studies are frequently larger than their neuroanatomical targets. Clearly this allows for differing interpretations in specifying which fiber tracts or

nuclear groups have been stimulated. Hence, it is understandable that Routtenberg and Malsbury (1969), Ellman et al. (1973), Crow et al. (1972), and Ritter and Stein (1973) could all have electrode placements in approximately the same area, yet attribute self-stimulation behavior to different structures.

For example, Routtenberg and Malsbury (1969) maintain that the brachium conjunctivum is a structure that supports self-stimulation. They have presented the hypothesis that the extrapyramidal system (with the red nucleus as the central component) mediates self-stimulation in dorsal brain stem areas; so it is not surprising that they concluded that the brachium conjunctivum is a self-stimulation site.

On the other hand, Crow et al. (1972) and Ellman and his co-workers (1973) attribute self-stimulation behavior in this area to the locus coeruleus. Thus, the neural systems which they postulate as subserving self-stimulation are those to which the locus coeruleus projects. Since Maeda and Shimizu (1972) and Olson and Fuxe (1972) have shown that the locus coeruleus has far-reaching projections, some of which ascend to, and terminate in, the hypothalamus, Ellman et al. (1973) suggested that self-stimulation in the area of the locus coeruleus is intimately related to hypothalamic self-stimulation.

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We do not think that the present paper conclusively demonstrates which structures mediate self-stimulation in dorsal brain stem areas. Rather, we compared behaviors elicited from dorsal brain stem and hypothalamic sites. Data from this type of comparison may be important in differentiating between hypotheses attempting to specify which structures mediate dorsal brain stem self-stimulation behavior.

Specifically, the present paper compares dorsal brain stem and hypothalamic sites with respect to 4 electrical brain stimulation phenomena: (a) intracranial self-stimulation rate-intensity functions; (b) the effects of *d*- and *l*-amphetamine upon intracranial self-stimulation rate-intensity functions; (c) escape latency-intensity functions; and (d) simultaneous stimulation of hypothalamic and dorsal brain stem self-stimulation sites.

EXPERIMENT 1

In this experiment, intracranial self-stimulation response rates were ascertained in both dorsal brain stem and hypothalamic sites. Several (at least 5) intensities of current were employed at both sites to avoid the possibility that any single arbitrarily chosen intensity might be either too low or too high to sustain reliable self-stimulation behavior. Routtenberg and Malsbury (1969) maintained that highest rates of self-stimulation are found only in ventral anterior midbrain sites. We hypothesized that very high rates in posterior midbrain and anterior pons sites were not obtained by Routtenberg and Malsbury because subjects were not tested over a wide range of current intensities. We felt that a more complete picture of self-stimulation in the dorsal brain stem could be obtained if it were compared with hypothalamic self-stimulation over at least 5 intensities.

Method

Eighteen male albino Holtzman Sprague-Dawley rats (375-500 gm.) were anesthetized with Equi-Thesin (Jensen) and stereotaxically (Kopf) implanted with 2 bipolar electrodes, each electrode made of 2 intertwined strands of stainless steel wire (.3-mm.-diam.) completely insulated except at the tips. Electrodes were fastened to the skull with dental cement, which was anchored to the

skull by means of 2 stainless steel screws. In each subject, electrodes were aimed at the hypothalamus and the dorsal brain stem in the area of the locus coeruleus. The incisor bar was set at -5 mm.

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. Dorsal brain stem coordinates were: (a) 1.5-2.0 mm. posterior to lambda, (b) 1.0 mm. lateral to a line extrapolated from the sagittal suture, and (c) 7.0 mm. from the top of the skull at the intersection of coordinates a and b.

After recovery from surgery (10 days), each subject was placed in an operant conditioning chamber and shaped to bar press by the method of successive approximations. The chamber, constructed of Plexiglas and stainless steel, was 20 × 20 × 22 cm. A 4 × 2-cm. retractable lever was located 4 cm. above the grid floor on 1 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 passed through one of the subject's bipolar electrodes on a continuous reinforcement schedule. Stimulation consisted of biphasic sinusoidal 60-Hz. waves. Train duration was held constant at .25 sec. Current intensity was varied between trials according to the demands of the experiment by placing a megohm resistor in series with the subject. 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 cathode ray oscilloscope.

Subjects were shaped for a minimum of 15 successive daily sessions at a variety of current intensities (5-200 μ a.) in each site. If, after 15 daily sessions, a rat did not self-stimulate, it was discarded. Animals which self-stimulated continued in testing on the following schedule: Each day, each rat was allowed to self-stimulate throughout a 42-min. session that was divided into 6 7-min. periods; changes in current intensity occurred during a 1-min. time out, which separated each successive 7-min. period. During the first 7-min. period, stimulation intensity was sufficiently low so that the animal's mean response rate over the last 5 min. of the period was below an arbitrarily defined response threshold of 10 responses/min. The second, third, fourth, and fifth intensities, presented in ascending order, sustained self-stimulation behavior at rates that approached or reached highest responding. We always attempted to ascertain peak response rates for each subject; at times, it was found that the fifth intensity resulted in lower than peak response rates. Generally, the decrement in response rates was not due to overt convulsions. Only 2 rats convulsed at the fifth intensity, and only hypothalamic stimulation produced convulsions. The animals never

TABLE I
COMPARISON OF DORSAL BRAIN STEM (DB) AND HYPOTHALAMIC (HYP) SELF-STIMULATION

Subject	Site	Peak rate (in responses/ min.)	Peak intensity (in μ a.)	Threshold intensity (in μ a.)	Routtenberg & Malsbury rating ^a	Electrode site ^b
65A	DB	20	85	57	moderate	locus coeruleus
S9C	DB	28	71	42	moderate	sub-coeruleus
2E	DB	40-45	85-127	57	high	midbrain medial longitudinal fasciculus at level of ventral tegmental nucleus
3E	HYP	200+	35-71	35	very high	lateral hypothalamus
	DB	365	18	11	very high	dorsal noradrenergic bundle
5E	HYP	200+	148-177	49	very high	lateral hypothalamus
	DB	136	71	—	very high	locus coeruleus
7E	DB	29	35	28	moderate	locus coeruleus
	HYP	165	141	28	very high	fortix
9E	DB	15	27-32	23	moderate	ventral tegmental nucleus
	HYP	170	177	64	very high	fornix
13E	DB	15	99-113	85	moderate	dorsal noradrenergic bundle
	HYP	45	156-177	57	high	fornix
14E	DB	22	212	156	moderate	dorsal noradrenergic bundle
14E	HYP	112	85	35	very high	lateral hypothalamus
17E	DB	23	85-106	85	moderate	anterior locus coeruleus
	HYP	136	99	71	very high	lateral hypothalamus
18E	DB	100	35-39	32	very high	dorsal noradrenergic bundle
	HYP ^c	60	38	14	high	fornix
28E	DB	100	28	14	very high	anterior locus coeruleus
	HYP ^c	65	120	35	high	zona incerta
54E	DB	35	84	50	high	dorsal noradrenergic bundle
23F	DB	30	98	64	moderate	dorsal noradrenergic bundle

^a Routtenberg and Malsbury classification system: Very high: >1,000 responses/15 min.; high: 501-1,000 responses/15 min.; moderate: 201-500 responses/15 min.; low: 51-200 responses/15 min.

^b Dorsal noradrenergic bundle refers to those fibers present in the posterior midbrain that emanate from the locus coeruleus and travel forward ventrally.

^c Animal convulsed.

convulsed when self-stimulation behavior was elicited from the dorsal brain stem electrode. Responses during the final 7-min. period resulted in no stimulation (extinction). Rate-intensity functions, averaged over 5 days, were determined for each electrode site in each rat.

After completion of all experiments, each rat was injected with an overdose of Equi-Thesin and perfused with .9% normal saline solution, followed by 10% formalin solution. Frozen serial sections were stained with luxol fast blue and cresyl violet (Klüver-Barrera procedure, 1953), and electrode locus determined by microscopic examination of the sections.

Results

Nine animals self-stimulated at both hypothalamic and dorsal brain stem sites, and 5 others self-stimulated only in a dorsal brain stem site. Table I shows each rat's highest response rate, the current intensity for this rate, and the arbitrarily defined threshold intensity. A response rate classification sys-

tem used by Routtenberg and Malsbury (1969) is included in Table I for direct comparison of our data with their results. Table I also lists the neuroanatomical locations of all electrode tips, while Figure 1 shows

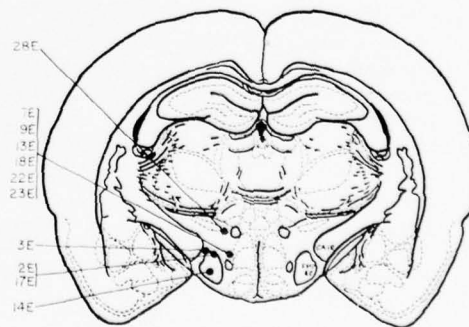


FIGURE 1. Location of electrode tips in hypothalamic sites.

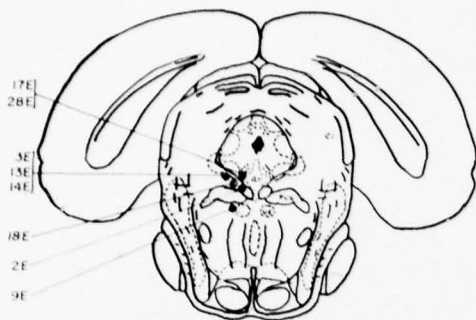


FIGURE 2. Location of electrode tips in posterior midbrain sites.

the neuroanatomical location for hypothalamic sites, and Figures 2 and 3 for dorsal brain stem sites (dots and triangles indicate electrode tips). The dots on all figures indicate animals that were self-stimulators; on Figure 3, the two triangles indicate rats that did not display self-stimulation behavior and that will be referred to in Experiment 4.

In general, self-stimulation rate-intensity functions for dorsal brain stem electrode placements were similar to rate-intensity functions generated from hypothalamic placements. Of the 9 animals which self-stimulated at both sites, 5 had lower threshold intensities in the hypothalamus and 4 had lower threshold intensities in the dorsal brain stem. The highest response rates generated from dorsal brain stem sites varied across subjects from a low of 22 responses/min to a high of 365 responses/min. (mean responses for a 5-min. period). This range encompasses the range of response rates generated from hypothalamic sites.

On the average, response rates were higher for hypothalamic than for dorsal brain stem placements. However, contrary to the results of Routtenberg and Malsbury (1969) who found no posterior (dorsal brain stem) placements which elicited very high (over 1,000 responses/15 min.) rates, 4 rats (3E, 5E, 15E, and 28E) self-stimulated at very high rates for dorsal brain stem stimulation (Table 1). The dorsal brain stem electrode of 1 animal (3E) elicited the highest sustained response rate ever recorded in this laboratory, 365 responses/min. over a 5-min. period. Two dorsal brain stem place-

ments (Rats 2E, 54E) elicited a high (501-1,000 responses/15 min.) rate; the remainder of dorsal brain stem placements elicited moderate (201-500 responses/15 min.) response rates. If the animal self-stimulated at all, it did so at least at moderate rates at its optimum intensity. Figure 4 shows representative rate-intensity functions for 3 rats in their hypothalamic and dorsal brain stem sites.

Discussion

Dorsal brain stem electrode placements can yield not only reliable intracranial self-stimulation behavior, but self-stimulation comparable to the highest rates elicited by anteriorly placed electrodes. This is contrary to Routtenberg and Malsbury (1969), who contend that highest rates of self-stimulation are found only in ventral anterior midbrain sites. We believe that our electrodes elicited higher response rates than theirs because our range of intensities invariably included that intensity which elicited peak rates at each site, while in Routtenberg and Malsbury's procedure, peak response rates were elicited only if their single, arbitrarily chosen current intensity happened to be that site's optimum self-stimulation intensity.

It is well-known that hypothalamic electrical brain stimulation frequently results in convulsions (Reid, Gibson, Gledhill, & Porter, 1964). In our experience, electrical brain stimulation through dorsal brain stem electrodes has never resulted in a convulsion.

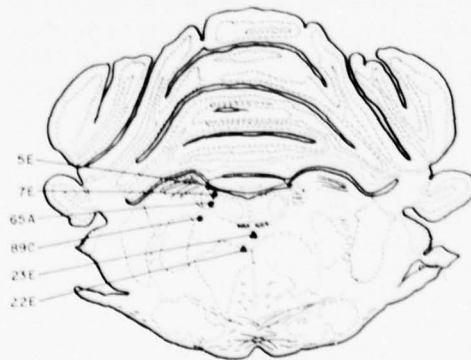


FIGURE 3. Location of electrode tips in anterior pons.

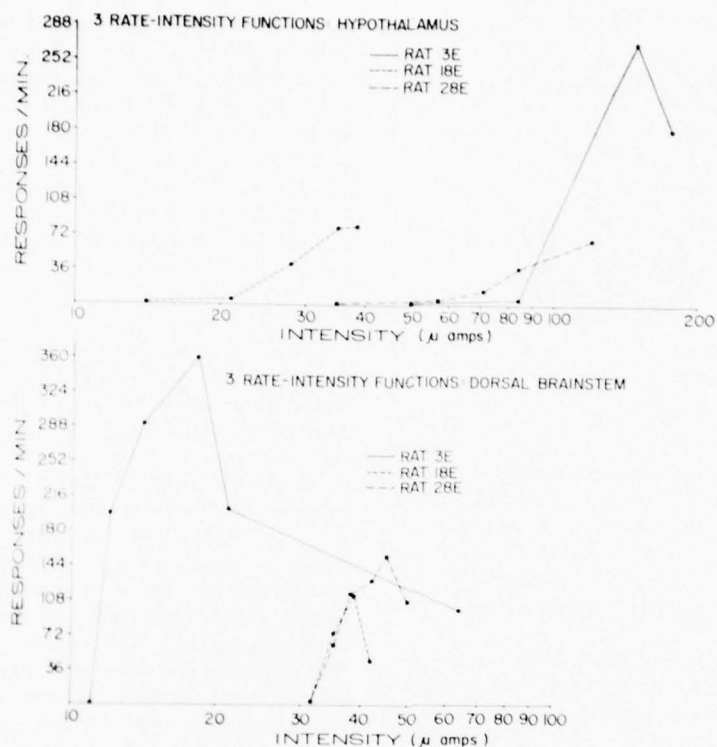


FIGURE 4. Rate-intensity functions for hypothalamic and dorsal brain stem sites in 3 representative animals.

Even in the 2 cases of multiple-implant animals in which hypothalamic electrodes elicited convulsions, dorsal brain stem electrical brain stimulation did not, even at relatively high intensities (200 μ a.). Furthermore, multiple-implant animals consistently demonstrated behavioral arousal when stimulated through their hypothalamic electrodes, but when stimulated through their dorsal brain stem electrodes, an absence of arousal was noted.

EXPERIMENT 2

Because it is our contention that the locus coeruleus or fibers emanating from the locus coeruleus account for at least some of the self-stimulation obtained in Experiment 1, we would predict that drugs known to affect noradrenergic areas (Stein, 1962, 1964) should have a significant effect on dorsal brain stem self-stimulation. To test this hypothesis, we compared the rats' responses

under *d*- vs. *l*-amphetamine. Phillips and Fibiger (1973) demonstrated that the hypothalamus, a noradrenergic self-stimulation site, shows a significantly larger effect with *d*- as opposed to *l*-amphetamine, whereas a dopaminergic self-stimulation area (substantia nigra) shows approximately equal effects with *d*- and *l*-amphetamine. We compared hypothalamic and dorsal brain stem sites within the same subject to demonstrate the noradrenergic nature of the dorsal brain stem site.

Method

Six rats (9E, 13E, 14E, 18E, 54E, 23F) were continued from Experiment 1. As described in that Experiment, baseline rate-intensity functions were determined for each electrode site in each rat. Thereafter, the animals were allowed to self-stimulate throughout daily 77-min. sessions which were divided into 11 7-min. periods. As before, intensity changes occurred during 1-min. time outs between successive 7-min. periods. However, in

this procedure, the rate-intensity functions of each rat's 2 electrode sites were interdigitated; i.e., delivery of the current was alternated between hypothalamus and dorsal brain stem in an ABBAABBAAB sequence, beginning with the dorsal brain stem electrode site.

Thus, throughout the first 7-min. period, responses resulted in stimulation through the dorsal brain stem electrode site. Throughout the following 2 7-min. periods, the current was delivered through the hypothalamic site. Responses in the next 2 7-min. periods led to dorsal brain stem stimulation, and so on until 5 intensities were run in ascending order in each site. The purpose of this procedure was to insure that both electrode sites would be tested equally in the same time interval and under the same conditions. Responses during the final 7 min. period resulted in no stimulation (extinction).

For each drug, data were averaged over 3 successive 3-day sequences. The drug was administered on the second day of each sequence; Day 1 and Day 3 served as pre- and postdrug controls. The *d*-amphetamine was administered on drug days of the first 9-day sequence, *l*-amphetamine in the second 9-day sequence. Finally, a single *d*-amphetamine sequence was run to determine if any changes in drug reaction had occurred since beginning the experiment.

On drug days, the rat was injected intraperitoneally with either *d*- or *l*-amphetamine (dosage: 1 mg/kg of body weight; concentration: 1 mg/ml .9% normal saline solution) 30 min. before the self-stimulation session. On pre- and postdrug control days, only the saline solution (concentration: 1 ml/kg of body weight .9% normal saline solution) was injected 30 min. before the self-stimulation session.

In addition, 2 rats were tested under the same procedure over a variety of drug doses (.5, 1.0, and 2.0 mg/kg) for both isomers.

Results

Figure 5 shows the proportional changes comparing rate-intensity curves from *d*- or *l*-amphetamine days to saline (baseline) days. These curves represent standardized scores for which response rates under saline are unity, and response rates under *d*- or *l*-amphetamine are expressed as a proportion of the saline rates. The *d*-amphetamine effect at every intensity at both hypothalamic and dorsal brain stem sites for each animal is significantly larger than the *l*-amphetamine effect. Both drugs show some elevation over the saline condition, but the *l*-amphetamine effect does not differ significantly from the saline condition (sign test, $p > .05$). The *d*-amphetamine effect is statistically significant, and differs from

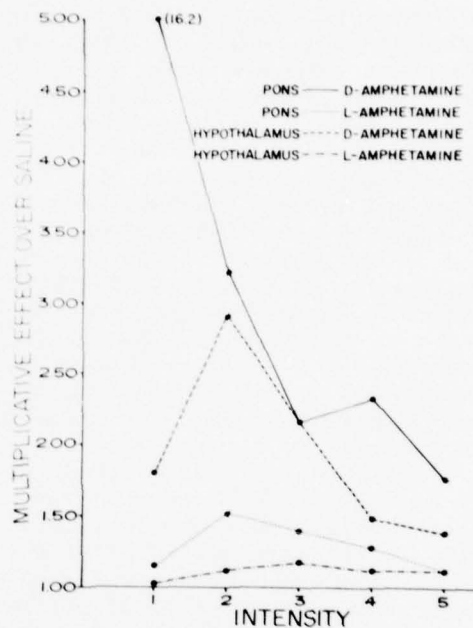


FIGURE 5. Proportional comparison of *d*- and *l*-amphetamine upon rate-intensity functions in hypothalamic and dorsal brain stem sites.

both saline and *l*-amphetamine conditions (sign tests, $p < .05$).

Figure 6 shows the proportional changes for dorsal brain stem sites comparing rate-intensity curves when different doses of *d*- and *l*-amphetamine were utilized. In Figure 6 one can see that rats have comparable effects at 2.0 mg/kg of *l*-amphetamine when these effects are compared with .5 mg/kg of *d*-amphetamine, indicating an approximate 4:1 ratio of *l*-amphetamine to *d*-amphetamine for this site.

Figure 7 shows the effects of the three doses of *d*- and *l*-amphetamine on the first intensity illustrated in Figure 6. Figure 7 also demonstrates an approximate 4:1 ratio of *l*-amphetamine to *d*-amphetamine. One can see this by comparing the effects of 2 mg/kg of *l*-amphetamine with .5 mg/kg of *d*-amphetamine.

Discussion

Previous research has indicated that a main action of *d*-amphetamine is to facilitate noradrenergic systems (Phillips & Fibiger,

1973; Stein, 1962, 1964; Steiner & Stokley, 1973). Dahlstrom and Fuxe (1964) and Ungerstedt (1971) have shown that there are noradrenergic locus coeruleus cell bodies and noradrenergic fibers emanating from the locus coeruleus with terminals in the hypothalamus. In a recent study, Anlezark, Crow, and Greenway (1973) demonstrated that bilateral lesions of the locus coeruleus lead to large decrements in brain norepinephrine. Our results are in accord with the hypothesis that noradrenergic fibers or cell bodies in the dorsal brain stem support self-stimulation in this area, and that these noradrenergic structures account for the large *d*-amphetamine effect in the present experiment.

Obviously, there are other explanations for our data that could involve nonnoradrenergic dorsal brain stem sites. It is possible that nonnoradrenergic fibers are stimulated which terminate in distant noradrenergic areas which, in turn, are responsible for the *d*-amphetamine effect. We can only comment that Phillips and Fibiger (1973) found specific pharmacological effects dependent on electrode site. Even under the assump-

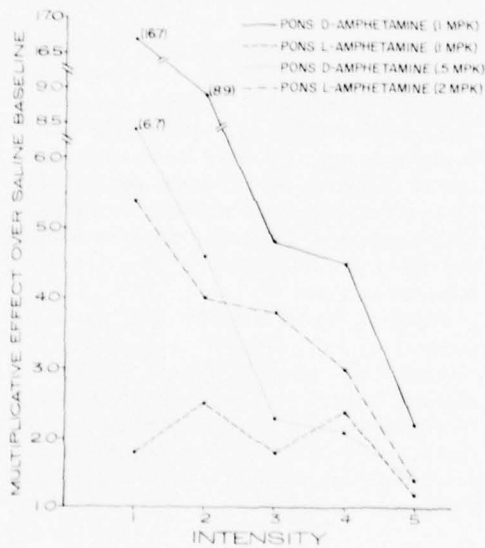


FIGURE 6. Proportional comparison of varying doses (.25, .5, 1.0, 2.0 milligrams per kilogram [MPK]) of *d*- and *l*-amphetamine upon rate-intensity functions in dorsal brain stem sites.

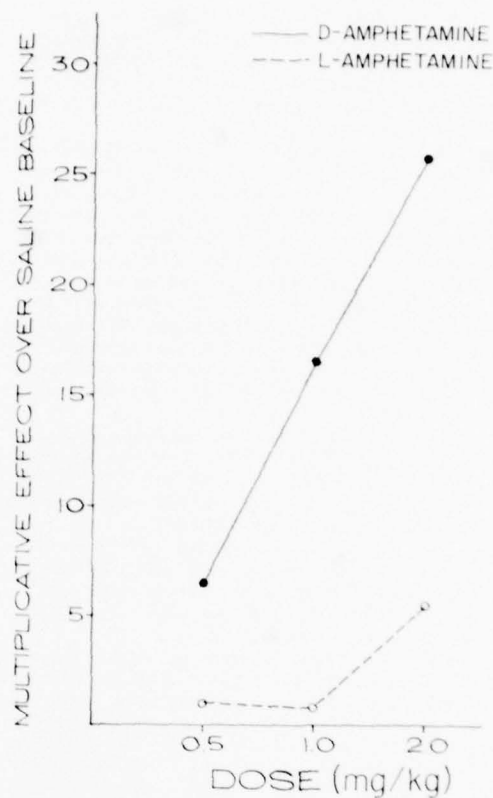


FIGURE 7. Proportional comparison of varying dose of *d*- and *l*-amphetamine upon threshold intensity in dorsal brain stem sites.

tion that the stimulated dorsal brain stem areas are nonnoradrenergic, we still conclude that the mechanism responsible for intracranial self-stimulation in this experiment is noradrenergic. Therefore, it seems difficult to explain our data on the basis of Routtenberg and Malsbury's "extrapyramidal" hypothesis, as the extrapyramidal motor system has been shown to be dopaminergic (Ungerstedt, 1971). Our contention is that stimulation of the noradrenergic cells and tracts surrounding our electrodes account for our results.

EXPERIMENT 3

In a previous experiment, Steiner, Bodnar, Ackermann, and Ellman (1973) demonstrated that escape behavior could be obtained from dorsal brain stem sites that also

elicit intracranial self-stimulation. In the present experiment, we systematically manipulated intensity (microamperes) to obtain escape latency-intensity functions from stimulation of dorsal brain stem sites in order to compare them with hypothalamic latency-intensity functions. Other investigators (Beer & Steiner, 1965; Bower & Miller, 1958) have demonstrated that escape latency from hypothalamic electrical brain stimulation varies as a function of current intensity. Thus, escape behavior would provide another useful comparison of dorsal brain stem and hypothalamic areas.

Method

Four of the animals (Rats 9E, 13E, 14E, and 15E) that participated in Experiments 1 and 2 were continued in Experiment 3. The rats were trained to escape from passive electrical brain stimulation in an operant chamber similar in size to the chamber used in Experiments 1 and 2, with the following exception: in place of a retractable lever, a treadle (4×10 cm.) was permanently inserted into the chamber on the wall opposite from the wall that had contained the retractable lever. The onset of a stream of electrical trains (60 cycles/sec. sine, .5-sec. duration, .5-sec. intertrain interval) delivered through 1 of the rat's 2 electrode sites marked the beginning of the first trial. Stimulation continued until either the rat depressed the treadle or 101 trains had been delivered. Either contingency constituted a single trial. After a 15-sec. intertrial interval, the stimulation was automatically reinitiated and the procedure repeated successively until a block of 10 trials was run. The animals were trained to make a treadle-press response to terminate stimulation, and all 4 rats learned the appropriate treadle-press response for stimulation in both dorsal brain stem and hypothalamic sites.

The rats were then tested at relatively high and low intensities (5-210 μ a.) to find ranges of escape latencies. For each site, 6 intensities, presented in descending order, were chosen to determine a latency-intensity function. At the highest intensity, the animals had to overcome stimulus-induced motor involvement in order to make the appropriate response; this was the first stimulus presented in the protocol. The next 3 lower intensities chosen were those from which the rat could escape reliably; that is, they were intensities which consistently elicited escape behavior but did not evoke involuntary movement. The fifth intensity was one to which the rat would give variable response latencies across days, whereas the sixth intensity was deliberately chosen to be below threshold. Responding for the sixth intensity was not noticeably different than at zero intensity.

The within-day procedure consisted of 80 trials;

the first 10 and the last 10 trials were control trials run at zero intensity. Rats were run at the 6 chosen intensities, 10 trials per intensity. Five consecutive days of data were taken for the dorsal brain stem site, followed by 5 consecutive days for the hypothalamus. Mean escape latencies were determined for each intensity at each site.

A final 3-day rate-intensity self-stimulation function was taken for each site for each subject to insure that self-stimulation behavior could still be elicited following the escape procedure.

The animals were either histologically prepared, as described in Experiment 1, or continued into Experiment 4.

Results

All subjects learned to escape reliably and differentially from the 5 highest intensities at each site (Figure 8). Both dorsal brain stem and hypothalamic functions approximated a straight line on a log-log plot in 7 (4 hypothalamic, 3 dorsal brain stem) out of 8 cases. This indicates that escape latency vs. stimulus intensity is a power function, suggesting that escape latency is a magnitude estimation of the intensity of the stimulus (Stevens, 1957). In the remaining case (a dorsal brain stem site), there was a gross motor artifact competing with appropriate escape responding at all intensities, which may explain why its latency-intensity function deviated from a power function plot.

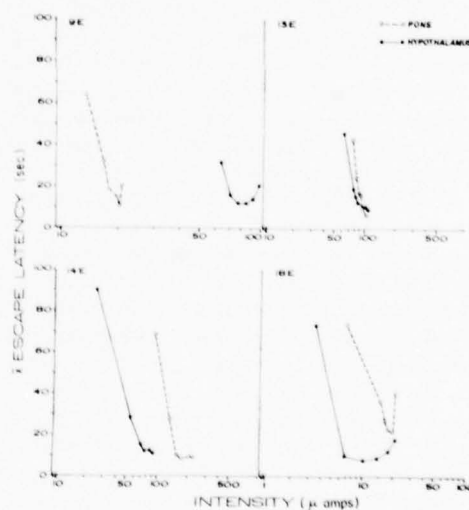


Figure 8. Escape latency intensity functions in hypothalamic and dorsal brain stem sites for four subjects.

Discussion

Bower and Miller (1958), Olds (1956, 1958, 1960), and Margules (1966) suggested separate neuronal systems for positive and for negative reinforcement. Olds (1960) and Margules (1966) maintained that they found discrete neuroanatomical areas in which electrical brain stimulation supports intracranial self-stimulation, but not escape behavior. However, Beer and Steiner (1965) and Steiner et al. (1973) have shown that any area in the hypothalamus that will yield intracranial self-stimulation will also yield escape behavior. In the present experiment, we found that all dorsal brain stem and hypothalamic self-stimulation sites that we tested yield escape behavior. In addition, the areas that Margules (1966) called pure positive areas are areas that were found to yield reliable escape behavior (Beer & Steiner, 1965; Steiner et al. 1973; the present study).

We believe that the conflicting findings are a function of relatively simple methodological differences between studies. In the studies that obtained escape behavior from all self-stimulation sites (Beer & Steiner, 1965; Steiner et al., 1973; the present study), the rats were actively shaped to emit escape responses. It is important to note that while initially shaping escape responses, low intensities (often below self-stimulation thresholds) are more effective in shaping escape responses than are high intensities. This may seem paradoxical since animals that have already learned to escape do so more quickly at high intensities than at low intensities. However, if one gives the rats high intensities early in training, they tend to become immobilized and little, if any, reliable behavior can be obtained. In Margules' (1966) study, no shaping was performed, and relatively high intensities were used early in the experimental run. Based on our experience, the surprising results from Margules' (1966) study is that any reliable escape behavior was obtained using this procedure. The "pure" positive reinforcement area, to our knowledge, remains undiscovered.

EXPERIMENT 4

Ungerleider and Coons (1970) stated that response enhancement between contralateral hypothalamic sites depends on the ability of each electrode site to support self-stimulation behavior; response enhancement does not occur when one electrode site supports self-stimulation but the other does not. Maeda and Shimizu (1972), using histochemical techniques, demonstrated a common neurohumoral transmitter substance (noradrenalin) and neuroanatomical connections between the hypothalamus and locus coeruleus. This experiment attempts to demonstrate a functional relationship between dorsal brain stem and hypothalamic intracranial self-stimulation sites. Specifically, we attempted to compare stimulation of dorsal brain stem and hypothalamic sites alone with the effects of simultaneous stimulation of these 2 sites. Our comparisons involved both response threshold and response rates.

Method

Five Holtzman Sprague-Dawley rats, 3 drawn from previous experiments, were used in this experiment. All 5 animals demonstrated sustained, reliable self-stimulation behavior from their hypothalamic electrodes. Three of the 5 subjects also demonstrated self-stimulation behavior for dorsal brain stem electrical brain stimulation (double pressers), while 2 subjects did not demonstrate self-stimulation from their dorsal brain stem electrodes, despite at least 15 shaping sessions (controls).

A rate-intensity function, as described in Experiment 1, was obtained for each electrode site that supported self-stimulation. Every rate-intensity function included a current intensity which yielded response rates below an arbitrarily defined threshold rate (a mean of 10 responses/min. over a 5 min. period). Upon determination of threshold intensities at each electrode site, the double pressers began the following schedule which is represented in Table 2.

Every day, each double presser had an opportunity to lever press during an 80 min. session which was divided into 10 min. periods. During 1 period, lever pressing led to delivery of subthreshold stimulation to 1 electrode site (single stimulation); during another period, lever pressing led to delivery of stimulation to the alternate electrode site (single stimulation); during a third period, lever pressing led to simultaneous delivery of subthreshold stimulation to both sites (simul-

taneous stimulation) at the same intensities as in the earlier periods. Each of these periods was separated by periods during which no stimulation was delivered (extinction); the purpose of the extinction periods was to reduce contrast effects between stimulation conditions. Data from the first 5 min. of each 10 min. period were disregarded; this also reduced contrast effects. The mean number of responses per minute over the last 5 min. of each period were calculated and compared.

The psychophysical method of limits was employed to determine current-intensity thresholds under the simultaneous condition. Current intensity was held constant at one site and varied at the alternate site. When simultaneous stimulation resulted in suprathreshold response rates, current intensity in the variable site was then reduced on successive days until simultaneous stimulation no longer supported suprathreshold response rates. This procedure was then reversed and current intensity at the variable electrode site was increased over successive days until suprathreshold response rates were once again attained during the simultaneous stimulation condition. Two alternate ascending and descending sequences were repeated, and an overall threshold was determined (Kling & Riggs, 1971).

This procedure was modified for the two rats that displayed self-stimulation behavior from only the hypothalamus. For these animals, hypothalamic rate-intensity functions were determined over a 5-day baseline period. Then, each intensity in the hypothalamic rate-intensity function was simultaneously paired with a constant dorsal brain stem intensity. The dorsal brain stem intensity was varied every fifth day until the hypothalamic rate-intensity functions had been paired with 5 intensities which included a range of intensities (35-175 μ a.) that normally supports self-stimulation. After the last dorsal brain stem current intensity had been tested, the baseline hypothalamic rate-intensity function was repeated over the next 5 days to see if it had changed. All rats were then histologically prepared as described in Experiment 1.

Results

Table 3 displays the subthreshold intensities for each rat's 2 sites, the rate elicited by a subthreshold intensity presented singly, the rate elicited by the subthreshold intensities presented simultaneously, and the peak hypothalamic rate as a comparison to the simultaneous stimulation rate. Threshold intensities (intensities eliciting fewer than 10 responses/min.) for simultaneous stimulation were 3-14 μ a. lower than threshold intensities for singly presented stimula-

TABLE 2
PROTOCOL FOR SIMULTANEOUS STIMULATION
SHOWING DATA FOR RAT 3E

Site	Stimulation intensity (in μ a.)	Mean response rate (first 5 min.)	Mean response rate (second 5 min.)
Extinction	0	2.6	2.2
Dorsal brain stem	11	2.8	7.0
Extinction	0	1.2	.6
Hypothalamus	53	1.6	0.0
Extinction	0	0.0	0.0
Dorsal brain stem/ hypothalamus	11-53	228.4	111.6
Extinction	0	6.4	.4
Dorsal brain stem	11	70.4	0.0

tion. Table 3 and Figures 1-3 note histological verifications of the electrode placements.

For each double-presser rat, response rates under simultaneous stimulation conditions were substantially above threshold at intensity levels which, when presented singly, yielded only subthreshold response rates. Figure 9 displays data for the determination of a simultaneous stimulation threshold for one such animal (Rat 3E). The 2 other double-presser rats replicated the results shown in all essential respects. All 3 subjects' simultaneous stimulation rates (elicited by intensities that were subthreshold when presented singly) were similar to maximum rates produced at any intensity at any site.

The 2 control subjects showed no evidence that simultaneous stimulation enhanced response rates in any way at any intensity. Figure 9 depicts Control Rat 22E contrasted with the effect of a double-presser (Rat 3E).

Discussion

We interpret these data to be evidence for neurophysiological interaction between neuroanatomically distinct and distant loci. Our hypothesis is that the behavioral data obtained in this experiment were the result of neurophysiological interaction between locus coeruleus and hypothalamic structures. This hypothesis is in accord with histofluorescent data (Maeda & Shimizu, 1972; Olson & Fuxe, 1972). However, at this point we cannot rule out the possibility that other neuroanatomical structures are involved in

TABLE 3
EFFECT OF SIMULTANEOUS STIMULATION IN HYPOTHALAMIC (HYP) AND DORSAL BRAIN STEM (DB) SITES
ON RESPONSE THRESHOLDS

Subject	HYP threshold intensity (in μ A.)	HYP rate (responses/min.)	HYP electrode localization	DB threshold intensity (in μ A.)	DB rate (responses/min.)	DB electrode localization	Peak HYP rate (responses/min.)	Simultaneous stimulation rate (responses/min.)
Double Pressers								
3E	53	0.0	lateral hypothalamus	11	0.0	dorsal noradrenergic bundle	200+	196.0
7E	23	1.0	fornix	141	1.5	locus coeruleus	165	132.8
9E	64	8.0	fornix	21	8.8	ventral tegmental nucleus	170	109.0
Controls								
22E	14	3.4	lateral hypothalamus	177	1.4	pontine reticular formation	219	6.0
23E	14	2.5	fornix	141	0.0	pontine raphe	127	8.8
				35	0.0			2

Note. Double pressers self-stimulate in both electrode sites; controls self-stimulate only in the hypothalamus.

this interaction, nor can we presently rule out the more unlikely possibility of some type of reinforcement summation that is relatively independent of neural pathways.

The data for the control animals demonstrate that simultaneous stimulation of a self-stimulation and a nonself-stimulation site does not result in increased response rates, allowing us to conclude that spread of

electrical current from dorsal brain stem to hypothalamic structures cannot account for the strong interaction found in double-presser subjects.

GENERAL DISCUSSION

The present group of experiments explored some of the types of behavior that can be elicited from dorsal brain stem self-stimulation sites. It has been demonstrated that animals with dorsal brain stem electrodes can: (a) self-stimulate at moderate to very high response rates; (b) have the type of differential effect to *d*- and *l*-amphetamine that one would expect from a noradrenergic site; (c) show escape latency functions that are similar to hypothalamic escape latency functions. In addition, simultaneous dorsal brain stem and hypothalamic self-stimulation lowers self-stimulation thresholds and raises response rates at least at relatively low intensities. The major difference noted between these self-stimulation sites is that behavioral arousal characterizes hypothalamic self-stimulation but not dorsal brain stem self-stimulation. (This is usually, but not invariably, the case.)

The data presented are in accord with the

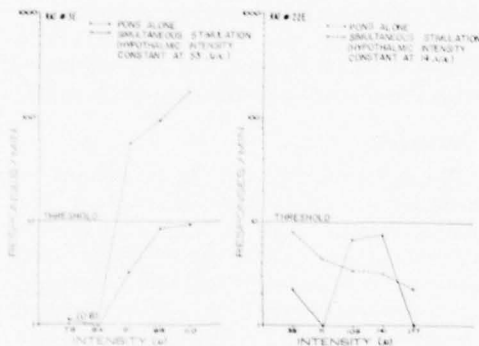


FIGURE 9. Comparison of simultaneous stimulation interaction between the dorsal brain stem and hypothalamic self-stimulation sites (Rat 3E) and simultaneous stimulation interaction between a hypothalamic self-stimulation site and a dorsal brain stem nonself-stimulation site (Rat 22E).

hypothesis that the locus coeruleus mediates dorsal brain stem self-stimulation. The fact that one can obtain a large increase in dorsal brain stem self-stimulation rates with *d*-amphetamine but not with *l*-amphetamine supports the notion that noradrenergic fibers or cell bodies are responsible for self-stimulation in this area. The fact that one can obtain interactions between dorsal brain stem and hypothalamic self-stimulation is not conclusive support of the argument that the locus coeruleus is a self-stimulation site, but it is congruent with the claim that the locus coeruleus has connections with the hypothalamus (Loizou, 1969; Maeda & Shimizu, 1972; Olson & Fuxe, 1972; Ungerstedt, 1971).

A last point that is of particular interest to the authors is the relationship of the present studies to rapid eye movement (REM) sleep research. Jouvet (1969) and Henley and Morrison (1969) have shown that the locus coeruleus is involved in triggering and maintaining tonic aspects of REM sleep. The results (Steiner & Ellman, 1972; Spielman, Mattiace, Steiner, & Ellman, 1973) showing a reciprocal relationship between intracranial self-stimulation and REM sleep is converging evidence for the contention that the locus coeruleus is an intracranial self-stimulation site.

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ATTACHMENT 5

BEHAVIORAL INTERACTIONS AMONG RAT BRAINSTEM AND HYPOTHALAMIC
SELF-STIMULATION SITES¹

by

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RUNNING HEAD: Self-Stimulation Interactions

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ABSTRACT

ACKERMANN, R. F., S. J. ELLMAN, R. J. BODNAR, F. JACKLER and S. S. STEINER. Behavioral interactions among rat brainstem and hypothalamic self-stimulation sites. PHYSIOL. BEHAV. Rats, with electrodes in hypothalamus, and one of: locus coeruleus, periaqueductal gray, substantia nigra or contralateral hypothalamus, were tested for intracranial self-stimulation (ICSS). If both of an animal's two electrode sites supported ICSS, simultaneous stimulation response rates, elicited at near-threshold intensities, were significantly greater than the sum of the rates elicited by single-site stimulation at the same intensities, indicating neurophysiological interaction between the ICSS sites. If only one site supported ICSS, no interaction took place. The magnitude of interaction between ICSS sites was estimated by comparing the current intensity necessary to support ICSS responding under single stimulation conditions to that under simultaneous stimulation conditions. Obtained interaction estimates were site-dependent; if both of an animal's ICSS sites were sensitive to d-, but not l-amphetamine, simultaneous stimulation threshold reductions were relatively small. If one site was sensitive only to d-amphetamine, but the other sensitive to both isomers, simultaneous stimulation threshold reductions were significantly larger. These results suggest the existence of two interacting ICSS systems.

intracranial self-stimulation

locus coeruleus

substantia nigra

hypothalamus

ventral tegmental area

periaqueductal central gray

d- and l-amphetamine

interactions

Studies of interaction among simultaneously stimulated intracranial self-stimulation (ICSS) sites have been few, and largely restricted to studies of inhibitory effects of stimulation at one site upon ICSS elicited from another site (24, 25, 33). Despite their obvious theoretical importance, studies of interaction between simultaneously stimulated ICSS sites have been rare, perhaps because of difficulties in interpreting their results (39). Such difficulties notwithstanding, Albino and Lucas (1) reported that when the ventral tegmentum and septum were stimulated nearly simultaneously, ICSS response rates were greater than the sum of response rates when either the tegmentum or septum was stimulated alone. From these data, they deduced that there was a physiological interaction between septal and tegmental ICSS sites. Subsequent ICSS studies demonstrated interactions between: (1) contralateral hypothalamic areas (14, 37); (2) amygdala and hypothalamus (35); (3) posterior hippocampus and hypothalamus (16); and (4) locus coeruleus and hypothalamus (10). Anatomical evidence (23) indicates that fibers of forebrain limbic structures have reciprocal connections among themselves and with such midbrain nuclei as the dorsal and ventral tegmental nuclei, the ventral tegmental area of Tsai, and ventral portions of the periaqueductal gray. Histofluorescence and pharmacological studies have shown that ascending catecholaminergic fibers innervate these forebrain limbic structures (9, 20, 26, 38). Subsequent studies have demonstrated that catecholamine-containing pontine and midbrain structures support ICSS; these structures include the locus coeruleus (LC) (8, 10, 12, 30), the dorsal noradrenergic

bundle (DNB) (10, 27), the ventral noradrenergic bundle (31), the periaqueductal noradrenergic bundle (5, 22), the mid-ventral periaqueductal gray (MV) (11, 19), and the substantia nigra (SN) (7, 32).

The present study, comprising two experiments, was an attempt to characterize interactions between hypothalamic (HYP) and tegmental self-stimulation sites. In Experiment 1, dorsal midbrain and pontine ICSS sites were surveyed for interaction with hypothalamic ICSS sites. In Experiment 2, an estimate of the magnitude of interactions was obtained. Finally, the data from Experiment 2 were analyzed together with previously published d- and l-amphetamine ICSS data (10, 11, 27, 28) in an attempt to determine if the magnitudes of interactions between sites corresponds with their sensitivities to the amphetamine isomers.

EXPERIMENT 1

Experiment 1 investigated interactions between the following combinations of electrode sites: (a) LC/HYP (or DNB/HYP); (b) MV/HYP; (c) SN/HYP; (d) HYP/contralateral HYP; and (e) LC/SN. Interactions between electrode sites were measured in terms of increased response rates when sites were stimulated simultaneously compared to the sum of the response rates when either site of a pair was stimulated alone.

Method

A. Subjects and Surgical Procedure

Twenty-six male Sprague-Dawley (Holtzman) albino rats (375-500 g) were anesthetized with Equithesin (Jensen; 1 ml/kg), placed in a Kopf stereotaxic instrument and implanted with either two or three bipolar electrodes (Plastic Products). Each bipolar electrode was made of two intertwined strands of stainless steel wire (0.3 mm diameter)

completely insulated except at the tips, which were 0.05-0.10 mm apart. After surgery, animals were housed individually and had access to food and water ad libitum.

Electrode implants, comprising two bipolar electrodes, were aimed at the following site combinations: (a) LC and HYP; (b) SN and HYP; (c) MV and HYP; (d) left and right HYP; (e) SN and LC. Implants comprising three bipolar electrodes were aimed at the following site combinations: (a) LC, MV and HYP; (b) LC, SN and HYP; and (c) LC, left HYP and right HYP.

With the incisor bar always set at -5 mm, HYP coordinates were 4.2-4.4 mm posterior to bregma, 1.5 mm lateral to the sagittal suture, and 8.7 mm from the top of the skull. LC coordinates were 1.5-2.0 mm posterior to lambda, 1.0 mm lateral to the sagittal suture, and 7.0 mm from the top of the skull. SN coordinates were 2.0 mm anterior to lambda, 2.0 mm lateral to the sagittal suture, and 8.2 mm from the top of the skull. MV coordinates were 0.6 mm anterior to lambda, 1.5 mm lateral to the sagittal suture, and 7.5 mm from the top of the skull and angled at 12° toward the mid-sagittal plane. In animals implanted with 3 bipolar electrodes, LC and HYP electrodes were ipsilateral to each other, while the SN electrodes were contralateral to the other two. MV electrodes entered the brain contralateral to HYP and LC electrodes, but their tips were located near the mid-sagittal plane.

B. Apparatus and Preliminary Testing

Ten days after surgery, each animal was shaped to lever-press in an operant conditioning chamber (20 × 20 × 23 cm), constructed of Plexiglas and stainless steel. A 2 × 4.5 cm retractable lever (Scientific Prototype) was located 4 cm above the grid floor on one wall of the

chamber. A force of 0.2 N was sufficient to depress the lever and constituted a response. Electromechanical and solid-state switching circuitry in an adjacent room monitored animals' behavior, recorded minute-by-minute response rates, and controlled contingencies of reinforcement. Reinforcements consisted of 250 msec trains of sinusoidal 60 Hz waves delivered on a continuous reinforcement schedule, and passed through either one or both of an animal's two bipolar electrodes, depending upon the experimental condition. Current intensity was held constant within trials and varied between trials according to the demands of the experiment. In addition, wave form and stimulus intensity were continuously monitored by observing on a differential input oscilloscope (Hewlett-Packard) the voltage drop across a 1,000 ohm resistor in series with the animal. Current fluctuations were maintained within one percent by placing a 100,000 ohm resistor in series with the animal.

Animals were shaped for a minimum of 15 successive daily sessions at a variety of current intensities (5-200 μ A) in each electrode site. Animals which did self-stimulate from at least one of their electrode sites were continued in testing on the following schedule in order to determine rate-intensity functions for each site. Rate-intensity functions were determined in daily 48-min sessions which were divided into six 7-min periods; changes in current intensity occurred during 1-min. timeouts between successive 7-min. periods. Data from the first 2 min. of each 7-min. period were disregarded. The mean response rates over the last 5 min. of each 7-min. period were recorded and constituted the dependent variable in all conditions. Current intensities utilized in determining rate-intensity functions were chosen in accordance with the following criteria: the first intensity was sufficiently low so

that the animal's mean response rate over the last 5 min. of the period was below an arbitrarily defined response threshold (usually 10 responses per min.). The fifth intensity sustained self-stimulation behavior at rates which approached or reached highest response rates. The second, third, and fourth intensities elicited response rates which were between threshold and peak intensity response rates. Responses during the final 7-min period resulted in no stimulation (extinction). Rate-intensity functions, averaged over 5 days, were determined for each electrode site in each animal.

C. Histology

After completion of the experiment, animals were overdosed with Equithesin (2 ml) and perfused with normal saline followed by 10% formalin. Serial frozen sections were cut at 40 microns thickness, stained with luxol fast blue and cresyl violet (17), and electrode locus was determined by comparison with the Konig and Klippel rat brain atlas (18).

Procedure

Animals demonstrating ICSS behavior in at least two electrode sites were termed double pressers; animals demonstrating ICSS 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 protocol, represented in Table 1 for a typical animal.

INSERT TABLE 1 ABOUT HERE

Every day, each double presser lever-pressed during a 70-min. session which was divided into ten 7-min. periods. As before, data from the last 5 min. of each 7-min. period were recorded.

During the first, third, fifth, and seventh 7-min. periods, responses resulted in no stimulation (extinction); the purpose of these interspersed extinction periods was to reduce any contrast effects between successive stimulation periods. During Period 2, responses resulted in delivery of subthreshold stimulation to one electrode site (single stimulation). During Period 4, responses resulted in delivery of subthreshold stimulation to the other electrode site (single stimulation). During Period 6, responses resulted in simultaneous delivery of subthreshold stimulation to both sites (simultaneous stimulation) at the same intensities as in Periods 2 and 4. During Period 8, responses resulted in delivery of subthreshold stimulation to the electrode site which had elicited the higher response rate during the earlier single stimulation periods. This condition was included to insure that the response rate elicited under the simultaneous stimulation condition was due to interaction between the two sites and not due to any spontaneous increase in responding by one site alone. During Periods 9 and 10, responses resulted in delivery of a stimulation intensity which elicited peak response rates for each respective electrode site. Peak response rates for each site were monitored in order to discern any shifts in rate-intensity functions, and also to maintain behavior in the face of the many extinction periods comprised by this paradigm. This procedure was repeated daily over five days for each animal.

INSERT TABLE 2 ABOUT HERE

The procedure was modified for control animals because they did not self-stimulate for any current intensity at one of their electrode sites. Table 2 presents the protocol for a typical control animal. Each control animal was tested in a 56-min. session divided into eight 7-min. periods. During Periods 1, 3, and 5, responses resulted in no stimulation (extinction). During Periods 2 and 6, responses resulted in delivery of stimulation to the animal's only ICSS site at an intensity which elicited 10-25 responses per min. (single stimulation). During Period 4, responses resulted in simultaneous stimulation comprising: (1) the same intensity as in Period 2 to the ICSS site, and (2) one of a variety of current intensities (5-200 uA) to the site which did not support ICSS. During Period 7, responses resulted in delivery of the same stimulation intensity to the non-ICSS site as in Period 4; this insured that this non-ICSS site still did not support ICSS following the simultaneous stimulation condition. During Period 8, responses resulted in delivery to the ICSS site of the stimulation intensity which elicited peak response rates. This procedure was repeated over approximately 40 days for each control animal; the current intensity delivered to the non-ICSS site was changed from day to day.

Results

Twenty-six animals completed Experiment 1; in 25 animals one pair of electrode sites was tested and in one animal two different electrode site combinations were tested. In 22 of the 27 electrode site combinations, both electrode sites supported ICSS. In the remaining 5 electrode site combinations, only one of the two electrodes supported ICSS. Table 3 summarizes the data collected in Experiment 1.

INSERT TABLE 3 ABOUT HERE

The data for each site combination were analyzed separately; for each of the 22 double presser combinations, the response rate under simultaneous stimulation was significantly higher (correlated difference score t-tests, $p \leq .05$) than the sum of the response rates of the two single stimulation conditions. These results were true regardless of the locus of the two ICSS electrodes (Table 3). By contrast, for each of the five control combinations, there was no significant difference (correlated difference score t-tests, $p > .05$) between the simultaneous stimulation condition and the single stimulation condition of the ICSS site regardless of the intensity delivered to the non-ICSS site; if one of the electrode sites was neutral, then simultaneous stimulation of both sites did not result in response rate enhancement. The control group included both tegmental and HYP non-ICSS placements, including one near the HYP (Rat 43F), one near the MV (Rat 8F), one near the SN (Rat 79F), and two near the LC (Rats 22E and 23E).

INSERT FIGURE 1 ABOUT HERE

Figure 1 illustrates the differential effects of simultaneous stimulation in a double presser (74E) as compared to a control animal (79F). Rat 74E, shown on the left, self-stimulated from both electrode sites, while Rat 79F, shown on the right, self-stimulated from the HYP but not from the tegmentum. Rat 74E, the double presser, shows clear enhancement in response rate in the simultaneous stimulation condition as compared to the response rates elicited in the single stimulation

conditions. It is equally clear that no matter which current intensity was employed at the non-ICSS site, the control animal, 79F, did not show enhancement in response rate during the simultaneous stimulation condition.

EXPERIMENT 2

Experiment 1 demonstrated that numerous tegmental ICSS sites interact with HYP ICSS sites at subthreshold intensities to support suprathreshold levels of ICSS. It could be argued that response rates under simultaneous stimulation are not an adequate measure of the magnitude of interaction between two ICSS sites because several studies (39, 40) have demonstrated that stimulation eliciting low response rates is, under some circumstances, chosen by rats over stimulation eliciting higher response rates. Also, different ICSS sites within the same animal can have disparate peak response rates, making it difficult to compare: (a) across sites within a given animal, and/or (b) across animals at the same sites. Therefore, a different measure of the rewarding properties of the interaction, independent of response rate, was necessary. The measure chosen was the reduction, under simultaneous stimulation, in the current intensity required to support the threshold response rate (10 responses/min.).

Procedure

Seventeen double pressers were continued from Experiment 1 into Experiment 2. The subthreshold intensities which would support simultaneous stimulation interactions were determined for each site. Then a modified psychophysical method of limits was employed to determine stimulation intensity thresholds under the simultaneous stimulation condition;

Table 4 illustrates this protocol in a representative animal. With one exception (7E) current intensity was held at a constant, subthreshold, intensity at the HYP (constant site) and systematically varied at the tegmental site (varied site). At the initial current intensities simultaneous stimulation resulted in suprathreshold response rates (>10 responses/min.). Then, current intensity at the varied site was reduced over successive days in steps of 1.4 μ A per day until simultaneous stimulation failed to support suprathreshold response rates. When this occurred, the same varied site intensity was repeated on the following day. If simultaneous stimulation supported suprathreshold response rates on the second day, the schedule of daily 1.4 μ A decrements was resumed on the following day until simultaneous stimulation ultimately failed to support suprathreshold responding on two successive days. This modification of the method of limits insured that a random failure to respond on any given day would not confound the threshold determination. Upon an animal's failure to respond at suprathreshold rates under the simultaneous stimulation condition on two successive days, the procedure was reversed and current intensity at the varied site was increased over successive days in steps of 1.4 μ A per day until suprathreshold response rates were once again attained on two successive days in the simultaneous stimulation condition, and then increased further until suprathreshold response rates were attained on two successive days at the varied site in the single stimulation condition. For each varied site, two alternating descending and ascending sequences of intensities were run, and an overall varied site single stimulation threshold and simultaneous stimulation threshold were determined for each pair of

sites. The magnitude of the difference between a varied site's single stimulation threshold intensity and simultaneous stimulation threshold intensity was considered a measure of the interaction between the two electrode sites.

INSERT TABLES 4 AND 5 ABOUT HERE

Results

In 16 of 17 animals, the interaction between one pair of electrode sites was tested and in one animal two different electrode site combinations were tested. The magnitude of the difference between each varied site's single stimulation and simultaneous stimulation thresholds ranged between 1.6 and 16.5 μ A. For every electrode site combination, the threshold intensity in the varied site was significantly lower (sign test, $p \leq .05$) in the simultaneous stimulation condition than in the single stimulation condition. Table 5 summarizes the data collected in Experiment 2.

INSERT FIGURES 2 AND 3 ABOUT HERE

Figures 2 and 3 show the differences between the varied sites' single stimulation and simultaneous stimulation threshold intensities across successive threshold determinations. Figure 2a indicates the differences over successive threshold determinations for a representative animal in the LC/HYP group; in this group varied site threshold reductions ranged between 1.6 and 8.5 μ A. Figure 3a displays similar data for a representative animal in the periaqueductal gray/HYP group;

threshold reductions in this group ranged between 3.4 and 14.4 μ A. Figure 3b shows a representative animal for the SN/HYP group for which reductions in varied site thresholds ranged between 3.2 and 16.5 μ A. Figure 2b shows a representative animal for the HYP/contralateral HYP group for which reductions in varied site thresholds ranged between 1.6 and 12.4 μ A.

Analysis

Based on pharmacological data, three different catecholamine-mediation hypotheses have been offered to account for ICSS: (1) ICSS is mediated primarily by norepinephrine (2, 30, 31, 34); (2) ICSS is mediated primarily by dopamine (4, 21); (3) ICSS is mediated by both norepinephrine and dopamine with either transmitter sufficient (11, 27, 28).

Snyder and his colleagues (6, 36) have suggested that behavioral effects elicited by d-amphetamine or l-amphetamine can be employed to differentiate between norepinephrine mediation or dopamine mediation of particular behaviors. Following them, Phillips and Fibiger (28) have suggested that norepinephrine-rich and dopamine-rich ICSS sites can be differentiated on the basis of their relative sensitivity to l-amphetamine. They found that norepinephrine-rich sites are insensitive to l-amphetamine while very sensitive to d-amphetamine; by contrast, at dopamine-rich sites the two amphetamine isomers are nearly equipotent because these sites are more sensitive to l-amphetamine and less sensitive to d-amphetamine as compared to norepinephrine-rich sites. Of the ICSS sites tested in the present study, the following were known from previous studies to be sensitive to d-amphetamine, but not to l-amphetamine ("d" sites): (a) the LC (DNB) (10, 11, 27); (b) an area adjacent to the

ventral tegmental nucleus (10); (c) the dorsal longitudinal fasciculus (DLF) (Ackermann et al., in preparation); (d) the medial forebrain bundle and perifornical area (10, 28); and (e) the crus cerebri/nigro-striatal tract (Farber et al., in preparation). Of the ICSS sites tested in the present study, the following were known from previous studies to be sensitive to both d-amphetamine and l-amphetamine ("l" sites): (a) the MV (11) and (b) SN, pars compacta (28). Therefore, on the basis of each electrode site's known sensitivity to d- and l-amphetamine, electrode pairs were grouped into the following three categories: Group A, comprising pairs in which both electrodes impinged on "d" loci; Group B, in which one electrode impinged on a "d" locus and the other electrode on an "l" locus; and Group C, consisting of only one electrode pair in which one electrode impinged on a "d" locus and the other electrode on a locus which has not been tested with d- and l-amphetamine.

The ten electrode pairs in Group A included all six LC (DNB)/HYP pairs, three of the four HYP/contralateral HYP pairs (Table 5A; Fig. 2a,b), and one of the periaqueductal gray area/HYP pairs (Rat 40E) (Table 5A; Fig. 2c) which sorted into Group A because its periaqueductal electrode was located in the lateral periaqueductal area from which response enhancements under d-, but not l-amphetamine were obtained. Group A electrode pairs had a mean threshold reduction of 4.5 μ A with a range of 1.6 to 8.5 μ A. The veracity of the assigned drug designation was confirmed by actually testing three of the ten animals in this group under the d- and l-amphetamine screening procedure (11); these three animals had electrode sites which were inclusive of all of the loci in Group A.

Group B comprised seven electrode pairs. One electrode of each Group B pair was in the lateral hypothalamus, a "d" site, and the second electrode was in one of two "l" sites, either the MV (n = 3) or the SN (n = 3) (Table 5B; Fig. 3a,b). A third "l" site in Group B was a single HYP electrode (Table 5B; Fig. 3c Rat 68F) located in the anterior nucleus of the hypothalamus, which, unlike all other HYP sites tested in this laboratory, was equally sensitive to d- and l-amphetamine. This result is perhaps accounted for by recent histofluorescent evidence that a dopaminergic incerto-hypothalamic fiber system traverses the anterior nucleus of the hypothalamus (3). Group B site combinations had a mean threshold reduction under simultaneous stimulation of 12.8 μ A with a range of 9.1 to 16.5 μ A. The veracity of the assigned drug designation was confirmed by actually testing three of the seven animals in this group under the d- and l-amphetamine screening procedure (11); these three animals had electrode sites which were inclusive of all of the loci in Group B.

There is a significant difference (Mann-Whitney U Test, $p \leq .05$) between the threshold reductions of Group A sites and Group B sites; in fact, there is no overlap in the two groups' values.

Group C comprised a single site combination in which one of the two electrode sites was the SN, pars reticulata, which has thus far not been tested under d- and l-amphetamine. The threshold reduction for the HYP/SN, pars reticulata site combination is considerably less than the other HYP/SN site combinations (Table 5C). This site combination was excluded from Group B because the SN, pars reticulata: (a) is not dopaminergic (38) and (b) differs from the pars compacta in its projections (29).

Discussion

These results indicate that tegmental ICSS sites interact with HYP ICSS sites and interact among themselves. Furthermore, such interactions are systematic; that is, each site combination has a characteristic threshold reduction under simultaneous stimulation which remains stable over several threshold determinations which require many weeks to obtain. This consistency is quite remarkable when one considers the difficulty of the discriminations required by the present experimental paradigm in which increments or decrements in current intensity are small ($1.4 \mu\text{A}$) and are made over days. Threshold reductions were consistent not only within pairs of sites within particular animals, but also across similar site combinations in different animals. For example, LC/HYP site combinations show consistently small threshold reductions (1.6 to $8.5 \mu\text{A}$), while SN, pars compacta/HYP site combinations show consistently larger threshold reductions (9.1 to $16.5 \mu\text{A}$).

When both of two electrode loci are reactive to d-, but not l-amphetamine, small threshold reductions occur under simultaneous stimulation. However, when one of the two electrode loci is equipotently reactive to d- and l-amphetamine, large threshold reductions occur under simultaneous stimulation. These results imply that physiological interactions between sites which are dissimilar in their reactivity to d- and l-amphetamine are greater than physiological interactions between sites which are similar in their reactivity to d- and l-amphetamine. These results cannot have been caused by passive current spread between electrodes because: (1) the magnitude of the threshold reduction under simultaneous stimulation was independent of the proximity of the two sites; i.e., the smallest threshold reductions were obtained from both

the least distant (HYP/HYP) and the most distant (LC/HYP) electrode site combinations; (2) the magnitude of the threshold reduction under simultaneous stimulation was independent of the total amount of administered current, i.e., the total current delivered to Groups A and B was not significantly different (Mann-Whitney U Test, $p > .05$); (3) control animals failed to demonstrate interactions under simultaneous stimulation with an ICSS site regardless of the proximity of the electrodes and regardless of the intensity delivered to the non-ICSS site; and (4) intensity increments ($1.4 \mu\text{A}$) had a much greater effect in increasing responding under the simultaneous stimulation condition than did the same increments under the single stimulation conditions; this result is the opposite of what would be predicted by a current-spread hypothesis.

Coyle and Snyder (6) reported that both amphetamine isomers increase reuptake by dopaminergic synaptosomes equally well while d-amphetamine increases reuptake significantly better than l-amphetamine in non-dopaminergic synaptosomes. Based on this finding, they suggested that behaviors could be characterized as being dopamine-mediated or norepinephrine-mediated, depending on the relative ability of the d- or l-isomers to facilitate them. Taylor and Snyder (36) found that d-amphetamine was ten times as effective as l-amphetamine in increasing locomotor behavior, while d-amphetamine was only twice as potent as l-amphetamine in inducing compulsive gnawing behavior. Thus, they asserted that locomotor activity is norepinephrine-mediated, while compulsive gnawing is dopamine-mediated. However, Coyle and Snyder's (6) original finding that noradrenergic and dopaminergic endings are differentially sensitive to d- and l-amphetamine has subsequently been contradicted (13, 15); d-amphetamine is only several times more effective than l-amphetamine

in blocking release and reuptake of norepinephrine, and the isomers show the same differential sensitivity for both noradrenergic and dopaminergic neurons. Nevertheless, Taylor and Snyder's (36) behavioral data are still unchallenged. The effect of d- and l-amphetamine on ICSS behavior depends on the locus of the electrode. ICSS elicited from norepinephrine-rich areas (LC, lateral hypothalamus) is very sensitive to d-amphetamine but almost refractory to l-amphetamine. On the other hand, ICSS elicited from dopamine-rich areas (SN, nucleus accumbens) is nearly equally sensitive to d- and l-amphetamine. Therefore, it seems that the d- and l-amphetamine isomers can be employed to differentiate between norepinephrine-rich and dopamine-rich areas, even though the mechanism for such differentiation must at present be considered unknown.

That large simultaneous stimulation threshold reductions are obtained from combinations of sites displaying dissimilar reactions to d- and l-amphetamine (MV/HYP, SN/HYP) suggests that larger threshold reductions are due to activation and interaction of two distinct systems, one d-amphetamine sensitive, l-amphetamine insensitive ("d" sensitive), the other sensitive to both isomers ("l" sensitive). That small threshold reductions are obtained when both electrodes of a site combination have similar reactions to d- and l-amphetamine (LC/HYP, DLF/HYP, HYP/HYP) suggests that small threshold reductions are due to activation and interaction of structures within the same (in this case, the "d") system.

Thus, we suggest that when either MV/HYP or SN/HYP site combinations are stimulated simultaneously, two systems, one "d" sensitive and therefore possibly noradrenergic, and one "l" sensitive and therefore

possibly dopaminergic, are activated; one system potentiates the effect of the other. Simultaneous activation of the two systems increases their mutual potentiation, thus resulting in large response enhancements.

In summary, the simultaneous stimulation technique further demonstrates differentiation of ICSS functioning and lends independent support to two hypotheses: (a) that at least two neurochemically coded ICSS systems exist and interact with each other, and (b) that the d- and l-amphetamine screening procedure can differentiate among ICSS sites.

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TABLE 1

PROTOCOL FOR SIMULTANEOUS STIMULATION SHOWING DATA FOR RAT 87F, AN ANIMAL WHICH SELF-STIMULATED AT BOTH OF ITS TWO ELECTRODE SITES (DOUBLE PRESSER)

Period	Stimulation Condition	Stimulation Intensity (μ A)	Responses/Minute (\bar{X} 5 min)
1	No Stimulation-Extinction	0.0	0.6
2	Single Stimulation-Hypothalamus	28.2	0.0
3	No Stimulation-Extinction	0.0	1.8
4	Single Stimulation-Substantia Nigra	21.2	0.2
5	No Stimulation-Extinction	0.0	0.4
6	Simultaneous Stimulation-Hypothalamus/Substantia Nigra	28.2/21.2	42.0
7	No Stimulation-Extinction	0.0	0.0
8	Single Stimulation-Substantia Nigra	21.2	0.0
9	Peak Intensity Stimulation-Substantia Nigra	63.6	62.8
10	Peak Intensity Stimulation-Hypothalamus	35.4	115.2

TABLE 2

PROTOCOL FOR SIMULTANEOUS STIMULATION SHOWING DATA FOR RAT 87F, AN ANIMAL WHICH SELF STIMULATED AT ONE OF ITS ELECTRODE SITES (HYPOTHALAMUS) BUT NOT AT ITS

OTHER ELECTRODE SITE (MEDIAL LEMNISCUS) (CONTROL SUBJECT)

Period	Stimulation Condition	Stimulation Intensity (μ A)	Responses/Minute (\bar{X} 5 min)
1	No Stimulation-Extinction	0.0	0.5
2	Single Stimulation-Hypothalamus	28.2	24.6
3	No Stimulation-Extinction	0.0	3.2
4	Simultaneous Stimulation-Hypothalamus/Medial Lemniscus	28.2/21.2	19.2
5	No Stimulation-Extinction	0.0	0.0
6	Single Stimulation-Hypothalamus	28.2	16.6
7	Single Stimulation-Medial Lemniscus	21.2	4.0
8	Peak Intensity Stimulation-Hypothalamus	127.3	56.0

TABLE 3

EFFECT OF SIMULTANEOUS STIMULATION ON RESPONSE RATES FOR DOUBLE PRESSER AND CONTROL SUBJECTS

Animal	Site 1		Site 2		Simultaneous Stimulation Response Rate (resp./min.)
	Response Rate (resp./min.)	Threshold Intensity (μ A)	Response Rate (resp./min.)	Threshold Intensity (μ A)	
I. Double Pressers					
Locus Coeruleus-Dorsal Noradrenergic Bundle (Site 1) / Hypothalamus (Site 2)					
3E	9.2	10.6	3.8	53.0	142.6
7E*	3.1	141.2	4.7	3.1	85.4
9E	3.6	21.2	8.5	63.6	45.1
25E	6.8	42.4	3.7	14.1	16.9
37E	7.0	21.2	0.0	14.1	23.0
74E	8.8	60.8	4.1	28.2	25.9
Periaqueductal Midbrain Central Gray Area (Site 1) / Hypothalamus (Site 2)					
40E	7.2	21.2	1.1	46.0	11.5
43E	6.1	81.3	0.2	21.2	20.4
19F	7.1	63.6	4.0	14.1	33.0
44F	5.0	89.1	7.9	33.9	20.2
51F	1.5	46.7	1.4	21.2	52.2
Substantia Nigra (Site 1) / Hypothalamus (Site 2)					
81E	7.5	106.1	2.0	50.9	71.2
58F	6.3	38.2	9.0	28.2	105.5
80F	9.8	36.8	4.5	18.4	58.7
82F	4.4	87.7	4.8	28.2	28.2
87F	5.4	28.2	2.0	21.2	30.5

TABLE 3, cont'd.

Hypothalamus (Site 1) / Contralateral Hypothalamus (Site 2)					
50E	0.3	12.7	5.5	22.6	12.6
74E	8.7	14.1	0.9	21.2	40.7
66F	3.8	17.0	9.7	18.4	40.1
68F	8.7	32.5	7.6	24.7	23.8
Locus Coeruleus (Site 1) / Substantia Nigra (Site 2)					
29F	6.0	63.6	0.0	127.3	9.6
90F	6.2	36.8	8.8	67.9	37.1
II. Controls					
Self-Stimulation Site (Site 1) / Control Site (Site 2)					
22E	3.4	14.1	1.4	177.0	6.0
			2.4	35.0	8.0
23E	2.5	14.1	0.0	141.0	8.8
			0.0	35.0	0.2
8F	10.7	106.1	0.3	177.0	2.2
			0.0	35.0	1.5
43F	6.5	46.0	0.2	177.0	9.3
			0.3	49.5	6.3
79F	14.2	28.2	0.5	177.0	5.1
			2.4	21.2	21.9

* This animal failed to sustain ICSS behavior in the locus coeruleus, but it is included in the double-presser group because of sustained behavioral interest in response to shaping. Control animals neither self-stimulated nor demonstrated behavioral interest in response to shaping.

TABLE 4

PROTOCOL FOR ESTIMATION OF THRESHOLD FOR SIMULTANEOUS STIMULATION AS COMPARED TO THE
TEGMENTAL SITE ALONE USING A MODIFIED METHOD OF LIMITS (RAT 3E)

	Intensity (μ A)		Response Rate ($\bar{X}/\text{min.}$)			
	Hypothalamic Site	Tegmental Site	Hypothalamic Site Alone	Tegmental Site Alone	Simultaneous Stimulation	Simultaneous Stimulation
First Run (Descending Series)	53.0	10.6	0.0	0.0	0.0	19.6
	53.0	9.2	3.8	3.6	3.6	127.6
	53.0	7.8	0.2	1.2	1.2	0.0
Second Run (Ascending Series)	53.0	7.8	0.0	1.0	1.0	2.0
	53.0	8.5	0.0	0.2	0.2	0.2
	53.0	9.2	0.0	4.8	4.8	0.0
	53.0	9.9	0.6	9.8	9.8	178.8
Third Run (Descending Series)	53.0	9.9	0.0	0.6	0.6	35.4
	53.0	9.2	1.8	4.6	4.6	98.6
	53.0	8.5	0.2	0.0	0.0	0.2
Fourth Run (Ascending Series)	53.0	8.5	0.2	1.8	1.8	1.0
	53.0	9.2	3.4	0.0	0.0	6.0
	53.0	9.9	0.0	15.0	15.0	59.8

TABLE 5

COMPARISON OF VARIED SITE SINGLE STIMULATION AND SIMULTANEOUS STIMULATION THRESHOLD INTENSITIES

Animal	CONSTANT SITE Electrode Locus	Threshold Intensity (µA)	VARIED SITE Electrode Locus	Threshold Intensity (µA)	Simultaneous Stimulation Threshold Intensity (µA)	Difference Between Varied Site and Simultaneous Stimulation Threshold Intensities (µA)
GROUP A: BOTH SITES REACTIVE TO d-AMPHETAMINE, BUT NOT TO l-AMPHETAMINE						
3E	lateral hypothalamus	53.0	dorsal noradrenergic bundle	11.3	9.2	2.1
7E	medial locus coeruleus	141.4	perifornical nucleus	25.1	22.6	2.5
9E	medial forebrain bundle/fornix	63.6	ventral tegmental nucleus	22.6	14.1	8.5
25E	medial forebrain bundle/lateral hypothalamus	14.1	ventral tegmental nucleus	42.8	40.7	2.1
37E	posterior hypothalamic nucleus of hypothalamus	14.1	dorsal noradrenergic bundle/locus coeruleus	20.5	18.9	1.6
74E	left lateral hypothalamus	28.2	locus coeruleus	59.0	53.7	5.3
40E	zona incerta	46.0	dorsal longitudinal fasciculus, pars tegmentalis	21.9	18.5	3.4

TABLE 5, cont'd.

50E	left medial fore- brain bundle	12.7	right lateral hypothalamus	24.0	21.7	2.3
74E	left lateral hypothalamus	21.2	right fornix	13.8	12.2	1.6
66F	left lateral hypothalamus	17.0	right fornix	17.7	11.3	6.4

GROUP B: ONE SITE REACTIVE TO BOTH d- AND l-AMPHETAMINE; ONE SITE REACTIVE TO d-AMPHETAMINE ONLY

19F	lateral hypothalamus	14.1	midline gray between dorsal tegmental nuclei	63.5	49.1	14.4
43E	lateral hypothalamus	21.2	oculomotor nucleus	82.0	72.1	9.9
51F	stria terminalis	21.2	oculomotor nucleus	51.3	40.3	11.0
68F	right lateral hypothalamus/ medial forebrain bundle	24.7	left anterior nucleus of hypo- thalamus, medial to fornix	30.4	18.0	12.4
81E	zona incerta/fields of Forel	50.9	substantia nigra, pars compacta	94.0	77.5	16.5
82F	dorsomedial nucleus of hypothalamus	28.2	substantia nigra, pars lateralis	99.7	83.4	16.3
87F	dorsolateral nucleus of hypo- thalamus/fields of Forel	21.2	substantia nigra, pars compacta	29.0	19.9	9.1

TABLE 5, cont'd.

GROUP C: ONE SITE NOT TESTED IN AMPHETAMINE SCREEN*			
80F	fields of Forel/ dorsomedial nucleus of hypothalamus	18.4	
	substantia nigra, pars reticulata*	3.54	32.2
			3.2

FIGURE LEGENDS

Figure 1

Number of ICSS responses per minute as a function of tegmental current intensity and stimulation condition for two animals: (1) Rat 74E, a double presser and (2) Rat 79F, a control animal. ICSS rates elicited by stimulating the non-ICSS site alone were always less than 10 responses per minute.

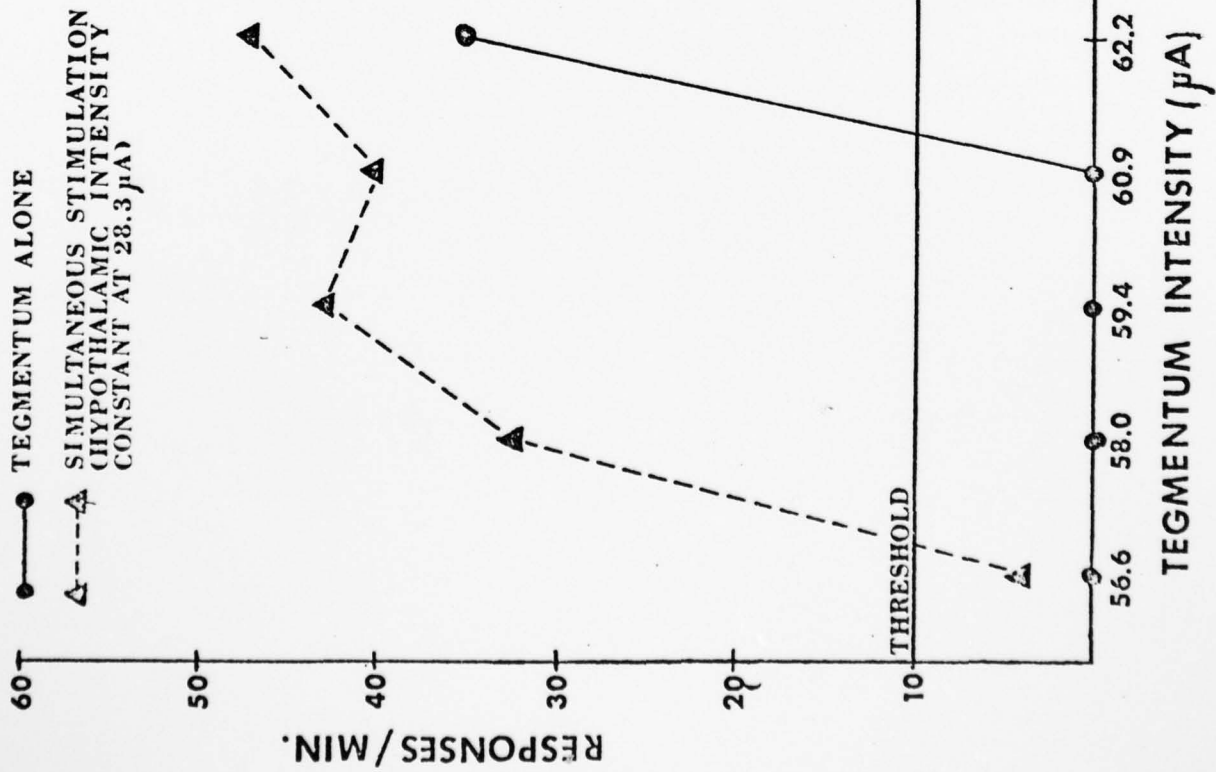
Figure 2

Current intensity at the varied site as a function of stimulation condition and threshold determination for three animals in which both electrodes were sensitive to d-, but not l-amphetamine. In the first and third threshold determinations current intensity was gradually reduced, while in the second and fourth threshold determinations current intensity was gradually increased.

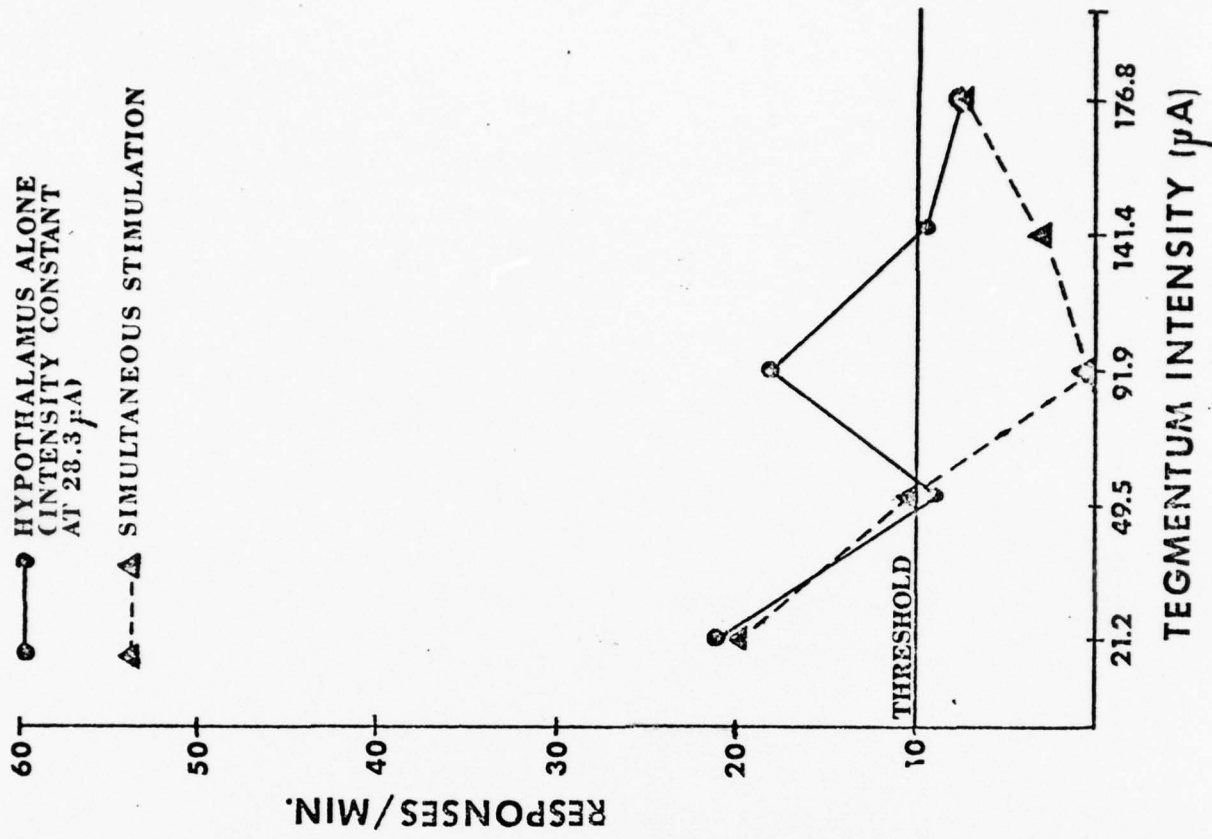
Figure 3

Current intensity at the varied site as a function of stimulation condition and threshold determination for three animals with electrode site pairs in which one site was sensitive to d-, but not l-amphetamine, while the other site was equally sensitive to both isomers. In the first and third threshold determinations current intensity was gradually reduced, while in the second and fourth threshold determinations current intensity was gradually increased.

RAT #74E DOUBLE PRESSER



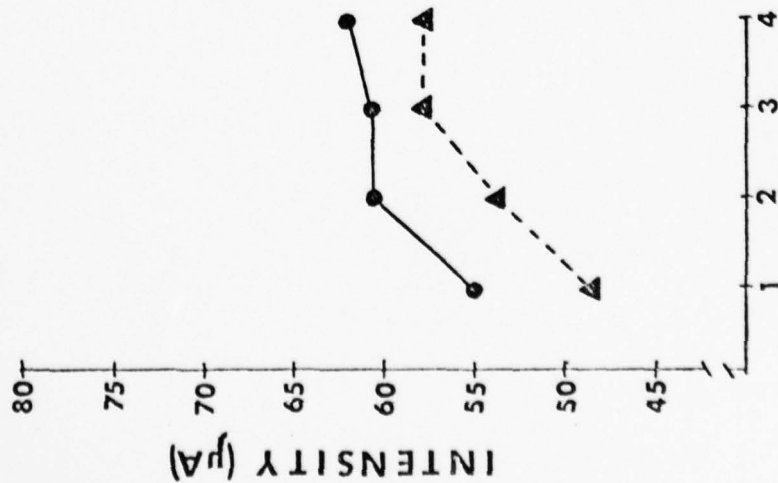
RAT #79F CONTROL



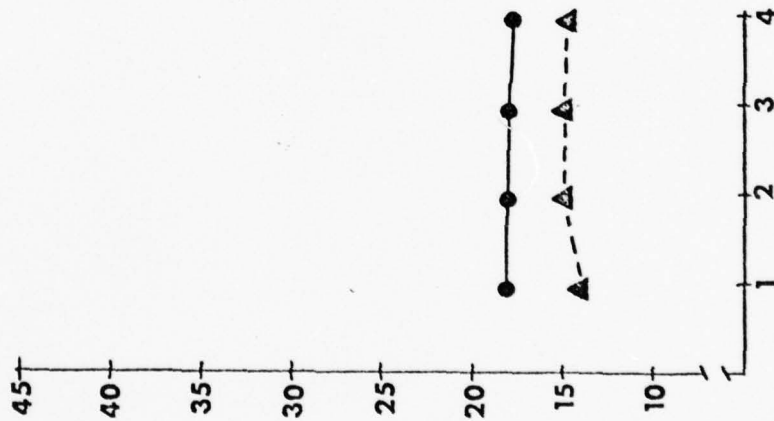
●—● VARIED SITE ALONE

▲- - - -▲ SIMULTANEOUS STIMULATION

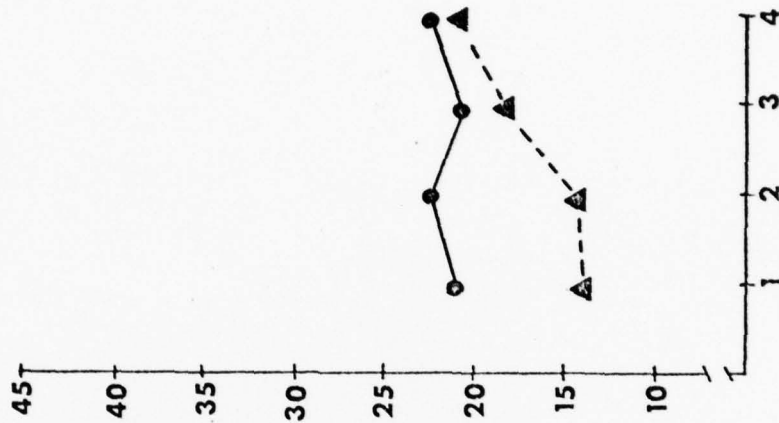
A. 74E·LC/HYP.



B. 74E·HYP./HYP.

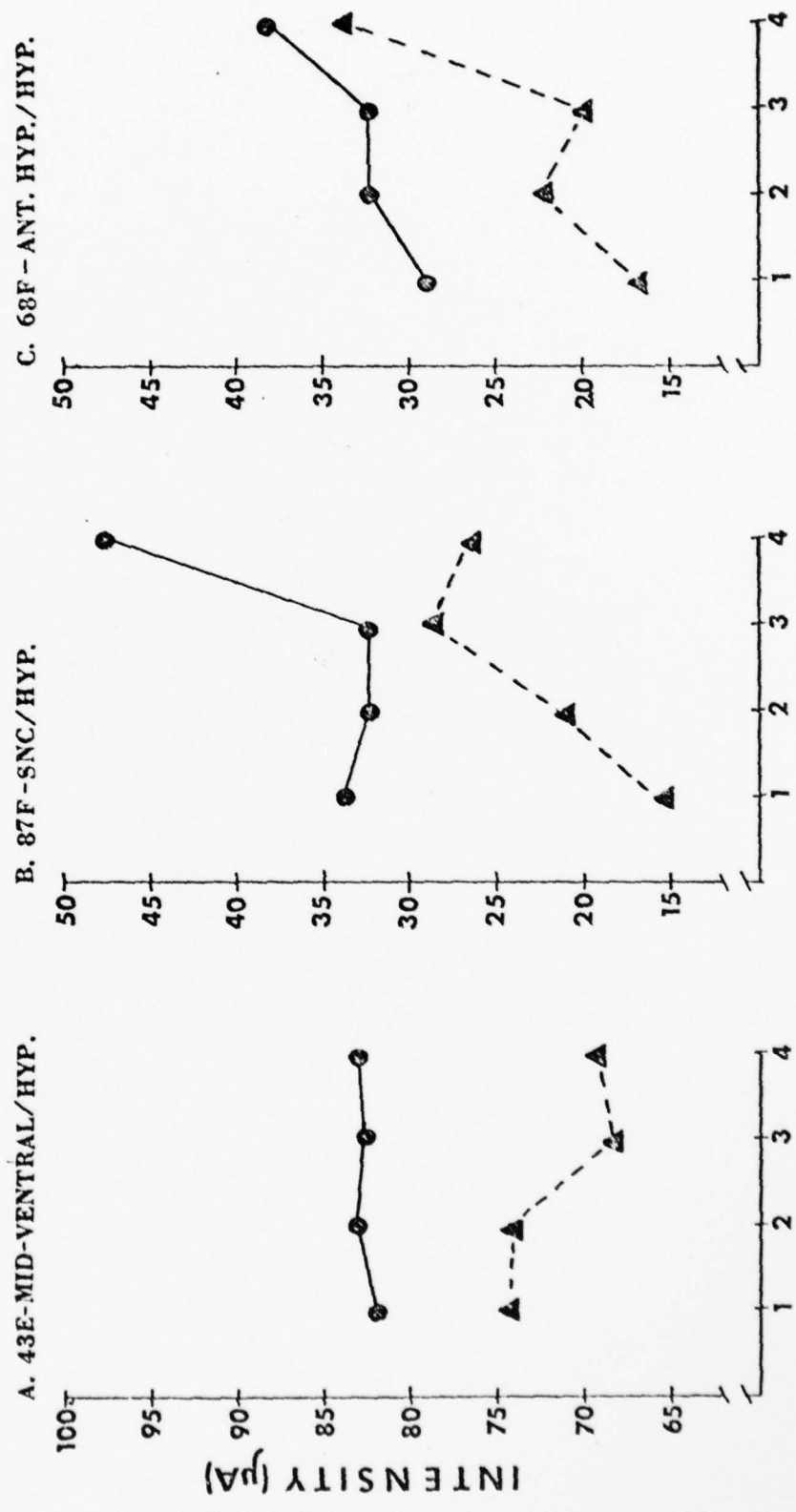


C. 40E·DLF/HYP.



SUCCESSIVE THRESHOLD DETERMINATIONS

● — VARIED SITE ALONE
 ▲ - - - SIMULTANEOUS STIMULATION



SUCCESSIVE THRESHOLD DETERMINATIONS

ATTACHMENT 6

DIRECTIONALITY OF NEUROPHYSIOLOGICAL INTERACTIONS BETWEEN BRAINSTEM AND HYPOTHALAMIC SELF-STIMULATION LOCI IN RATS. Richard J. Bodnar*, Steven J. Ellman, Robert F. Ackermann*, Edward R. Greenblatt*, Solomon S. Steiner* (Dept. Psych., CUNY, New York, N.Y. 10031) and Edgar E. Coons (Dept. Psych., New York Univ., New York, N.Y.).

Ellman et al. (JCPP 88:816, 1975) using sinusoidal stimulation found that locus coeruleus (LC) and hypothalamic (HYP) intracranial self-stimulation (ICSS) response rates are enhanced when the two sites are stimulated simultaneously at threshold intensities as compared to the sum of response rates elicited by each site alone at the same intensities, suggesting neurophysiological interaction between the two sites. Farber et al. (Science, in press) demonstrated that LC lesions reduce or abolish ICSS in HYP sites with nigro- and neo-striatal influences, but do not affect medial forebrain bundle ICSS. Ungerleider and Coons (Science 169:785, 1970) using Deutsch's (JCPP 58:1, 1964) C-T technique found that with bilateral HYP stimulation [C(conditioning) pulse in the left HYP, T (test) pulse in the right HYP or vice versa], a neurophysiological interaction occurs such that refractoriness is eliminated. In order to determine the neurophysiological relationship between brainstem and HYP ICSS sites, twelve rats were stereotaxically implanted with one electrode aimed at the HYP and a second at either the LC or periaqueductal midbrain central gray (PMCG). Each rat was trained to bar-press for monophasic square-wave electrical stimulation; a voltage was chosen which would optimally support ICSS rates at C-T intervals outside the refractory period, but yield operant level responding when the T pulse was omitted. To determine refractoriness for each site, nine C-T intervals, ranging from 0 to 5.0 msec., were randomly presented in each of nine days; refractory period duration for each site ranged between 0.5 and 1.5 msec. The C and T pulses were then split between the two sites at their respective voltages; nine days of C-HYP, T-LC/PMCG and nine days of C-LC/PMCG, T-HYP, alternated in an abba manner, were randomly tested over the nine C-T intervals each day. In nine of twelve animals stimulated in this manner, individual site refractoriness was eliminated. The remaining three animals did not self-stimulate at one electrode site and when either the C or T pulse was delivered to their neutral sites, these animals pressed at only operant levels, as though they were receiving pulses only in their ICSS sites. Of the nine animals in which refractoriness was eliminated, four animals had significantly higher response rates for the C-LC/PMCG, T-HYP combination than for the C-HYP, T-LC/PMCG combination, suggesting that the interaction between LC/PMCG and HYP is predominantly an ascending excitatory influence of the LC/PMCG upon the HYP. In the other five animals in which refractoriness was eliminated, response rates were similar for both combinations. However, the C-HYP, T-LC/PMCG combination generated slightly higher rates, suggesting a weak descending excitatory influence of HYP upon LC/PMCG. These results are discussed with respect to the neuroanatomical placement of electrodes.

ATTACHMENT 7

INTRACRANIAL SELF-STIMULATION SITE SPECIFICITY:
MONOPOLAR ACTIVATION OF BIPOLAR ELECTRODES

By

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W. T. Nelson and Steven J. Ellman

Department of Psychology, City College of New York

Paper presented at the 84th Annual Convention of the American Psychological Association, September 1976, Washington, D. C., and currently being prepared as a manuscript for publication.

Intracranial self-stimulation (ICSS) behavior has been demonstrated to be a reliable and consistent phenomenon elicited from many sub-cortical brain loci; however, the substrate(s) of ICSS behavior have been a source of continual debate for several reasons. First, ICSS is usually elicited only by activation of many reward-relevant neurons and only by electrodes with large cross-sectional areas, often greater than the diameter of the specified reward-relevant site (33). Second, it is often difficult to estimate the amount of tissue which is directly activated by the stimulation and the extent of the current spread. Third, response-rate is often used as an ICSS criterion and is subject to across-animal ceiling effects. Fourth, the bipolar stimulation methods usually employed make it difficult to identify the appropriate structure, particularly in instances in which opposing theories suggest different, yet adjacent, neuroanatomical structures as the substrate for ICSS (23).

Several theories have alternatively suggested that ICSS is mediated by 1) catecholamines in general (12), 2) norepinephrine in particular (28), 3) dopamine in particular (3), or 4) the extrapyramidal system and prefrontal cortical sites (25, 26). Hence, according to alternate interpretations, ICSS behavior elicited from the dorsal pontine tegmentum has been attributed to the activation of either the noradrenergic locus coeruleus (6, 9, 10, 13, 21, 24, 29) or the brachium conjunctivum and mesencephalic V (4, 25), since negative results were claimed for ICSS from locus coeruleus electrodes (2, 27).

Another area of dispute in ICSS behavior is the mesencephalon in which ICSS has been reported in both lateral and midventral areas of the periaqueductal gray by some investigators (1, 5, 10, 19). In

contrast, other reports implicated only midventral central gray ICSS, while claiming that the rest of the area is aversive (17). Both catecholaminergic and extrapyramidal theories predicted substantia nigra ICSS (1, 6, 18, 22, 25), but only an extrapyramidal ICSS hypothesis implicated the red nucleus in ICSS behavior.

The present study attempted to delimit the rewarding loci in dorsal pontine, mesencephalic and hypothalamic ICSS sites and to overcome the problems cited above. To accomplish this, a monopolar stimulation technique (31) was used in which either pole of a bipolar electrode could act as the cathode, which allowed comparison of ICSS response rates elicited from each pole of the same bipolar electrode in the same animal. Any differences in response rate between the two poles would then be used in conjunction with histological verification to specify further the sources of ICSS reward.

Method.

Forty-six male, albino Holtzman Sprague-Dawley rats, weighing between 350 and 500 grams, were anesthetized with Chloropent (2 ml/kg, Fort Dodge) and stereotaxically implanted with two bipolar electrodes (Plastic Products MS 303) aimed at two of the following sites: locus coeruleus, midbrain periaqueductal gray, substantia nigra and hypothalamus. Each bipolar electrode was insulated except at the tips, aligned in a medial-lateral direction perpendicular to the mid-sagittal plane, and each tip was separated from the other by 0.3 mm. Two stainless steel cortical screws were attached to the skull and connected to a third electrode by uninsulated wires; these screws served as anodal indifferents as well as anchors to hold a cap of dental acrylic to the skull.

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INSTITUTE FOR BEHAVIORAL RESEARCH INC SILVER SPRING MD F/G 6/15
LOCALIZATION OF SITES OF ACTION OF HIGH-ABUSE-LIABILITY DRUGS I--ETC(U)
SEP 75 S S STEINER DADA17-73-C-3072

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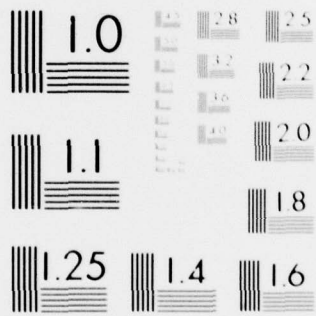
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MICROCOPY RESOLUTION TEST CHART
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With the incisor bar set at five mm. below the interaural line, the locus coeruleus electrode coordinates were: a) 1.5 - 2.0 mm. posterior to the lambda suture, b) 1.0 mm. lateral to a line extrapolated from the sagittal suture, and c) 7.0 mm. from the top of the skull. Periaqueductal midbrain central gray coordinates were: a) 0.6 mm. anterior to the lambda suture, b) 1.5 mm. lateral to the sagittal suture, c) 7.5 mm. from the top of the skull, and d) inserted at a 12 degree angle to the mid-sagittal plane. Substantia nigra coordinates were: a) 2.0 mm. anterior to the lambda suture, b) 2.0 mm. lateral to the sagittal suture, and c) 8.2 mm. from the top of the skull. Hypothalamic coordinates were: a) 4.2 - 4.4 mm. posterior to the bregma suture, b) 1.5 mm. lateral to the sagittal suture, and c) 8.7 mm. from the top of the skull.

After ten days recovery, each animal was tested for ICSS behavior from each site. Each animal was placed in a Plexiglas and stainless steel operant conditioning chamber (20 cm. x 20 cm. x 22 cm.). A 4 cm. by 2 cm. retractable lever was located 4 cm. above the grid floor on one wall of the chamber; a force of 0.2 Newtons was sufficient to depress the lever and this constituted a response. Electromechanical and solid-state switching circuitry, located in an adjacent room, monitored the animal's response rate, recorded minute-by-minute response rates on a data tape, controlled the amount of time the lever was available, and controlled contingencies of reinforcement.

Reinforcements were negative-going pulses of electrical stimulation delivered to the animal's electrode site from a stimulator constructed from Digi-bit solid-state logic circuitry, which allowed the experimenter

to manipulate independently interpulse intervals and pulse durations. All parameters of stimulation were preset before the stimulation period by monitoring on a cathode ray oscilloscope across a 10,000-ohm precision resistor which substituted for the animal, thus giving a ratio between the amplitude of that resistor and the actual resistance of the animal.

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

An animal could press for the following electrical stimulation parameters. Pulse duration was set at 0.1 msec. and the interpulse interval was set at 5 msec. (200 pulses/sec.) unless the animal exhibited motor artifacts which interfered with its lever pressing. In such cases, the interpulse intervals were lengthened in order to eliminate these artifacts. The train duration was set at 700 msec., a value comparable to those of previous studies which used response rate as a measure (8, 14). Current intensities ranged from 150 - 700 μ A. The current intensity was varied in 50 microampere steps until the lowest intensity was found which would elicit peak responding from one of the poles of the bipolar electrode. When this intensity was set, the animal was tested for specificity of response rate in the following manner.

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

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

After completion of the experiment, each rat was injected with an overdose of Chloropent and intracardially perfused with 0.9% normal saline solution, followed by 10% Formalin solution. Frozen serial sections (40 μ .) were stained with luxol fast blue for fibers, and cressyl violet for cell bodies (15). Microscopic examination of each electrode site with precise localization of each pole of the bipolar electrode was done by comparing the stained sections with available rat atlases (16, 34). All electrode calls were made by two independent

raters. One rater knew the data of each animal, while the second rater was blind with respect to the data. The independent rating had a 0.98 correlation with one another; in cases in which the raters were in slight disagreement, the call was remade by the rater who was blind to the data.

Results.

Sixty-six electrode placements in 46 animals were tested for ICSS responding, with each electrode tip serving as cathode.

Hypothalamic placements were compared in three ways: 1) medial forebrain bundle (MFB) vs. perifornical area, 2) lateral aspect of the MFB vs. MFB, and 3) perifornical area vs. dorsomedial hypothalamic area.

First slide.

In seven of 14 animals, the tip in the medial forebrain bundle exhibited response rates which were significantly greater than the tip in the perifornical area (α less than .05). In the remaining seven animals, electrode tips in the medial forebrain bundle elicited higher rates than those from tips in the perifornical area in five of seven instances, although these differences did not attain significance. Perifornical ICSS rates were never significantly greater than MFB ICSS rates in any animal tested.

Second slide.

In six of eleven animals, the tip in the MFB exhibited response rates which were significantly greater than those from the tip lateral to it, whereas in no case was the reverse true. These lateral areas border on the hypothalamic aspects of the crus cerebri and internal capsule.

Third slide.

In three of eight animals, the tip in the perifornical area exhibited response rates which were significantly greater than those from tips either dorsal or medial to the perifornical area. In the remaining animals, four of five animals produced higher response rates when stimulating from the perifornical area than when stimulating from an area dorsal or medial to it, although these differences did not attain significance. In no case did dorsal or medial placements elicit significantly higher response rates than those from perifornical placements. These medial areas include the dorsomedial, ventromedial, periventricular, and anterior hypothalamic nuclei.

Locus coeruleus placements were compared in two ways: medial aspect of the locus coeruleus vs. locus coeruleus area, and lateral aspect of the LC vs. LC area.

Fourth and fifth slides.

In nine of thirteen animals, the tip either impinging upon or closer to the locus coeruleus or dorsal noradrenergic bundle elicited response rates which were significantly greater than those from the tip medial to it. Of the remaining four animals, three had higher response rates for the tip in the locus coeruleus than for the tip medial to it, although these differences did not attain significance. No animals in this study elicited significantly higher rates for stimulation delivered to areas medial to the locus coeruleus as compared to the locus coeruleus itself.

Sixth and seventh slides.

In three cases, the tip impinging upon or closer to the locus coeruleus elicited ICSS responding, while the tip medial to it did

not elicit any ICSS responding at any set of parameters.

In three of five animals, the tip either impinging upon or closer to the locus coeruleus or dorsal noradrenergic bundle elicited response rates which were significantly greater than those from the tip lateral to it. In one case in which ICSS rates were significantly greater for the medial tip closer to the locus coeruleus, the lateral tip did not sustain ICSS behavior.

Periaqueductal midbrain central gray placements were compared on the basis of one tip's impinging upon midline structures while the other tip was lateral to midline structures.

Eighth and ninth slides.

In ten of twelve animals, the tip impinging upon midline central gray structures elicited response rates which were significantly greater than those from the tip lateral to it. In all instances, the electrode tip impinging upon midline central gray structures yielded higher response rates than the tip lateral to it. In three cases, the tip impinging upon midline central gray structures elicited ICSS responding while the tip lateral to it did not elicit ICSS responding for any set of parameters.

Tenth and eleventh slides.

In one instance, both electrode tips straddled the midline; in this instance, the response rates were equipotent. These midline structures included the oculomotor/Edinger-Westphal nuclei, the medial longitudinal fasciculus, nucleus linearis, and the dorsal and ventral tegmental decussations. Most of these sites correlate with mesencephalic A-10 of Dahlstrom & Fuxe (7), and are considered to be dopaminergic (32).

Substantia nigra placements were compared on the basis of impinging upon or proximity to the pars compacta of the substantia nigra. In all three cases, electrode tips located in or close to the pars compacta elicited response rates which were significantly greater than rates elicited by electrode tips ventral or medial to the pars compacta.

Twelfth and thirteenth slides.

In one case, both tips were in the red nucleus, an extrapyramidal structure, and neither tip supported ICSS.

Discussion.

In 42 of 66 placements tested, response rates elicited under one tip serving as cathode were significantly greater than response rates elicited by the other tip serving as cathode 0.3 mm. away. This fact suggests very strongly that the areas supporting the ICSS phenomenon are very discrete, and small changes in electrode placement produce significant changes in ICSS behavior.

Hypothalamic ICSS, particularly medial forebrain bundle ICSS, has been most widely studied by researchers (see 12 for review). In most studies, hypothalamic ICSS has been thought of as uniform. However, recent lesion (11) and pharmacological (30) studies have demonstrated different influences on various aspects of hypothalamic ICSS. This study provides strong evidence that hypothalamic ICSS can be differentiated on the basis of response rate. Since the hypothalamus has been extensively mapped, comparisons of the present and previous studies' results are a means of validating this particular procedure. German & Bowden (12) reviewed over 500 hypothalamic placements described in over 30 separate studies and reported the same response differentiations

as noted in the present study. Therefore, this procedure should be an accurate indicator of differences in responding for brain loci where controversy exists as to the source of ICSS from a given area.

This study provides direct evidence for involvement of the locus coeruleus and dorsal noradrenergic bundle in ICSS behavior. Several laboratories (6, 9, 10, 21, 24) report that ICSS behavior can be elicited from the locus coeruleus and dorsal noradrenergic bundle electrode sites. On the other hand, other studies (2, 25, 27) report that no ICSS behavior could be elicited from locus coeruleus cell bodies. In this study, 18 pontine placements supported ICSS behavior; in all cases, one or both of the electrode tips was located in the region of the locus coeruleus. On the basis of our findings that proximity to the locus coeruleus produces higher ICSS response rates, it is apparent that ICSS is elicited from only a discrete dorsal pontine area, and that this area overlaps the locus coeruleus.

Periaqueductal placements exhibited the same degree of response specificity with respect to electrode tip location. These data correlate well with reports of both ICSS and non-ICSS behavior reported previously (6, 10, 17). Most of these sites correlate with mesencephalic a-10 of Dahlstrom & Fuxe (7), and are considered to be dopaminergic (32).

Substantia nigra placements also demonstrate differentiation between electrode tips in close correlation with previous studies (6, 25).

This technique seems to overcome four criticisms. First, current spread cannot be used as a criticism because the electrode tips and hence cathodal sources are 0.3 mm. apart and any difference between

the two tips should necessarily be attributed to activity beneath the tip, since each tip has an equipotent chance of spreading current to equivalent-sized areas. Under a current-spread hypothesis, electrode tips in such proximity should exhibit similar response rates, whereas in fact, 75% of the placements tested elicited significantly different response rates from their electrode tips.

A second criticism was the use of response rate as a measure, where ceiling effects in some animals might confound the results. Most mapping studies (12, 20) in the past have used a separate-groups design; that is, only one electrode site was tested in each animal. Therefore, if particular animals had idiosyncratic response limitations, then response rate would not be affected by locus of stimulation, but rather by some peculiarity of the animal. In this study, comparisons are made within a particular electrode locus within a particular animal. Thus, any between-animal differences are controlled.

A third possible criticism is differences in tissue conductivity under each electrode tip, as measured by differences in resistance level. As described in the methods section, the amplitude of the current intensity delivered to the animal is pre-set by comparing the animal's resistance level to a 10,000-ohm resistor for each electrode tip. Large differences in resistance between electrode tips would be mirrored in large differences in the ratios established across the resistor. In every case, there was no difference between the resistance levels of the two tips, even though there may have been a significant difference in response rate.

A fourth possible criticism is the use of bipolar stimulation methods. Ranck (23) provides some discussion of the problems inherent

in such a technique, the most prominent of which is the problem of anodal surround. A fiber is depolarized near a cathode by outward current, and hyperpolarized on either side by inward current ("anodal surround"). If the hyperpolarization is large enough, an action potential initiated under the cathode may not be able to propagate through the region of hyperpolarization. We have circumvented this problem by aligning the electrode tips in a medial-lateral axis to take advantage of the fact that the neural substrates of ICSS traverse in an ascending-descending axis, rather than in a medial-lateral one. We have also employed a monopolar stimulation arrangement, with the anodal indifferent anterior and distant from the electrode tips.

In conclusion, ICSS seems to be a discrete phenomenon, delimited by and corresponding closely to catecholaminergic systems.

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