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The Effects of Intracranial Administration of Hallucinogens on Operant Behavior in the Rat. I. Lysergic Acid Diethylamide[1]

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MOKLER, D. J., K. W. STOUDT, L. C. SHERMAN AND R. H. RECH. *The effects of intracranial administration of hallucinogens on operant behavior in the rat. I. Lysergic acid diethylamide.* PHARMACOL BIOCHEM BEHAV **25**(4) 717–725, 1986.—Lysergic acid diethylamide (LSD) was infused in one μl volumes into discrete brain regions of rats trained to press a bar for food reinforcement. The sites were chosen as major areas of the brain 5-hydroxytryptamine (5HT) system: the dorsal and median raphe nuclei, dorsal hippocampus, lateral habenular nuclei, and the prefrontal cortex. Following training in a fixed ratio-40 (FR-40) operant behavior rats were implanted with stainless steel cannulae aimed at the brain area to be examined. Bilateral cannulae were implanted for the lateral habenular nuclei, dorsal hippocampus and the prefrontal cortex. Following recovery from surgery, LSD (8.6 to 86 μg) or vehicle was infused immediately before a daily operant session. Infusion of vehicle was inactive. LSD produced a dose-dependent decrease in reinforcements and an increase in 10-sec periods of non-responding (pause intervals). LSD was significantly more potent when infused into the dorsal raphe nucleus than following intracerebroventricular (ICV) administration, whereas LSD was less potent when infused into the median raphe, lateral habenula or dorsal hippocampus. ED50s for increases in pause intervals were 9, 13, 23, 25, and 54 μg for infusion into the dorsal raphe, prefrontal cortex, dorsal hippocampus, median raphe, and lateral habenular nuclei, respectively. The ED50 for ICV administration in a previous study was 15 μg. The ED50 of LSD placed into the prefrontal cortex did not differ significantly from that of the ICV infusion. The time-course of effects of equivalent doses of LSD was shorter than the 40-min operant session for IP, ICV, dorsal raphe, lateral habenula, and dorsal hippocampus administrations. However, median raphe and prefrontal cortex infusions of comparable doses increased pausing throughout the 40-min session. The dose-response curve for IP administration of LSD was shifted to the left in animals with cannulae in dorsal raphe or prefrontal cortex, whereas no changes were seen in the response to IP administration of LSD in animals cannulated in the other sites. These data suggest that the disruption of operant behavior by LSD may have important components of activity in the dorsal raphe, median raphe and the prefrontal cortex. Nevertheless, it seems likely that LSD acts at multiple brain sites simultaneously in order to induce these behavioral effects.

Intracranial LSD Operant behavior 5HT brain sites

INVESTIGATIONS of the mechanisms by which lysergic acid diethylamide (LSD) produces its effects have predominantly shown an interaction with 5-hydroxytryptamine (5HT) neuronal systems in the brain [15,34]. Aghajanian and coworkers [1] have demonstrated by electrophysiological techniques that LSD inhibits the firing of 5HT neurons in the dorsal raphe nucleus. Trulson and Jacobs [35,36] reported that the effects of LSD on discharge of cells in the dorsal raphe nucleus follows a similar time course as the effects of LSD on a behavioral model of hallucinogenic activity.

Lesion of 5HT systems by intraventricular (ICV) administration of 5,7-dihydroxytryptamine (5,7-DHT) or into the medial forebrain bundle produces a potentiation of the disruption of fixed ratio-40 (FR-40) behavior induced by LSD [9,11]. Other effects of LSD on behavior are potentiated following depletion of 5HT by administration of 5,7-DHT ICV

[1]This data was presented in a thesis submitted by D.J.M. to Michigan State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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[3,16]. Infusion of 5,7-DHT intracranially, however, into discrete brain regions (IC into septal nuclei, nucleus accumbens and hippocampus) has failed to alter the response to IP administration of LSD [8]. In a drug discrimination paradigm, White *et al.* [38] showed a shift to the left in the dose-response curve to LSD following treatment with ICV 5,7-DHT.

The purpose of the present experiments was to examine the effects of direct infusion of LSD into brain areas which receive 5HT input and which may be involved in the disruption of behavior by the hallucinogens. Brain areas chosen for study included the dorsal and median raphe nuclei, since these are sites of the cell bodies of ascending 5HT neurons. Minnema *et al.* [22] have reported that LSD infused into the dorsal raphe was slightly more potent than intraperitoneal LSD in producing appropriate responding in rats trained to discriminate systemically administered LSD from saline. The prefrontal cortex and dorsal hippocampus were included because they are major forebrain areas of termination of 5HT neurons. The effects of infusion of LSD into lateral habenular nuclei were also studied; these nuclei receive cholinergic input from forebrain areas as well as projections from the dorsal raphe nucleus [5,27]. Furthermore, the lateral habenular nuclei have collaterals that project back to the dorsal and median raphe via the interpeduncular nucleus [29].

METHOD

Animals

Male, Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing between 275–325 g at the beginning of the experiment were used. Animals were housed individually in Plexiglas cages with wire tops in a room with a natural light cycle and controlled temperature and humidity. Rats were food-restricted to attain 75–80% free-feeding body weights; supplemental food (Standard Lab Bloks) was given after behavioral sessions to maintain the desired weight range. Tap water was available ad lib.

Apparatus

Operant behavioral equipment consisted of 4 standard operant cages (Lehigh Valley Electronics, Lehigh Valley, PA), each with one lever and food pellet dispenser, that were placed in sound-attenuating chambers. The operant lever required a force of 10–15 g to activate. Programming was controlled with electro-mechanical units (Lehigh Valley Electronics, Lehigh Valley, PA).

Training and Behavioral Procedures

Acquisition of the operant response was accomplished using the method of autoshaping. Programming was initially set on continuous reinforcement (CRF) with each bar-press delivering one 45 mg food pellet (Bio-Serv, Inc., Frenchtown, NJ). Once the animal demonstrated sufficiency on CRF the schedule progressed until a fixed-ratio (FR) 40 was achieved (i.e., the animal was required to make 40 responses to obtain each reinforcer). Rats were placed into operant chambers six days a week for daily 40-min sessions. Drug treatments were tested on Wednesdays and Saturdays, with the remaining days serving as control days.

The number of reinforcers earned and the number of 10-sec periods of non-responding during the session (pause intervals) were counted for each of four 10-min periods. Data is presented as the percent of control reinforcers or the change in pause intervals following drug treatment compared to those of the control session on the preceding day.

Stereotaxic Procedures

Guide cannulae were implanted after the animals had shown stability on the FR-40 schedule. Anesthesia consisted of 3 ml/kg Equithesin. Animals were placed in a standard Kopf stereotaxic apparatus and cannulae were placed according to the coordinates of Pellegrino and Cushman [31]. Figure 1 shows the coordinates used for cannula placement; guide cannulae were placed one mm above these coordinates. Bregma was used as stereotaxic zero for cannulae implanted into the frontal cortex and dorsal hippocampus; interaural zero was used for the remaining areas. The use of bregma or interaural zero for reference was contingent upon the proximity of the zero to the area to be examined. Our experience and that of others [30] has been that this reduces the error in implanting cannulae at a precise site. Cannulae aimed at the dorsal or median raphe were placed as a single cannula at an angle of 30 or 20 degrees from the vertical, respectively, to avoid interference with the cerebral aqueduct. Bilateral placements were used for the lateral habenula, dorsal hippocampus and prefrontal cortex. Cannulae were secured to the skull with dental acrylic and small stainless steel screws placed into the skull.

Guide cannulae were constructed of 23-gauge stainless steel hypodermic tubing (Small parts, Inc., Miami, FL). A small bead of solder was placed on the upper portion of the guide cannula to facilitate anchoring of the cannula to the dental acrylic. Guide cannulae were 10 mm in length except for those aimed at the dorsal and median raphe, which were 15 mm in length. Following surgery and for the remainder of the experiment (except during infusions) the guide cannula was occluded with a length of 0.016″ stainless steel wire, which extended 1 mm beyond the tip of the guide cannula. The infusion cannulae (see below) also extended 1 mm beyond the guide cannulae when put in place. Histological studies showed that this procedure allowed for less gliosis and more viable neurons at the site of infusion as compared with the procedure of making the wire occluder and infusion cannula flush with the guide cannula.

Infusion cannulae were constructed from 30-gauge hypodermic needles. The hub was removed using needle-nosed pliers and the tubing bent at an obtuse angle (approximately 150 degrees) at either 11 or 16 mm from the tip for use with either 10 or 15 mm guide cannulae, respectively.

FACING PAGE

FIG. 1. Sites of cannulae placements (from [31]). Guide cannulae were implanted to 1 mm above the site shown. In the cases of the dorsal and median raphe cannulae, the cannula tract is shown by a dashed line. B indicates A.P. coordinate with reference to bregma, DeG indicates A.P. coordinate with reference to deGroot zero, i.e., interaural zero.

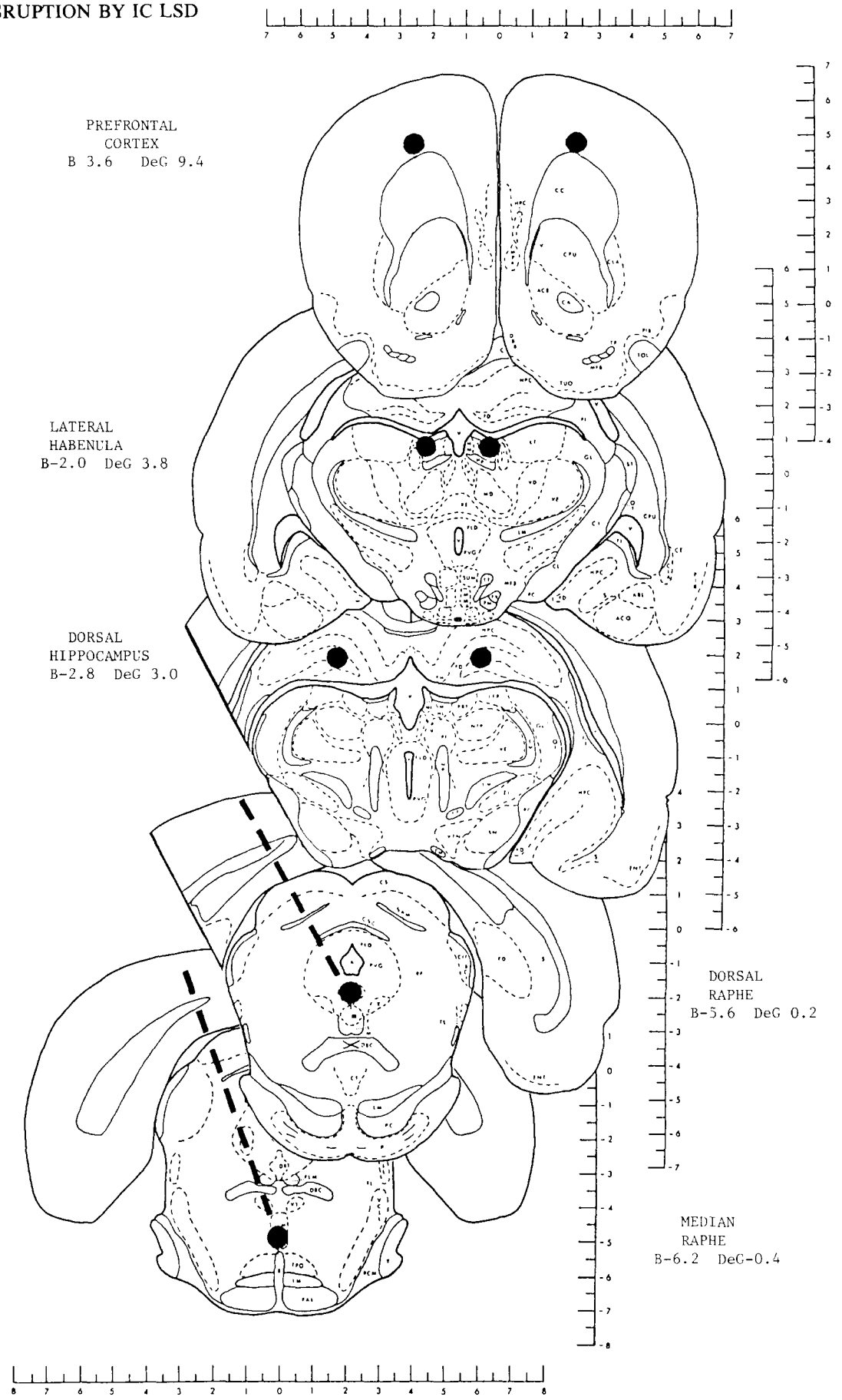
PREFRONTAL
CORTEX
B 3.6 DeG 9.4
LATERAL
HABENULA
B-2.0 DeG 3.8
DORSAL
HIPPOCAMPUS
B-2.8 DeG 3.0
DORSAL
RAPHE
B-5.6 DeG 0.2
MEDIAN
RAPHE
B-6.2 DeG-0.4

TABLE 1

BASELINE RESPONDING IN FR-40 BEHAVIOR OF RATS IMPLANTED WITH IC CANNULAE

Site of Cannulation	n‡	Reinforcers	Pause Intervals
Unimplanted*	25	132 ± 9†	42 ± 5
Dorsal Raphe	5	102 ± 15	25 ± 4
Median Raphe	8	106 ± 18	20 ± 3
Lateral Habenula	6	119 ± 19	21 ± 5
Dorsal Hippocampus	12	105 ± 8	51 ± 8
Prefrontal Cortex	6	117 ± 5	26 ± 5

*Data taken from Mokler *et al.* [23].
†Values represent mean ± S.E.M. for data on control days.
‡n=No. of animals with cannulae implanted at site or included in study.

TABLE 2

F VALUES FOR PAUSE INTERVAL DATA

	Dose	Site	Interaction
Intracranial Administration	52.7 (3, 86)†	5.34 (5, 86)†	1.99 (15, 86)†
Intraperitoneal Administration	95.7 (2, 56)†	4.42 (5, 28)†	61.8 (10, 56)†
	Time Course		
Site	Dose	Site	Interaction
Dorsal Raphe	9.3 (4, 14)†	11.9 (3, 42)†	21.5 (12, 42)†
Median Raphe	10.1 (4, 16)†	9.1 (3, 48)†	32.6 (12, 48)†
Lateral Hebenula	4.0 (3, 12)*	5.2 (3, 36)†	19.0 (9, 36)†
Dorsal Hippocampus	20.4 (5, 25)†	10.1 (3, 75)†	129.5 (15, 75)†
Prefrontal Cortex	11.8 (5, 16)†	8.8 (3, 48)†	22.7 (15, 48)†

Values represent F values for ANOVAs as outlined in the Method section. Numbers in parentheses indicate degrees of freedom. *$p<0.05$; †$p<0.01$.

Drug Infusion Procedure

For infusions, an infusion cannula was connected to PE-10 tubing (Clay-Adams, Parsippany, NJ) which, in turn, was connected to a 5 μl syringe (Hamilton Co., Reno, NV). The syringe and tubing were filled with distilled water except for the volume of IC drug to be injected plus two μl. Infusions were controlled with a Harvard Infusion Pump (Harvard Apparatus, Mills, MA) connected to a Gra-Lab Universal Timer (Gray Co., Dayton, OH). The occluder pin was removed and the infusion cannula placed into a guide cannula while the rat was being held under gentle hand restraint. The rat was put back into its home cage and LSD was infused for one min in a volume of one μl. After the infusion was completed an additional minute was allowed to pass before removal of the infusion cannulae to allow for diffusion from the site. Infusions into bilateral structures were done simultaneously, one μl on each side. Any fluid appearing at the top of the guide cannula as well as behaviors during infusion were noted.

Following completion of all behavioral experiments each animal was again anesthetized with Equithesin. Under a surgical level of anesthesia the rat was perfused with 50 ml 0.9% saline via intracardiac catheter, followed by 100 ml of 10% buffered formalin. The cannula cap was then gently removed and the brain removed from the skull. The brain was post-fixed in 10% formalin. Cannula placement was verified by visual inspection of cannula tracts in slices made with a microtome with a freezing stage. Site of injection was determined by the most ventral point of the guide cannula tract and measuring 1 mm beyond or, if present, the tract made by the infusion cannula itself. If cannulae were verified as being placed greater than 0.5 mm from the designated coordinates (Fig. 1), the data for that animal was not used.

Drugs

d-Lysergic acid diethylamide tartrate (LSD) was obtained from the National Institute on Drug Abuse. For intraperitoneal injections LSD was dissolved in 0.9% saline. All IP injections were made in a volume of one ml/kg. For intracranial infusions LSD was dissolved in a solution of 2.3 mM $CaCl_2$ in sterile saline. The solution contained calcium concentrations similar to cerebrospinal fluid, while affording low concentrations of other salts to allow for maximal solubility of drugs. All weights of drugs refer to the weight of the salt. Doses administered IC and ICV are reported as actual total amount infused. Doses for IP injections are extrapolated on the basis of the mean body weight for each group of identically-treated subjects.

Statistics

Analysis of dose-response data for IC LSD was done using a two-way ANOVA [19]. All sites were included as well as data from intracerebroventricular administration of LSD in the same paradigm [24]. Dose and site were used as factors in the analysis. The time course of disruption was examined statistically for each site of infusion by two-way ANOVA with time and dose as factors. The effects of IP administration of the drug in animals implanted at various sites were analyzed using a two-way ANOVA with dose and site of cannula placement as factors. Least significant differences tests were used for post-hoc tests. The level of significance was set at $p<0.05$. ED50s were determined using probit analysis. Data for probit analysis was transformed using an arcsin transformation to normalize the data.

RESULTS

Baseline performances of rats with intracranially-placed cannulae are listed in Table 1, along with values for reinforcers earned and pause intervals recorded from unimplanted subjects (data from [23]). Infusion of LSD into various areas of the brain produced a disruption of behavior characterized by a decrease in reinforcers with a concomitant increase in pause intervals. In previous work we have shown that a reciprocal increase in pausing is associated with the decrease

TABLE 3

TIME-COURSE OF THE EFFECTS ON PAUSE INTERVALS OF LSD INFUSED INTO VARIOUS BRAIN REGIONS

Site	Dose (μg)*	n‡	Change in Pause Intervals/ 10-min Period 1	2	3	4
Dorsal Raphe	8.6	4	24 ± 12†	29 ± 13†	6 ± 4	4 ± 5
	17.2	4	45 ± 4†	47 ± 4†	33 ± 8†	16 ± 11
	43	3	42 ± 4†	52 ± 1†	41 ± 1†	14 ± 14
Median Raphe	34.4	3	43 ± 4†	27 ± 12†	28 ± 11†	16 ± 9†
	43	4	52 ± 1†	43 ± 4†	26 ± 9†	20 ± 7†
Lateral Habenula	34.4	5	30 ± 9†	38 ± 7†	17 ± 9	6 ± 4
	86	3	36 ± 6†	33 ± 11†	26 ± 11†	22 ± 7†
Dorsal Hippocampus	17.2	4	1 ± 3	10 ± 3†	8 ± 6	9 ± 13
	34.4	4	45 ± 4†	27 ± 6†	10 ± 7	−6 ± 7
	86	5	51 ± 1†	47 ± 5†	35 ± 9†	2 ± 7
Prefrontal Cortex	17.2	4	27 ± 12	24 ± 10†	9 ± 7	11 ± 5
	34.4	5	44 ± 2†	38 ± 5†	25 ± 5†	22 ± 4†
	86	3	47 ± 4†	50 ± 3†	44 ± 3†	35 ± 10†

*Though more doses were tested, only those that caused a significant increase in pause intervals in at least one period are listed.

†Significantly different from infusion of vehicle into that area. Values represent mean ± S.E.M. Two-way ANOVA, least significant difference test, $p<0.05$.

‡n=number of animals tested at this dose; not all animals were tested at all doses.

in reinforcers from the lower range of IP doses of 5HT agonists, but that this relationship does not hold for other types of psychoactive agents that disrupt the FR-40 schedule. Therefore, the data for both reinforcers and pause intervals are presented here to demonstrate that this reciprocal relationship also applies to LSD administered by the ICV or IC routes. Overall, there was a significant difference in the effects of LSD placed into these brain areas (Table 2). The time-course of the effects varied at the different sites (Tables 2, 3). Infusion of LSD (4.3 to 43 μg) into the dorsal raphe produced a peak effect during the first two ten-minute periods (Table 3, Fig. 2). By the fourth period the disruption of behavior was no more than that caused by vehicle. The dose-response pattern for LSD during the ten-minute period of greatest disruption following infusion into the dorsal raphe is compared with that by ICV administration in Fig. 3 (values on the ordinate are absolute μg doses; see the Method section). LSD infused into the dorsal raphe (IC) was significantly more potent than following ICV administration; the ED50 for dorsal raphe infusion was 9 μg as compared to 15 μg for ICV infusion (Table 4). Also shown in Fig. 3 is the response to IP administration of LSD in the same animals with cannulae implanted into the dorsal raphe nucleus.

In contrast to dorsal raphe administration, infusion of LSD into the median raphe, dorsal hippocampus or lateral habenular nuclei was less potent than infusion into the lateral ventricles (Table 4). Figure 5 compares the dose-response pattern for the peak 10-min effect of LSD placed into the prefrontal cortex with the dose-effect curves of ICV and IP administrations. Infusion of LSD into the prefrontal cortex resulted in the same potency range as for ICV administration (13 vs. 15 μg, Fig. 5, Table 4).

The time-course of the effects after infusing LSD into the prefrontal cortex (Fig. 4) or median raphe differed from those after administration into the dorsal raphe, lateral habenula, dorsal hippocampus, or by ICV or IP administration. Operant behavior was disrupted throughout the 40-min session at the larger doses infused into the prefrontal cortex (Table 3, Fig. 4) or the median raphe (Table 3). The maximal disruption generally occurred during the first two periods.

The effects of IP administration of LSD in animals cannulated in various brain regions were also determined. Animals with cannulae placed into the dorsal raphe and prefrontal cortex were significantly more sensitive to IP administration than animals with cannulae placed into other areas or the lateral ventricles (Table 2), as well as more sensitive than unimplanted rats.

DISCUSSION

The disruption of operant behavior by infusion of LSD into discrete brain regions is qualitatively similar to the disruption produced by IP administration [10,23] or ICV administration [24]. This disruption is characterized by a dose-dependent decrease in reinforcers and concomitant increase in pause intervals. That is, the ED50s for these two measures are similar (85 μg/kg for reinforcers and 81 μg/kg for pause intervals) following IP administration [23].

The potency of LSD to disrupt operant behavior when infused into brain areas does not differ to any great extent from that after administration by either the IP or ICV routes. The ED50s for central administration range from 9 μg (dorsal raphe) to 54 μg (lateral habenula). The ED50 for disruption

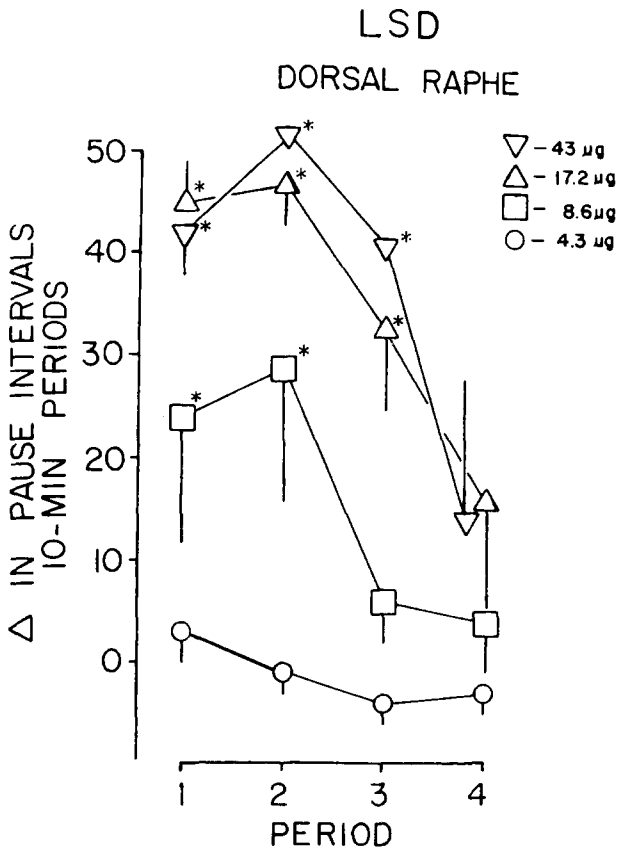

FIG. 2. Time-course of the effects on pause intervals of LSD administered into the dorsal raphe nucleus. Periods represent successive 10-min intervals of 40-min operant sessions. *$p<0.05$, significantly different from control, least significant differences test, two-way ANOVA.

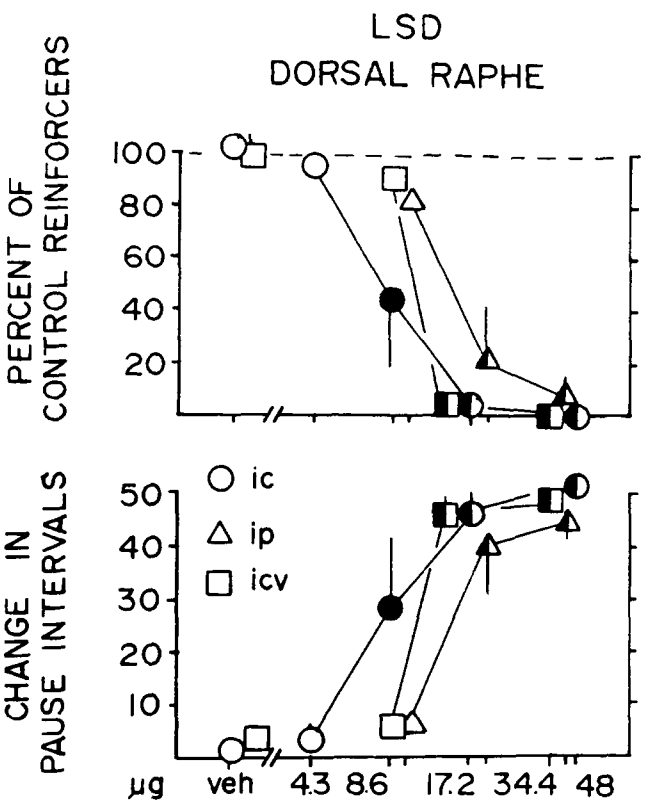

FIG. 3. Peak 10-min effects of LSD following ICV administration (taken from [24]) and IC infusion into the dorsal raphe nucleus or IP injection in the same subjects. Shading of the left half of a symbol signifies a significant difference from control ($p<0.05$, least significant differences test, one-way ANOVA). Shading of the right half of a symbol indicates a significant difference from ICV LSD ($p<0.05$, least significant differences test, two-way ANOVA). Doses on the ordinate are µg of LSD administered per animal as indicated in the Method section.

of this operant behavior after IP administration has been reported as 19 µg [24] and 25 µg (81 µg/kg) [23]. It would be expected that LSD infused into an active site in the brain would show a much greater potency than this, if one or a few local active sites determined the effect. One explanation for the lack of differences in potency observed here is that LSD may produce a potent response initially but may distribute rapidly away from the site; the large time periods (10 min) examined here may not allow the sensitivity to detect a maximal effect during the early part (2–3 min) of the session. Another possible explanation is that multiple sites may be involved in the disruption and, therefore, some redistribution would be necessary to observe any effects. In any case, the small differences between IP and ICV doses indicate the rapid access that systemic LSD has to crucial central sites of action.

A third possibility is that LSD is not acting at central sites to produce this disruption. Several lines of evidence argue against this. Destruction of 5HT neurons in the CNS by ICV administration of the neurotoxin 5,7-dihydroxytryptamine potentiates the disruption of operant behavior by LSD [9,11]. In addition, pretreatment with centrally-acting 5HT antagonists attenuates the effects of peripherally administered LSD [10, 23, 25]. Furthermore, pretreatment IP with xylamidine tosylate, a peripheral 5HT antagonist, does not alter the effects of IP LSD, whereas ICV administration of xylamidine tosylate antagonizes the effects of IP LSD or mescaline ([37]; Mokler *et al.*, unpublished observations).

Despite the lack of potency differential when administered into the cerebral ventricle, LSD infused into discrete brain areas did show a differential response in the potency and time-course of the effects. LSD infusion into the dorsal raphe in the present study was more potent than ICV administration [24], which suggests that the dorsal raphe is a primary site of action. The time-course of the effects, however, was similar to that of ICV administration, with peak effects occurring during the first two 10-min periods. This contrasts with the time-course of the effects after IP administration, for which peak disruption occurs during the second 10-min period [24]. In all three methods of administration the effect on behavior did not differ from control performance by the fourth period.

Minnema *et al.* [22] have reported a similar potency for LSD (20 µg) infused into the dorsal raphe nucleus in rats trained in an operant discrimination paradigm to discriminate LSD from vehicle. The time-course of this effect closely resembled that which we found for disruption of FR-40 behavior following infusion of LSD into the dorsal raphe. A number of

TABLE 4

ED50s FOR CHANGE IN PAUSE INTERVALS: ICV VS. IC ADMINISTRATION

	LSD (μg)
ICV	15* (10-19)
IC	
Dorsal Raphe	9 (2-20)
Median Raphe	25 (21-28)
Lateral habenula	54 (29-249)
Dorsal Hippocampus	23 (14-37)
Prefrontal Cortex	13 (0-47)

*Values are ED50s for increases in pause intervals using probit analysis. Values in parenthesis are 95% confidence limits. ICV values were taken from Mokler and Rech [24].

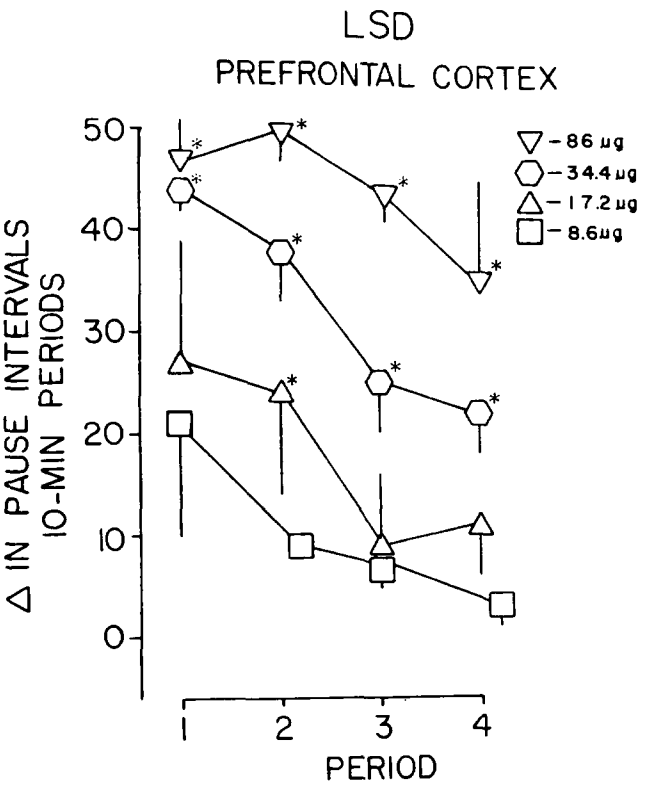

FIG. 4. Time-course of the effects on pause intervals of LSD administered bilaterally into prefrontal cortex. Periods represent successive 10-min intervals of 40-min operant sessions. *$p<0.05$, significantly different from control, least significant differences test, two-way ANOVA.

investigators have reported that microiontophoretic application of LSD into the dorsal raphe mimics IV administration in suppressing 5HT cell firing in the dorsal raphe [1, 2, 13, 14, 26]. These data also support a hypothesis that activity of LSD in the dorsal raphe nucleus may be important for its behavioral as well as electrophysiological effects.

Infusion of LSD into the median raphe and prefrontal cortex produced a disruption of behavior with a rapid onset and extended time-course. Thus, at the larger doses of LSD examined in these two sites, the disruption was maximal during the first and second period but was still significantly greater than vehicle during the fourth period. This disruption is of greater duration than the disruption produced by infusion of LSD into other sites or following administration ICV or IP [24]. Therefore, LSD infused into the median raphe and prefrontal cortex has prolonged effects which would suggest these as other important sites of action. The concept that behavioral effects of LSD involve activity at brain sites other than the dorsal raphe was proposed by Trulson and Jacobs [36]. They demonstrated that repeated administration of LSD induced tolerance to the behavioral effects of the drug without inducing tolerance to the suppression of dorsal raphe cell discharge. Furthermore, the drug lisuride is even more potent than LSD in suppressing dorsal raphe discharge, but fails to mimic the electrophysiological effects of LSD on certain forebrain neurons [21], and lisuride does not share the hallucinogenic effects of LSD in man. Interest in the prefrontal cortex has developed from the observation that this brain region is relatively rich in $5HT_2$ receptors [6,33], and the behavioral effects of LSD and other hallucinogenic agents appear to be mediated at least in part by actions on $5HT_2$ receptors [7, 12, 25].

Animals with cannulae implanted into the dorsal raphe or prefrontal cortex showed an enhanced sensitivity to IP administered LSD. Cannulation may result in nerve terminal damage and a consequent supersensitivity to the effects of LSD. Lesion of forebrain 5HT systems by ICV or medial forebrain bundle administration of the neurotoxin 5,7-DHT [9,11] results in a similar enhancement of the effects of IP LSD on FR-40 behavior. This suggests more critical involvement of these sites in the actions of LSD to disrupt this behavior.

These data are also consistent with previous reports concerning the interactions of LSD with the $5HT_1$ and $5HT_2$ subtypes of receptors. Binding studies have shown that LSD has a high affinity for both subtypes of 5HT receptors; ratios for $5HT_1/5HT_2$ vary from 1.1 [32,33] to 2.4 [17] to 8 [18]. The behavioral effects of LSD have been shown to be antagonized by the $5HT_2$ receptor antagonist pirenperone [7,25]. This blockade of the effects of LSD by pirenperone is similar to that seen with metergoline, a 5HT antagonist with $5HT_1$ and $5HT_2$ properties [6, 10, 20, 23, 33]. Furthermore, in drug discrimination experiments, LSD generalized completely to 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), an indolealkylamine hallucinogen relatively more active at $5HT_2$ sites [6]. LSD only partially generalized to the $5HT_1$ agonist MK-212 [4]. Binding studies have shown that differences do exist in the distribution of $5HT_1$ and $5HT_2$ binding sites in the brain [6, 28, 33]. Blackshear and coworkers [6] reported that the number of binding sites for the two ligands in the frontal cerebral cortex of the rat is about 30% higher for $5HT_2$ than $5HT_1$ receptors. Peroutka and Snyder [32,33] reported that the receptor numbers for $5HT_1$ and $5HT_2$ in this region are similar. These differences may be attributable to binding assay conditions. In any case, the frontal cerebral cortex has the highest density of $5HT_2$ receptors of any brain region. The highest relative density of $5HT_1$ receptors in the rat

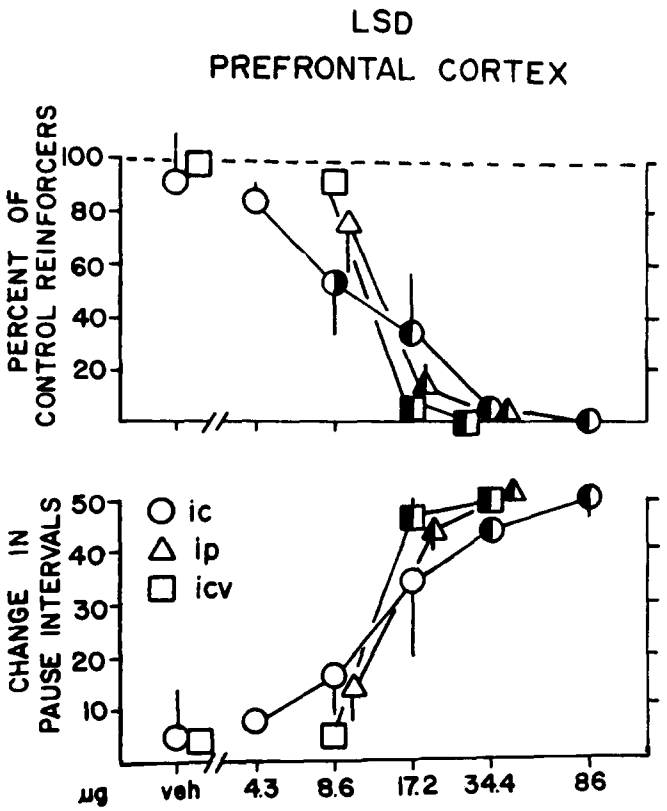

FIG. 5. Peak 10-min effects of LSD following ICV administration (taken from [24]) and IC infusion into prefrontal cortex or IP injection in the same subjects. Meanings of the shading of symbols and notation on doses are indicated in the legend for Fig. 3.

brain is found in the hippocampus [6,33], $5HT_1$ binding in this region exceeding $5HT_2$ binding by 5-fold. The findings of the present study, that LSD produces a greater disruption of behavior following infusion into the prefrontal cortex and is relatively impotent in the hippocampus, suggests that activity at $5HT_2$ receptors may be relatively more important in the actions of LSD to disrupt behavior. However, the lowest ED50 for LSD occurred after infusion into the dorsal raphe, and median raphe infusion resulted in a prolonged disruption of FR-40 responding. Therefore, the $5HT_2$ activity may not be sufficient to disrupt behavior, but LSD effects may also involve actions at autoreceptors on 5HT cell bodies or $5HT_1$ receptors at other brain sites (see [15,21]).

In conclusion, infusion of LSD into discrete areas of the brain produces a disruption of FR-40 behavior which is qualitatively similar to the disruption following IP or ICV administration. The potency and time-course of the effects of IC LSD differed regionally. Infusion of LSD into the dorsal raphe nucleus is more potent than infusion into any other site, or ICV or IP administration. The time-course of the disruption following infusion into either the median raphe or prefrontal cortex was prolonged in contrast to infusion into other sites or by other routes of administration. These data suggest that the prefrontal cortex and the dorsal and median raphe nuclei are important in the disruption of behavior by LSD. However, the small differences in potency of LSD infused into these sites also suggest that multiple sites are involved in the disruption of this operant behavior.

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