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The Effects of *d*-Lysergic Acid Diethylamide (LSD), 2,5-Dimethoxy-4-Methylamphetamine (DOM) and *d*-Amphetamine on Operant Responding in Control and 6-Hydroxydopamine-Treated Rats

R. L. COMMISSARIS[1], W. H. LYNESS, J. J. CORDON, K. E. MOORE AND R. H. RECH

Department of Pharmacology and Toxicology, Michigan State University East Lansing, MI 48824

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COMMISSARIS, R. L., W. H. LYNESS, J. J. CORDON, K. E. MOORE AND R. H. RECH. *The effects of* d-*lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM) and* d-*amphetamine on operant responding in control and 6-hydroxydopamine-treated rats.* PHARMAC. BIOCHEM. BEHAV. 13(5)621–626, 1980.—The purpose of the present study was to determine the role of central catecholaminergic neuronal systems in the effects of LSD, DOM and *d*-amphetamine on fixed ratio (FR) operant responding in rats. Food-deprived male rats were trained to press a bar for food reinforcement on a FR-40 schedule. Control responding on this schedule is characterized by a rapid, constant rate of responding (approximately 100 responses/min) throughout a 40 min test session. LSD and DOM, as with other hallucinogens, produced dose-dependent periods of nonresponding or "pausing," followed by reinstatement of responding at or near the control rate. Administration of the non-hallucinogen, *d*-amphetamine, did not produce "pausing," but caused the response rate to slow and become erratic. In animals pretreated intraventricularly with 6-hydroxydopamine (6-OHDA; 200 μg/10 μl×2), the response to LSD and DOM was unchanged, while the response to *d*-amphetamine was significantly diminished. The neurotoxin significantly decreased brain catecholamines to less than 25 percent of control in all regions examined, without altering 5-HT concentrations in these same regions. These data demonstrate that the effects of LSD and DOM on FR-40 responding are quite different from those of *d*-amphetamine, and that this difference may be due to the extent of catecholamine involvement in the effects of these agents.

Catecholamines 6-OHDA LSD DOM *d*-Amphetamine and hallucinogens

THERE is considerable evidence to suggest that many of the behavioral effects of hallucinogens are mediated through catecholaminergic neurons in the brain. Members of both the indolealkylamine and phenethylamine classes of hallucinogens stimulate motor activity [8, 12, 15] and, at higher doses, produce stereotyped responding ([8], Commissaris *et al.*, unpublished results). Hallucinogens of both classes produce rotational behavior following unilateral destruction of dopaminergic neurons in the substantia nigra produced by the neurotoxin 6-hydroxydopamine (6-OHDA; [9,13]). These behavioral effects of hallucinogens, which are also observed following administration of the psychomotor stimulant *d*-amphetamine [12–14], are presumed to be mediated by activation of catecholaminergic systems in the brain.

The purpose of the present study was to determine the role of catecholaminergic neuronal systems in the effects of hallucinogens and *d*-amphetamine in another behavioral paradigm, fixed ratio 40 (FR-40) operant responding. *d*-Lysergic acid diethylamide (LSD) was chosen as the prototype indolealkylamine hallucinogen. The amphetamine analogue 2,5-dimethoxy-4-methylamphetamine (DOM) was chosen as a representative member of the phenethylamine class of hallucinogens. These hallucinogens induce a pattern of disrupted responding with long pausing interspersed between periods of normal rates as the predominant pattern [3–5, 10], as opposed to graded decreases in response rate being more common with *d*-amphetamine and neuroleptics. Quantitation of the extent of pausing [3–5] has facilitated the analysis of pausing in relationship to overall response rates. The use of this method in the present study demonstrates its utility in differentiating patterns of response characteristics induced by hallucinogens and *d*-amphetamine.

[1]Send reprint requests to R. L. Commissaris, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

TABLE 1

THE EFFECTS OF INTRAVENTRICULAR 6-OHDA ADMINISTRATION ON THE CONCENTRATIONS OF 5-HT, DA AND NE IN VARIOUS BRAIN REGIONS

	5-HT		DA		NE	
	Vehicle	6-OHDA	Vehicle	6-OHDA	Vehicle	6-OHDA
Cortex	0.43 ± 0.03	0.37 ± 0.02 (86)	0.30 ± 0.02	0.07 ± 0.02* (23)	0.40 ± 0.02	0.03 ± 0.01* (8)
Hippocampus	0.46 ± 0.05	0.32 ± 0.05 (70)	n.d.	n.d.	0.31 ± 0.01	0.04 ± 0.01* (13)
Hypothalamus	1.01 ± 0.04	1.09 ± 0.06 (108)	n.d.	n.d.	1.67 ± 0.10	0.10 ± 0.01* (6)
Striatum	0.77 ± 0.03	0.64 ± 0.11 (83)	5.78 ± 0.17	1.37 ± 0.27* (24)	n.d.	n.d.

Data are expressed in μg amine/g wet tissue weight; each value represents the mean ± SEM obtained from six 6-OHDA-treated (200 μg/10 μl × 2) or eight vehicle-treated animals. Numbers in parentheses represent concentration of amine in 6-OHDA-treated animals expressed as a percentage of vehicle-treated animals.

n.d.=amine concentration not determined.

*$p<0.05$, Student's t-test.

METHOD

Subjects

The subjects were 16 male Sprague-Dawley (Spartan Farms, Haslett, MI) rats weighing 200–250 g at the start of the experiment. All subjects were housed individually in a room with 12 hr light-dark cycle (lights on 0700–1900 hr). The subjects had not received any drugs prior to the start of the experiment.

Apparatus

Behavioral testing was conducted between 1500 and 1800 hr in four standard operant chambers (LVE 143-20-215) equipped with food pellet dispensers; these chambers were located in sound-attenuating boxes. Each chamber contained a single lever which required a force of 10–15 g to activate. All experimental events were controlled by electromechanical programming circuits and responses were recorded on electromagnetic counters and cumulative recorders. Two parameters were monitored in the operant sessions: (1) the number of reinforcements obtained, a reflection of the average response rate, and (2) the period of non-responding or "pausing". To quantify the period of non-responding during operant sessions, a pause interval timer (3–5) was incorporated into the program as described below.

Each response by the subject reset a 10-second timer. If the animal responded before 10 seconds elapsed, the timer reset and the program continued. If the animal failed to respond during this 10-second interval, a count was registered and the timer automatically reset. Therefore, the number of counts registered by the pause interval timer was an index of the extent of non-responding in terms of cumulated 10-second pause intervals.

Neurochemical Lesions

The subjects were randomly assigned to one of two groups of eight animals each and administered either 6-OHDA HBr (200 μg/10 μl; 136 μg 6-OHDA free base) or its vehicle intracerebroventricularly (ICV) through a 30 gauge cannula; the coordinates for the injection were (from Bregma): anterior-posterior 0.0, 1.5 mm lateral; 3.2 mm below the surface of the brain. To insure substantial destruction of catecholamine neurons, this procedure was repeated after a three-day recovery period. All subjects were anesthetized with Equithesin (3 ml/kg) and placed in a standard stereotaxic apparatus prior to vehicle or 6-OHDA treatments.

Behavioral Procedure

Following recovery from surgery (approximately one week) and for the remainder of the experiment, all subjects were maintained at approximately 70–80% on their free-feeding weights. The subjects were first trained to respond on a continuous reinforcement (CRF) schedule for food reinforcement (45 mg Noyes pellets). Daily sessions were 40 minutes in duration. Each animal was run at the same time of day and in the same cage seven days a week. After the subjects were responding on the CRF schedule (approximately 2–4 days) a FR schedule was introduced and gradually (2–3 weeks) increased to FR-40. After an additional 2–3 weeks of control FR-40 sessions, behavioral testing was begun.

Two of the 6-OHDA treated subjects never obtained stable baseline performance on the FR-40 schedule and were therefore excluded from the study. In the initial phase of the experiment (5–6 weeks) the effects of various doses of LSD (25, 50, 100 and 200 μg/kg) and DOM (0.25, 0.5, 1.0 and 2.0 mg/kg) were determined in each subject. In the second phase of the experiment (2–3 weeks) the effects of various doses of *d*-amphetamine (0.25, 0.5, 1.0 and 2.0 mg/kg) were determined in these subjects. In both phases the order of doses (and drugs) administered was completely randomized for each subject. All test drugs were administered IP immediately prior to the start of the FR-40 session. In these studies all drug test days were preceded by at least three non-drug days to avoid the development of tolerance.

Biochemical Measurements

After completion of the behavioral testing (3-1/2 months

TABLE 2

THE EFFECTS OF VEHICLE OR 6-OHDA ADMINISTRATION ON CONTROL FR-40 OPERANT RESPONSE PARAMETERS

	Reinforcements	Pause intervals
Vehicle	96.2 ± 14.9	56.3 ± 11.9
6-OHDA	50.6 ± 7.2* (53)	81.2 ± 12.1 (144)

Each value represents the mean ± SEM obtained from six 6-OHDA-treated or eight vehicle-treated animals. Average response parameters for each animal were determined as the mean of the 30–40 control (no injection) FR-40 sessions throughout the study. Numbers in parentheses represent percent of control (vehicle-treated) values.
*$p<0.05$, Student's t-test.

after administration of the neurotoxin) the subjects were sacrificed and the concentrations of 5-hydroxytryptamine (5-HT), dopamine (DA) and norepinephrine (NE) in various brain regions were determined fluorometrically [1,6].

Statistical Analysis

Drug effects were assessed by comparing the data from test days to the average of the three days prior to the test day (baseline). The Student's t-test for paired data was used to evaluate the effects of individual doses of the drugs. Dose-response relationships were compared by analysis of variance in a block design. The effects of 6-OHDA on regional biogenic amine concentrations were determined by Student's t-test for unpaired data. In all statistical evaluations $p<0.05$ was used as the criterion for statistical significance.

Drugs

d-Amphetamine sulfate was purchased from Sigma Chemical Co. DOM hydrochloride and LSD tartrate were obtained from NIDA; doses of these agents refer to the salts. 6-OHDA hydrobromide was obtained from Sigma Chemical Co.; dose refers to the salt in vehicle (0.1% ascorbic acid in saline).

RESULTS

Table 1 summarizes the results of the neurochemical determinations in these subjects. 6-OHDA treatment significantly decreased DA and NE concentrations in all regions tested, without altering the concentrations of 5-HT in any of the regions examined.

Table 2 indicates the effects of 6-OHDA-induced destruction of catecholamine neurons on control FR-40 responding. Control FR-40 responding in vehicle-treated subjects was characterized by a rapid, constant rate of responding (approximately 100 responses/min) throughout the session. 6-OHDA treatment decreased this rate of responding,

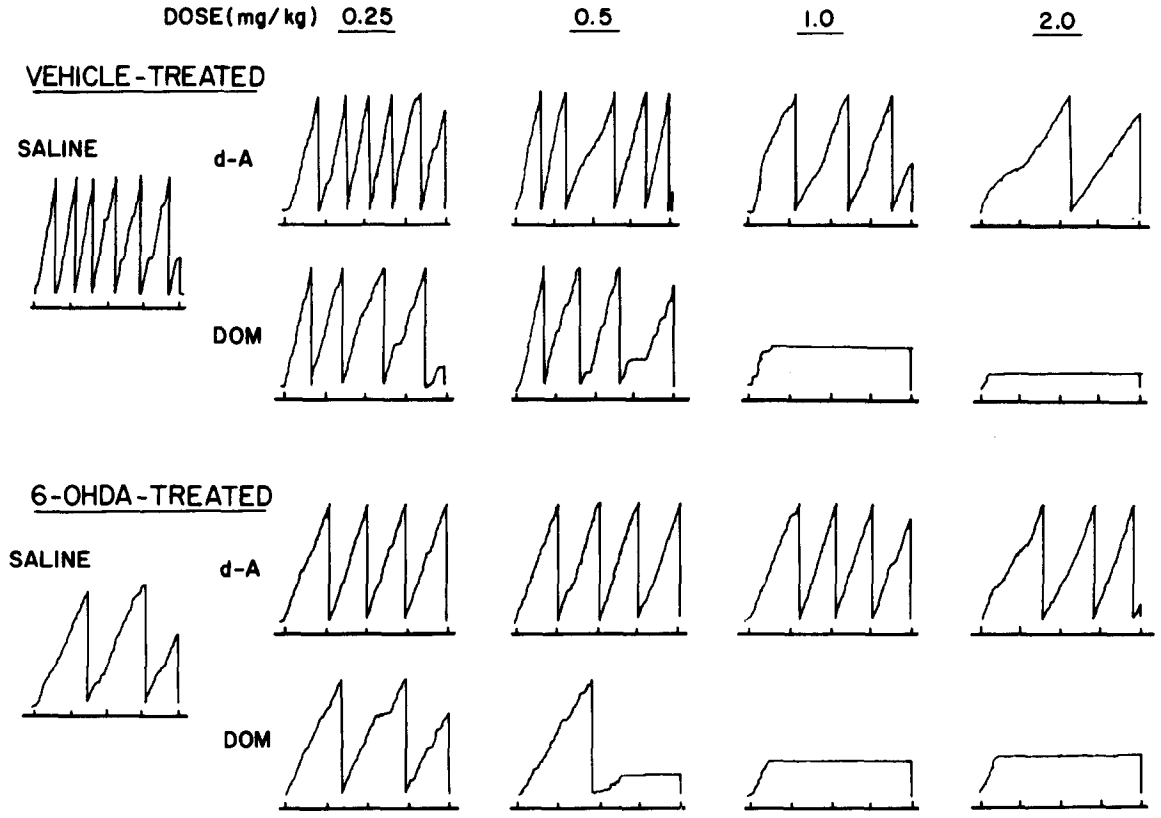

FIG. 1. Cumulative recordings illustrating the effects of saline, *d*-amphetamine (d-A) and DOM on FR-40 responding in one vehicle-treated and one 6-OHDA-treated rat. Top two rows: The effects of various doses of d-A (first row) and DOM (second row) administered to a vehicle-treated subject. Bottom two rows: The effects of various doses of d-A (third row) and DOM (fourth row) administered to a 6-OHDA-treated subject. Saline-treatment recordings illustrated on far left.

TABLE 3

RELATIONSHIP OF REINFORCEMENTS RECEIVED AND THE CHANGE IN PAUSE INTERVALS IN THE FR-40 SCHEDULE AFTER VARIOUS DRUG TREATMENTS

Treatment	N	Percent of control reinforcements	Change in number of pause intervals
0.5 mg/kg DOM, IP	8	44 ± 8*	94 ± 13*
100 μg/kg LSD, IP	8	45 ± 11*	102 ± 21*
1.0 mg/kg *d*-A, IP	8	54 ± 9*	18 ± 16
200 μg/10 μl 6-OHDA, ICV	6	53 ± 13*	25 ± 17

Each value represents the mean ± SEM. Percent of control reinforcements and change in pause intervals determined as described in Methods. See Methods for details of 6-OHDA treatment.

*$p<0.05$, Student's *t*-test for paired values.

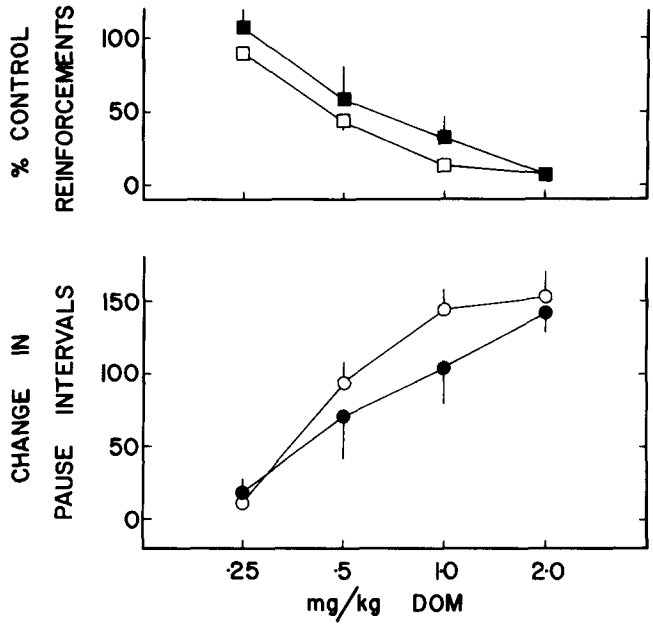

FIG. 2. The effects of DOM on FR-40 responding in vehicle- and 6-OHDA-treated rats. The change in pause intervals (circles) and percent of control reinforcements obtained (squares) produced by various doses of DOM during FR-40 operant sessions in vehicle (open symbols, n=8) or 6-OHDA-treated (filled symbols, n=6) subjects are illustrated. Change in pause intervals and percent of control reinforcements were determined by comparing data obtained on test days to the average of the 3 days prior to the test day (baseline). Percent of control reinforcements was chosen because of different baselines for reinforcements obtained in the vehicle- and 6-OHDA-treated subjects (see Table 2 for control FR-40 response parameters). Each symbol and vertical bar represents the mean±SEM. There were no significant effects of 6-OHDA pretreatment on the DOM dose-response pattern.

significantly decreasing the number of reinforcements obtained, withoug significantly altering the number of pause intervals produced.

Cumulative records illustrating the effects of saline, *d*-amphetamine and DOM in a vehicle- and a 6-OHDA-treated subject are depicted in Fig. 1. In the vehicle-treated subject *d*-amphetamine and DOM produced dose-dependent disruptions of FR-40 operant responding which are both qualitatively and quantitatively different. *d*-Amphetamine typically produced erratic intrasession response rates, while DOM produced "pausing." Destruction of catecholamine neurons with 6-OHDA decreased the rate of responding relative to control, but produced neither pausing nor erratic intrasession response rates. In the 6-OHDA-treated subjects the disruptive effects of *d*-amphetamine were greatly attenuated, while the effects of DOM were not altered.

Table 3 quantitates the "pause-producing" effects of LSD, DOM, *d*-amphetamine and 6-OHDA treatments at approximately ED_{50} doses in regard to their effects on reinforcements obtained. The hallucinogens (100 μg/kg LSD, 0.5 mg/mg DOM) produced dramatic increases (90–100 over baseline), whereas *d*-amphetamine (1.0 mg/kg) and 6-OHDA treatments produced only modest increases (18–25 over baseline) which were not significant.

Quantitative assessment of the effects of DOM on FR-40 responding in vehicle- and 6-OHDA-treated subjects is illustrated in Fig. 2. DOM produced a dose-dependent increase in pause intervals and a dose-dependent decrease in reinforcements obtained; these effects were not altered by 6-OHDA treatment.

Figure 3 illustrates the effects of LSD on FR-40 responding. As with DOM, this hallucinogen produced a dose-dependent increase in pause intervals and a decrease in reinforcements obtained. Again, 6-OHDA treatment failed to alter these effects.

Figure 4 quantitates the effects of *d*-amphetamine on FR-40 operant responding. In vehicle-treated subjects *d*-amphetamine produced a dose-dependent decrease in reinforcements obtained. Unlike the hallucinogens, this drug did not produce a significant change in pause intervals. Moreover, the effects of *d*-amphetamine on response rate were significantly attenuated by 6-OHDA treatment, $F(1,31)=6.73$; $p<0.05$.

DISCUSSION

In control subjects DOM and LSD produced disruptions of FR-40 responding characterized by periods of non-responding. The non-hallucinogen *d*-amphetamine also disrupted FR-40 responding, but this disruption was characterized by erratic intrasession response rates and not by pausing. Although both *d*-amphetamine and the hallucinogens produced dose-dependent decreases in the number of reinforcements obtained, only the hallucinogens produced a dose-dependent increase in the number of pause intervals. Thus, the pause interval timer can be used to differentiate

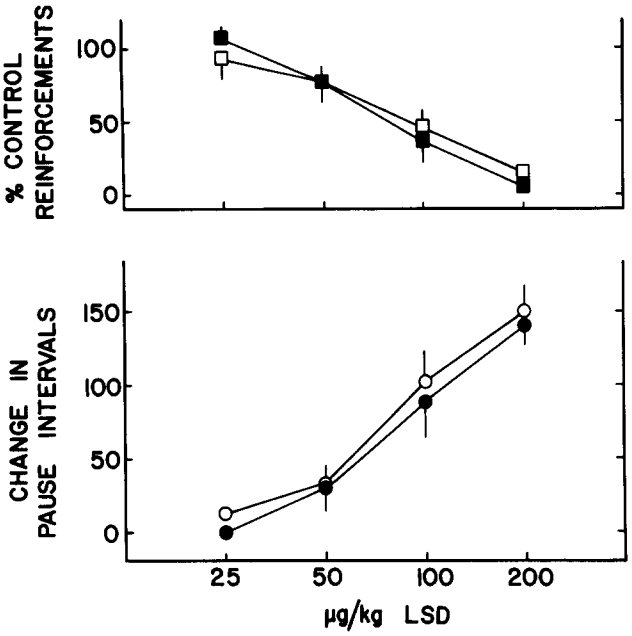

FIG. 3. The effects of LSD on FR-40 responding in vehicle- and 6-OHDA-treated rats. See Fig. 2 legend for other details. There were no significant effects of 6-OHDA on the LSD dose-response pattern.

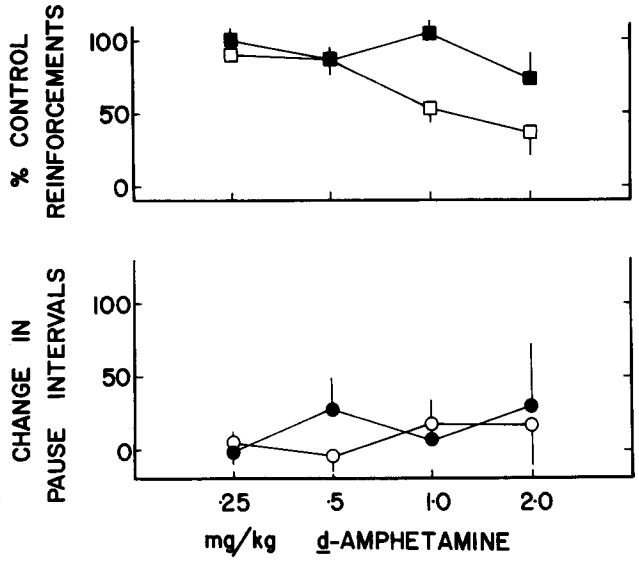

FIG. 4. The effects of *d*-amphetamine on FR-40 operant responding in vehicle- and 6-OHDA-treated rats. See Fig. 2 legend for other details. 6-OHDA treatment significantly attenuated the effects of *d*-amphetamine on the number of reinforcements obtained.

the behavioral effects of the hallucinogens from those of *d*-amphetamine in rats. Results from additional studies in our laboratory (unpublished) have revealed that the central nervous system depressant phenobarbital and the neuroleptic chlorpromazine also disrupt FR-40 responding by altering the rate of responding and not by producing "pausing." As with *d*-amphetamine, these agents produce a dose-dependent decrease in reinforcements obtained without a significant change in pause intervals until highly-sedative doses are administered.

Destruction of catecholaminergic neurons with 6-OHDA decreases the rate of FR-40 responding during control sessions, but does not result in "pausing." This effect is reflected in a decrease in the number of reinforcements obtained without a significant change in the number of baseline pause intervals produced relative to vehicle-treated animals. These data suggest that central catecholamine neuronal systems are important for maintaining rapid response rates.

The disruptive effects of *d*-amphetamine were significantly attenuated by 6-OHDA administration, while the pause-producing effects of DOM and LSD were unaltered by this treatment. Since 6-OHDA pretreatment altered baseline rates of reinforcement, there is a possibility that rate-dependency factors are involved. We examined potential rate-dependency for all the agents studied in both vehicle-control and 6-OHDA-lesioned rats. Tendencies for rate dependency were observed at a number of doses, but these effects were not statistically significant nor were they different in the 6-OHDA-treated animals compared to vehicle controls. These data suggest that the catecholamines are not involved in the FR-40 disruptive effects of these hallucinogens, but that normal catecholamine neuronal activity is required for the disruptive effects of *d*-amphetamine. The data regarding *d*-amphetamine are consistent with the established mechanism of action of this drug (i.e., release of catecholamines in the brain; [2,10]).

Although hallucinogens of both the phenethylamine and indolealkylamine classes are capable of producing some behavioral effects which are likely to be mediated by catecholaminergic neurons [8, 9, 12–14], the observation that destruction of catecholaminergic neurons fails to alter the FR-40 pause-producing effects of either DOM or LSD suggests that catecholamines are not involved in these behavioral effects of the two hallucinogens. Pretreatment with intraventricular 5,7-dihydroxytryptamine [3,7] or systemic *p*-chlorophenylalanine [5,7], on the other hand, does enhance the sensitivity of rats to the pause-inducing effects of LSD and DOM. Since these pretreatments are rather specific for disrupting central 5-HT neurons, the FR-40 "pausing" appears to relate better to these neurons. In addition, pretreatment with 5,7-dihydroxytryptamine did not alter the *d*-amphetamine dose-response pattern for disruption of FR-40 behavior (Commissaris *et al.*, unpublished results), suggesting that this latter agent does not exert its effects in this behavior via brain 5-HT pathways.

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