



A Characteristic Effect of Hallucinogens on Investigatory Responding in Rats

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Abstract. The disruption of the temporal distribution of investigatory responses by rats in a novel hole-board following lysergic acid diethylamide-25 (LSD), as described in a companion paper (Geyer and Light, 1979), was found to be a characteristic effect of a variety of hallucinogens. Similar effects were produced by indoleamine hallucinogens, such as LSD, N,N-dimethyltryptamine, and psilocin, and by phenylethylamine hallucinogens, such as mescaline or 2,5-dimethoxy-4-methylamphetamine (DOM). Congeners of DOM that are inactive in humans had no significant effects. Furthermore, of a variety of other psychoactive drugs tested, only apomorphine produced an effect similar to that of the hallucinogens. These results suggest that a simple behavioral measure of exploration in a hole-board may provide a useful animal model with which to examine the common effects of hallucinogens.

Key words: Hallucinogens — Hole-board — Exploration — Orienting response

In a companion paper (Geyer and Light, 1979) we reported that injections of a D-lysergic acid diethylamide-25 (LSD) produced a characteristic alteration in the distribution of investigatory responses over time during a 24-min test in a novel hole-board in which investigatory responses were quantified as the number of 'nose-pokes' into holes in the floor. When first placed in a novel hole-board rats readily poked their noses in the holes; the frequencies and the durations of these responses were monitored as the operational measure of investigatory behavior. Responses to other stimuli in the chamber have been usually ignored by investigators, although locomotor activity is sometimes monitored simultaneously (Ljungberg and Ungerstedt, 1976). The reliability and validity of the hole-board as a measure of exploration has been demonstrated through high degrees of test-

retest predictability, sensitivity to novelty, and rapid habituation (File and Wardill, 1975a, b). Compared to controls, rats treated with 40–160 µg/kg LSD exhibited fewer responses during the first 8 min in the hole-board and a greater percentage of their responses during the third 8-min segment. In some experiments, LSD-treated rats made significantly more nose-pokes during the third 8-min segment than did controls. These effects resulted in a significant interaction between the drug and time in the hole-board which was shown to be independent of changes in general activity and unrelated to the time course of LSD's effect. Additional experiments demonstrated that the change produced by LSD in the temporal distribution of responding was due primarily to an interaction between the drug effect and the stress or other stimuli associated with the animal being handled and placed in the novel hole-board. The results were interpreted as reflecting an LSD-induced increased sensitivity to handling stress rather than a specific effect of LSD on exploratory behavior itself.

Here we describe a series of studies designed to assess the generality of this phenomenon with respect to other hallucinogens. Using a standard paradigm, we compared the effects of a variety of hallucinogens, including both indoleamine and phenylethylamine derivatives, with several representatives of disparate drug classes. The results indicate that the characteristic interaction between the effect of LSD and time in the hole-board is also found with other hallucinogens. Of the nonhallucinogenic drugs tested, only apomorphine produced a similar alteration in the distribution of nose-pokes over time.

Materials and Methods

Animals. Experimentally naive male Sprague-Dawley rats (250–400 g), (Hilltop Labs, Scottsdale, PA) were housed in groups of five in a temperature regulated (25 ± 2 °C) animal room on a 12–12 h light-dark cycle (lights on 6 a.m.). Food and water were continuously available. After 5–9 days in the animal room the rats were transferred to the laboratory for at least 1 h before testing. All behavioral testing was performed 7 a.m. – 7 p.m.

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Apparatus. Exploratory activity was monitored in two wooden hole-board chambers (27.5 × 66 × 47.5 cm). The walls extended 20.5 cm below the floor. Three 3.7 cm holes were cut 16.5 cm apart along the long axis of the floor so that the animal must leave the perimeter of the chamber to investigate the holes. Two infrared light-emitting diodes were aligned under the holes 9 mm below the surface of the floor. Interruption of either light beam triggered both cumulative duration and response counters and lit a small 0.36 W white light bulb located under the floor. The light provided the rat with a visual feedback stimulus for the duration of the nose-poke. A dim red light centered on the chamber ceiling illuminated the interior.

Procedure. The typical experiment involved 40 rats randomly divided into four groups of ten. Each rat received an IP injection of 1.0 ml/kg isotonic saline or one of three doses of the test drug. All animals in a given experiment were tested on the same day according to a predetermined counterbalanced order. After injection each animal was placed in an individual cage for the pretest interval (see Table 1). The 24-min test began when the animal was gently placed into a corner of the hole-board chamber and the lid was closed. Care was taken to be consistent in the handling of the animals, and the experimenter placing an animal generally had no knowledge of the animal's treatment. The hole-boards were cleaned with soap and water between tests.

Data Analysis. The cumulative number of responses and total duration were automatically recorded on a print-out counter and on magnetic tape. The tape was read by a Burroughs 6700 computer and the data were condensed into three 8-min segments. One-way analyses of variance (ANOVA) were performed on the number of responses in each 8-min segment and the total number of responses. Specific comparisons of each drug group with the common control

group were made by Dunnett's method (Winer, 1971). Two-tailed sampling distributions were used for determining significance unless otherwise stated.

To test for differential patterns of responding across the 24-min session, two-way mixed ANOVAs were performed with three 8-min segments being repeated measures.

To describe further the distribution of responding over time, we calculated for each rat the percentage of its total responding that occurred in each eight 8-min segment. These percentages have been found to be consistent for control groups, independent of the more variable overall rate of responding. Comparisons between these percentage scores were made by the non-parametric Mann-Whitney U-test (Siegal, 1956).

Drugs. The following drugs were tested: LSD Tartrate; N,N-dimethyltryptamine fumarate (DMT); psilocin; mescaline HCl; 2,5-dimethoxy-4-methylamphetamine HCl (DOM); 2,5-dimethoxy-4-ethylamphetamine HCl (DOET) (Research Technology Branch, NIDA); 2,5-dimethoxy-4-propylamphetamine HCl (DOPR); 2,5-dimethoxyamphetamine HCl (DMA); 2,5-dimethoxy-4-amylamphetamine HCl (DOAM); *d*-amphetamine SO₄ (Sigma); apomorphine HCl (Merck); clonidine HCl; chlorimipramine HCl (CMI) (Geigy Pharmaceuticals); methysergide maleate (Sandoz); *dl*-parachlorophenylalanine methyl ester HCl (Sigma); and scopolamine HBr (Sigma). Doses (see Table 1) refer to the salt form. Drugs were generally prepared and dissolved in isotonic saline immediately before use.

Results

The results are summarized in Table 1 as the percentage of total responses in each 8-min segment and the total

Table 1. Responses in the hole-board test. Each drug and dose condition was tested in ten animals (nine in experiments 4 and 13, and 14 in experiment 14). Pretest interval is in min and dose is in mg/kg unless otherwise specified

Drug	Pretest interval (min)	Dose	%Total/8-min interval			Total	Drug- × -time (F)
			1	2	3		
Experiment 1							
LSD (lysergic acid diethylamide)	10	0	58	29	13	52	4.9
		40 µg	49	22	29	41	
		80	36	15	39 ^b	30	
		160	46 ^a	21	33	18 ^a	
Experiment 2							
DMT (N,N-dimethyl- tryptamine)	3	0	57	21	22	42	NS ^c
		0.25	49	33	18	38	
		0.50	50	26	24	26	
		1.0	48	20	32	46	
Experiment 3							
DMT	3	0	59	22	19	68	7.0
		2	35 ^a	24	41 ^b	22 ^a	
		4	36 ^a	28	38	29 ^a	
Experiment 4							
Psilocin	10	0	59	26	15	58	4.9
		2.5	63	18	19	24 ^a	
Experiment 5							
Mescaline	15	0	58	28	14	49	2.5
		10	56	19	25	26	
		20	54	20	26 ^b	22 ^a	

Table 1 (continued)

Drug	Pretest interval (min)	Dose	% Total/8-min interval			Total	Drug- × -time (F)
			1	2	3		
Experiment 6							
DOM (2,5-dimethoxy-4-methylamphetamine)	10	0	54	27	19	54	5.1
		0.25	61	28	11	52	
		1.0	39	34	27	28 ^a	
Experiment 7							
Saline	10	0	56	27	17	58	5.5
DOET (2,5-dimethoxy-4-ethylamphetamine)		1.0	42	20	38 ^b	40	
DOPR (2,5-dimethoxy-4-propylamphetamine)		1.0	43	25	32	40	
Experiment 8							
Saline	10	0	47	32	21	79	NS ^c
DMA (2,5-dimethoxy-amphetamine)		4	58	30	12	60	
DOAM (2,5-dimethoxy-4-amylamphetamine)		4	41	36	23	75	
Experiment 9							
Amphetamine	5	0	49	32	19	56	NS ^c
		0.5	39	32	29	104	
		1.0	37	35	28	137 ^b	
		2.0	28 ^a	36	36 ^b	114	
Experiment 10							
Apomorphine	3	0	51	31	18	72	3.0
		0.25	57	21	21	38 ^a	
		0.50	47	32	21	43 ^a	
		1.0	37 ^a	24	39 ^b	33 ^a	
Experiment 11							
CMI (chlorimipramine)	15	0	49	30	21	78	NS ^c
		5	58	22	20	40	
		10	54	36	10	36 ^a	
		20	64	22	14	33 ^a	
Experiment 12							
Clonidine	3	0	57	28	15	72	NS ^c
		12.5 μg	75 ^b	17	8	65	
		25	73 ^b	23	4	48	
		50	87 ^b	13 ^a	0 ^a	30 ^a	
Experiment 13							
Methysergide	15	0	61	26	13	67	NS ^c
		1.5	57	16	27 ^b	37	
		3.0	55	31	14	67	
Experiment 14							
PCPA (parachloro-phenylalanine)	24 h	0	54	26	20	47	NS ^c
		2 × 250	56	30	14	23 ^a	
Experiment 15							
Scopolamine	15	0	66	27	7	45	NS ^c
		0.5	40	32	28	56	
		1.0	45	35	20	79 ^b	
		2.0	44	30	26 ^b	86 ^b	

^a Significantly below corresponding control ($P < 0.05$)^b Significantly above corresponding control ($P < 0.05$)^c Not significant

number of responses. Our criterion for concluding that a test drug mimics the effect of LSD is the presence of a significant drug $\times$ time interaction term from the two-way mixed ANOVA. Such an interaction indicates that the temporal distribution of nose-poke responding by the drug-treated groups differs from that of the saline control group. The source of this interaction, for drugs having an LSD-like effect, can typically be recognized by the significant reduction in nose-poke frequency during the first 8 min coupled with a small increase in response frequency from the second to the third 8-min segment, a pattern that has occurred in only 1 of 22 control groups (experiment 2, Table 1).

The first four experiments listed in Table 1 involve hallucinogens containing an indoleamine structure: LSD; DMT; and psilocin. All three of these drugs produced significant drug $\times$ time interactions. The LSD experiment is the same as that shown in Fig. 1 of Geyer and Light (1979). The second experiment shows that low doses of DMT (≤ 1.0 mg/kg) had no significant effects on any measure. However, the higher doses of DMT, shown in experiment 3, produced the same pattern of responding as seen with LSD: A significant drug $\times$ time interaction and a significant reduction in response frequency during the first 8 min (cf. File, 1977a). Both the 2.0 and 4.0 mg/kg doses of DMT also reduced overall response frequency. Similarly, psilocin produced a significant overall reduction and a significant drug $\times$ time interaction at a dose of 2.5 mg/kg. Higher doses of psilocin were tested (5.0 and 10.0 mg/kg), but the data are not included because the animals became ataxic.

The effects of four phenylethylamine-derived hallucinogens are shown in the next three experiments of Table 1. With all four drugs there were significant reductions in the first 8 min and significant interactions with time. Only the highest dose of mescaline (20 mg/kg) significantly reduced the total number of responses. The other three drugs (DOM, DOET, and DOPR) are substituted phenylisopropylamines that differ only in the number of carbons in the alkyl side chain at the 4-position of DOM. We also tested two other congeners of DOM, having zero (DMA) or five (DOAM) carbon chains. At 4.0 mg/kg neither congener had any significant effect on hole-board responding.

The next six drugs listed in Table 1 are not considered to be hallucinogens. Low doses of *d*-amphetamine significantly increased the overall frequency of nose-pokes, especially at a dose of 1.0 mg/kg, but did not produce an interaction with time. Apomorphine significantly reduced the number of nose-pokes. Although we did not quantify locomotor activity, routine observation of the animals through wide angle lenses suggested a moderate increase in

ambulation concomitant with the reduction in nose-pokes (cf. Ljungberg and Ungerstedt, 1976). Frequently rats were seen to walk near or across the holes without taking any apparent notice of them. Apomorphine, however, did produce a significant drug $\times$ time interaction, especially apparent in the increase in response frequency from the second to third segment exhibited by the animals given 1.0 mg/kg.

Three of the nonhallucinogenic drugs we tested reduced the initial response frequency without yielding a significant interaction with time. Both the alpha-adrenergic agonist clonidine and the serotonergic reuptake inhibitor CMI produced dose-dependent reductions in nose-pokes throughout the 24-min session. Doses of clonidine above 50 μ g/kg were not tested because they produce marked ataxia (D. S. Segal, personal communication). We have also found recently that 20 mg/kg desmethylinipramine HCl has the same effect as 20 mg/kg CMI.

In addition to CMI, two other drugs known to affect serotonergic systems were tested. Both methysergide, a putative serotonergic receptor blocker (Segal, 1976), and PCPA, an inhibitor of tryptophan hydroxylase (Koe and Weissman, 1966), failed to produce significant interactions with time. The significant effect of 1.5 mg/kg methysergide during the first 8 min was not reproduced either by higher doses (Table 1) or by a replication experiment (not shown). PCPA consistently reduced the frequency of nose-pokes relative to controls throughout an extended 48-min test.

The last experiment shown in Table 1 shows the effects of scopolamine on hole-board responding. Scopolamine produced a dose-dependent increase in the frequency of nose-pokes throughout a 54-min test session, but did not produce a significant drug $\times$ time interaction.

Discussion

Our results demonstrate that a variety of hallucinogens share a common effect in altering the distribution of responses during a 24-min test in a hole-board. Rats treated with either indoleamine or phenylethylamine hallucinogens exhibit a reduced frequency of nose-pokes during the first 8 min in the hole-board which is not maintained throughout the test. Rather, their frequencies generally increase from the second to third 8-min segment, resulting in a significant drug $\times$ time interaction. This increase in the percentage of responses in the third segment clearly distinguishes this effect of hallucinogens from an impairment of movement or other forms of sedation produced by drugs such as clonidine, CMI, and PCPA, or by lesions of the noradrenergic locus coeruleus (Geyer et al., 1976). With regard to LSD, and presumably the other halluci-

nogens, this pattern of hole-board responding is unrelated to either locomotor activity levels or the time course of the drug action and is due primarily to an increased sensitivity of the drug-treated animals to the stimuli associated with being handled and placed into the novel environment (Geyer and Light, 1979).

In addition to the indoleamine hallucinogens LSD, DMT, and psilocin, we tested a homologous series of phenylethylamines having known potencies in humans. Based on the structure of DOM, these congeners vary only in the number of carbons in the alkyl chains at the 4-position on the phenyl ring; only those with from one to three carbon chains are active as hallucinogens in man (Shulgin and Dyer, 1975). In our animal test also, only DOM, DOET, and DOPR produced significant alterations in the temporal distribution of responding in the hole-board. With either zero (DMA) or five (DOAM) carbons in the 4-position, the compounds are inactive in man and in the hole-board test. Mescaline, another phenylethylamine-derived hallucinogen, also produced effects similar to those of LSD on hole-board responding.

In general, the effects we have found with the other psychoactive drugs included for comparison confirm the previous results of others. Scopolamine increased nose-poke frequency in a manner consistent with an impairment of habituation in both our hole-board and in the slightly different device used by Feigley and Hamilton (1971). Low doses of amphetamine are reported to increase hole-board responding (Ljungberg and Ungerstedt, 1976; Mekanjuola et al., 1977), although reductions with amphetamine, particularly at higher doses, have also been noted (File, 1977b). Treatment with PCPA reportedly has only marginal effects on the frequency of nose-pokes during a 10-min test (File, 1977b), but consistently reduced nose-poking throughout our 48-min test (Table 1). We tested PCPA for 48 min because it increases locomotor activity only after 30 min in the test chamber (Tenen, 1967; Fibiger and Campbell, 1971). As with other manipulations in which changes in locomotion do not parallel changes in nose-poking (Geyer and Light, 1979; Ljungberg and Ungerstedt, 1976), the PCPA-treated rats did not show any late increase in responding to the holes.

Of the nonhallucinogenic drugs tested, only apomorphine produced a pattern of behavioral effects similar to that of the hallucinogens. We have also found recently that apomorphine, like phenylethylamine hallucinogens, increases tactile startle responding (Geyer et al., 1978). In a study of three other potential screening tests for hallucinogens, Silva and Calil (1975) reported also that this dopaminergic agonist was the most difficult to distinguish from the hallucinogens. Furthermore, Nichols (1976) has discussed parallels in chemical structure between LSD and apomorphine.

The possibility that dopaminergic systems are involved in some of the behavioral affects of hallucinogens is indicated by several lines of evidence (Trulson et al., 1977; Pieri et al., 1974; Kelly and Iverson, 1975). It is possible that the characteristic effect of hallucinogens on behavior in the hole-board is related to some common effect of these drugs and apomorphine on dopaminergic function.

Neither electrolytic nor 5,7-dihydroxytryptamine lesions of the midbrain raphe nuclei produce similar effects in the hole-board, nor do they preclude the effects of either LSD or apomorphine on hole-board behavior (Petersen and Geyer, unpublished observations). Likewise, lesions of the noradrenergic locus coeruleus reduce the frequency of nose-pokes, but do not produce an LSD-like change in the distribution of responses (Geyer et al., 1976). Rather, the effects of locus coeruleus lesions are similar to those of clonidine, at doses reported to inhibit firing of cells within the locus coeruleus (Svensson et al., 1975). Clonidine and locus coeruleus lesions also have comparable effects on tactile startle responding (Geyer, 1978; Geyer et al., 1976). The only manipulation reported to produce an interaction with time in the chamber similar to that following hallucinogens is lesions of the septal nuclei (Feigley and Hamilton, 1971). Septal lesioned rats are known to be hyperreactive to handling stress (Fried, 1972; Bernard et al., 1975). As in the case of LSD (Geyer and Light, 1979), the effects of septal lesions on the distribution of responding in the hole-board may be attributable to an interaction with handling. The neurochemical and neuroanatomical systems involved in the effects of hallucinogens on hole-board responding cannot yet be specified, but further investigation of the relevance of dopaminergic systems seems warranted.

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