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The Relative Effectiveness of Several Hallucinogens in Disrupting Maze Performance by Rats*

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Summary. The effects of hallucinogens and their nonhallucinogenic analogs on the performance of rats on the Lashley-III underwater maze were evaluated. After the rats were trained to swim through the maze without an error, they were injected i.p. with test compounds or saline solution and tested at various intervals after the injection. A substantial number of the treated animals made one or more errors. The time of peak effect of lysergic acid diethylamide (LSD-25) and mescaline was 15 min after the injection; that of 2-brom-lysergic acid diethylamide (BOL-148) and of several trimethoxyamphetamines (TMA) was 30 min; and that of d-amphetamine was 45 min. The dose-response curves showed that the effect of the compounds was a function of dose. The median effective doses (ED 50) in μmol/kg were: LSD-25 (0.3); TMA-2 (12.8); BOL-148 (15.9); d-amphetamine (32.0); TMA (33.4); TMA-6 (46.0); TMA-3 (52.1); mescaline (91.0); and TMA-4 (94.0). The rank order of potency of the compounds is, in general, similar to that derived from the data on man.

Key-Words: Lysergic Acid Diethylamide — Mescaline — Trimethoxyamphetamines — Potency — Animal Behavior.

Several studies have shown that hallucinogens are more effective than their respective nonhallucinogenic analogs in disrupting performance of animals. For example, Appel and Freedman (1965) found that lysergic acid diethylamide (LSD-25) is approximately ten times more effective than its nonhallucinogenic analog, 2-brom-lysergic acid diethylamide (BOL-148), in disrupting the lever-pressing performance of the rat deprived of food. Moreover, LSD-25 was found to be more effective than BOL-148 in inhibiting aggressive behavior of isolated mice toward non-isolated mice (Uyeno and Benson, 1965; Uyeno, 1966a), and of rats in a

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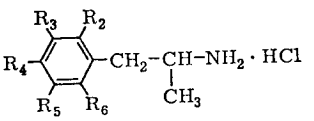
food competition situation (Uyeno, 1966b). Furthermore, LSD-25 and hallucinogenic trimethoxyamphetamines (Shulgin, 1964) were reported to be more effective than their respective nonhallucinogenic analogs in increasing the starting latency of rats tested in an underwater swim alley (Uyeno, 1968) and in disrupting the visual size-discrimination performance of squirrel monkeys, tested in the Wisconsin General Test Apparatus (Uyeno *et al.*, 1968).

The present experiment was designed to study the effects of the compounds on the performance of a previously learned maze-habit of the rat. The compounds were LSD-25 and hallucinogenic trimethoxyamphetamine hydrochlorides (TMA) (Peretz *et al.*, 1955: Shulgin, 1964; Shulgin *et al.*, 1961), and their nonhallucinogenic analogs, BOL-148 and 2,3,4-trimethoxyamphetamine hydrochloride (TMA-3) (Shulgin, 1964). Mescaline sulfate dihydrate and d-amphetamine hydrochloride were evaluated as standard reference compounds. The structural formulae of d-amphetamine hydrochloride and its derivatives used in the present study are shown in Table 1.

Table 1. *d-Amphetamine and its derivatives*



Compound	R_2	R_3	R_4	R_5	R_6	Hallucinogenic Properties
d-Amphetamine HCl	H	H	H	H	H	No
TMA · HCl	H	CH_3O	CH_3O	CH_3O	H	Yes[a]
TMA-2 · HCl	CH_3O	H	CH_3O	CH_3O	H	Yes[a]
TMA-3 · HCl	CH_3O	CH_3O	CH_3O	H	H	No[a]
TMA-4 · HCl	CH_3O	CH_3O	H	CH_3O	H	—
TMA-6 · HCl	CH_3O	H	CH_3O	H	CH_3O	Yes[b]

[a] Shulgin, 1964; [b] Shulgin, personal communication.

Methods

The Lashley-III underwater maze, a modification of Lashley-III maze (Lashley, 1929), consisted of a black sheet metal tank (115 cm by 62 cm by 18 cm) with removable sectioned plastic covers. At one side, a part of the tank (20 cm by 12 cm by 18 cm) jutted out to form an entrance area. Similarly, on the opposite side, the tank jutted out to form an exit. In the tank three black partitions were erected parallel to the sides to form four channels and four culs-de-sac at each end of the channels. Each partition had an opening (12 cm by 15 cm), allowing the animals to traverse from one channel to the next. A detachable plastic

start box with a wire mesh floor and a guillotine door at the front end, was used to submerge each animal. Lifting the door activated a timer that stopped when the animal swam past a photoelectric beam at the escape point. This measure of time was considered to be swimming time. The tank was filled with 23° C water to the height of 18 cm, submerging the channels completely.

Two hundred eighty male rats of the Wistar strain weighing 180 to 200 g were housed individually and fed ad libitum. During the first stage of training, each animal was placed in the start box, submerged in the last channel near the exit, and trained to avoid only one blind alley as it searched for the exit. They were given distributed trials (with an inter-trial interval of approximately 45 min) until they reached the criteria of two or less errors on three successive trials and a swimming time of 30 sec or less during each of the three criterion trials. During the second stage, they were submerged further away from the exit (i.e., at the entrance to the last channel) and trained to avoid two blind alleys. During the third, fourth, fifth, sixth, and seventh stages, the animals were submerged further away from the exit and trained to avoid three, four, five, six, and seven blind alleys, respectively. During these training stages, the criteria of learning was the same as the one selected for the first stage. During the eighth (final) stage each animal swam the full length of the maze with eight blind alleys. They were given training trials until they reached the criteria of four successive errorless trials and a swimming time (during each of the four errorless criterion trials) of 15 sec or less. When they achieved the criteria, they were assigned to nine main experimental groups (based on 9 compounds) of 30 animals each and a control group of 10 animals. The 30 animals in each main experimental group were assigned to three experimental subgroups (three dose levels) of 10 animals each.

Each experimental animal was injected i.p. with a predetermined dose of a compounds according to the main experimental group and to the dose level to which it was assigned (Table 2). A standard volume of 2.5 ml/kg of drug-saline solution or saline solution alone was injected in each experimental or control animal, respectively. All animals were given the maze test at 5, 15, 30, 45, 60, 75, 90 and 120 min after the injection.

Results

A substantial number of animals in each main experimental group made one or more errors, but none of the control animals made an error. For each dose level, the percentage of animals that made one or more errors is shown in Table 2. The time of peak effect was 15 min for LSD-25 and mescaline; i.e., the percentage of animals that made one or

Table 2. *Percentage of animals that made one or more errors in the Lashley-III underwater maze*

Compound	Dose[a] (i.p.)		Postinjection Time (min)							
	μmol/kg	mg/kg	5	15	30	45	60	75	90	120
LSD-25	0.2	0.063	0	40	20	10	0	0	0	0
	0.4	0.125	40	50	20	10	0	0	0	0
	0.8	0.25	80	80	40	30	10	0	0	0
BOL-148	6.2	2.5	10	10	20	20	0	10	10	0
	12.4	5.0	30	40	30	20	20	20	10	20
	24.9	10.0	20	40	70	70	60	40	50	40
d-Amphetamine · HCl	17.5	3.0	0	10	0	30	10	20	0	0
	35.0	6.0	0	10	40	50	30	20	10	0
	52.4	9.0	10	30	50	70	70	40	40	20
TMA · HCl	12.0	3.13	10	10	20	40	20	10	20	0
	23.9	6.25	10	40	40	40	30	10	0	0
	47.8	12.5	20	70	60	20	10	0	0	0
TMA-2 · HCl	6.0	1.56	20	0	20	20	0	10	0	0
	12.0	3.13	40	40	50	30	30	30	10	0
	23.9	6.25	20	70	70	50	30	10	0	0
TMA-3 · HCl	23.9	6.25	10	40	30	30	20	20	10	10
	47.8	12.5	10	40	40	30	30	10	0	0
	95.6	25.0	40	70	70	40	20	0	0	0
TMA-4 · HCl	38.2	10	20	30	20	40	20	0	0	0
	76.5	20	30	30	40	30	10	0	0	0
	114.7	30	10	50	60	10	10	0	0	0
TMA-6 · HCl	38.2	10	20	50	40	30	10	0	0	0
	76.5	20	20	60	70	30	10	30	20	0
	114.7	30	30	70	80	60	20	10	0	0
Mescaline	72.4	25	20	30	30	30	20	10	0	20
Sulfate Dihydrate	115.8	40	40	70	70	50	30	50	20	20
	144.8	50	40	90	70	70	70	60	40	30
Saline	—	—	0	0	0	0	0	0	0	0

[a] Ten animals were tested at each dose level.

more errors was highest during the trial given 15 min after the injection, The time of peak effect was 30 min for BOL-148 and TMA compounds. and 45 min for d-amphetamine.

The mean swimming times of the experimental animals, administered a low dose of one of the compounds, were not significantly different from those of the control animals. The medium dose of TMA-4, of TMA-6, and of mescaline increased significantly the mean swimming times at the time of peak effect. At the high doses, all but TMA and TMA-2, increased significantly the mean swimming times.

Since the error score was more sensitive than the swimming time, the former was used to plot dose-response curves and to determine the

the Lashley-III

nin)		
75	90	120
0	0	0
0	0	0
0	0	0
10	10	0
20	10	20
40	50	40
20	0	0
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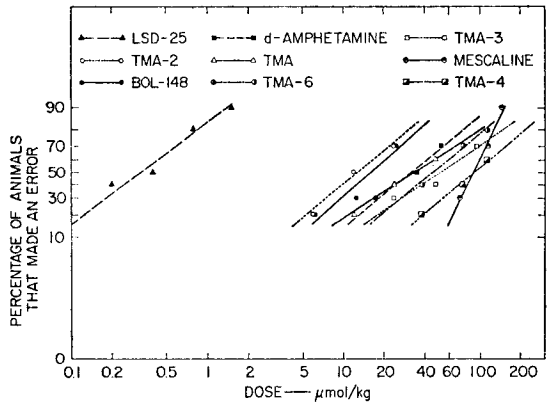

Fig. 1. Dose-response curves, showing effects of the compounds on performance of rats in the Lashley-III underwater maze

Table 3. *An analysis of error score in the Lashley-III underwater maze*

Compound	ED50[a]	95% Confidence Limits
	μmol/kg	
LSD-25	0.3	0.2— 0.6
TMA-2	12.8	7.5— 21.8
BOL-148	15.9	9.8— 25.9
d-Amphetamine	32.0	18.9— 54.1
TMA	33.4	17.6— 63.5
TMA-6	46.0	27.7— 76.4
TMA-3	52.1	27.6— 98.5
Mescaline	91.0	71.1—116.5
TMA-4	94.0	57.0—155.1

[a] The ED50 refers to the dose which disrupt an errorless performance in 50% of the animals.

median effective doses (ED50). The percentage of animals that made an error at the time of peak effect was plotted against dose on logarithmic probability paper (Fig. 1). The dose-response curves show that the effect of the compound was a function of dose. Table 3 presents the ED50 and the 95% confidence limits for each compound, calculated according to the graphic probit method of Litchfield and Wilcoxon (1949).

The relative effectiveness of the compounds was determined by derivation of potency ratios, according to the method of Litchfield and Wilcoxon (1949). The potency ratio (P.R.) between two compounds was calculated by dividing the ED50 of the less potent compounds (i.e., the one with the larger ED50) by the ED50 of the more potent compound. The potency ratio was compared with the factor for the 19/20 error of the

Table 4. *Relative effectiveness of compounds*

Compound		TMA-2	BOL-148	d-Amphet-amine	TMA	TMA-6	TMA-3	Mescaline	TMA-4
LSD-25	P. R.[a]	38.8	48.2	97.0	101.2	139.4	157.9	275.8	284.9
	Sig.[b]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	C. L.[c]	(17.8—84.6)	(22.7—102.1)	(44.5—211.4)	(42.9—238.9)	(64.5—301.1)	(66.9—372.6)	(148.3—512.9)	(133.1—609.6)
TMA-2	P. R.		1.2	2.5	2.6	3.6	4.1	7.1	7.3
	Sig.		No	Yes	Yes	Yes	Yes	Yes	Yes
	C. L.			(1.2—5.3)	(1.1—6.0)	(1.7—7.5)	(1.8—9.3)	(4.0—12.8)	(3.6—15.2)
BOL-148	P. R.			2.0	2.1	2.9	3.3	5.7	5.9
	Sig.			No	No	Yes	Yes	Yes	Yes
	C. L.					(1.4—5.9)	(1.5—7.3)	(3.3—9.9)	(2.9—11.9)
d-Ampheta-mine	P. R.				1.0	1.4	1.6	2.8	2.9
	Sig.				No	No	No	Yes	Yes
	C. L.							(1.6—5.1)	(1.4—6.1)
TMA	P. R.					1.4	1.6	2.7	2.8
	Sig.					No	No	Yes	Yes
	C. L.							(1.4—5.4)	(1.3—6.3)
TMA-6	P. R.						1.1	2.0	2.0
	Sig.						No	Yes	Yes
	C. L.							(1.1—3.5)	(1.0—4.2)
TMA-3	P. R.							1.8	1.8
	Sig.							No	No
	C. L.								
Mescaline	P. R.								1.0
	Sig.								No
	C. L.								

[a] Potency Ratio = $\frac{ED_{50} \text{ of compound in the top row}}{ED_{50} \text{ of compound in the first column}}$.

[b] Significance at the 5% level.

[c] 95% Confidence Limits.

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Compound	
TMA-2	
TMA-6	
TMA	
TMA-4	
TMA-3	

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(Shulgin

ratio (i.e., f P.R. obtained from a monograph of Litchfield and Wilcoxon). If the potency ratio exceeded the value of f P.R., the two compounds being compared, differed significantly in potency. The lower value of the 95% confidence limits of the potency ratio was calculated by dividing the potency ratio by f P.R. and the upper value was computed by multiplying the potency ratio by f P.R. In Table 4 the compounds are arranged in descending order of potency in the first column and again in the top row from left to right. LSD-25 was significantly more effective than any of the other compounds. Of the three hallucinogenic trimethoxyamphetamines (TMA, TMA-2, and TMA-6), only TMA-2 was significantly more effective than the nonhallucinogenic analog, TMA-3.

TMA-3	P.R.	1.8	1.8						
	Sig.	No	No						
	C.L.								
Mescaline	P.R.			1.0	1.8				
	Sig.			No	No				
	C.L.								

[a] Potency Ratio = $\frac{ED_{50} \text{ of compound in the top row}}{ED_{50} \text{ of compound in the first column}}$.

[b] Significance at the 5% level.

[c] 95% Confidence Limits.

Discussion

The present finding that the hallucinogens and their nonhallucinogenic analogs disrupt learned maze performance in a dose-dependent manner, is consistent with the previous finding that these compounds disrupt learned visual size-discrimintaion performance of the squirrel monkey in a dose-dependent manner (Uyeno *et al.*, 1968). The present study is also in accord with earlier studies which demonstrated that LSD-25 is more effective than BOL-148 in decreasing the bar-pressing response of food-deprived rats (Appel and Freedman, 1965), and in inhibiting the aggressive and dominance behavior of mice (Uyeno and Benson, 1965; Uyeno, 1966a) and rats (Uyeno, 1966b). In general, the rank order of potency of the compounds based on the disruption of learned maze performance is consistent with that based on the effectiveness in increasing the starting latencies in straight channel under water swimming, reported by Uyeno (1968). However, the ED50 of LSD-25 (0.3 μmol/kg), based on the error scores in the present study is

Table 5. *Comparative potencies of trimethoxyamphetamines based on mescaline = 1*

Compound	Rat[a] (Lashley-III maze)	Squirrel Monkey[a,b] (Size-Discrimination) (Easy Problem)	Human[c]
TMA-2	7.1	5.4	17.0
TMA-6	2.0	3.2	10.0
TMA	2.7	1.8	2.2
TMA-4	1.0	1.1	4.0
TMA-3	1.8	0.6	< 2.0

[a] Potency ratio for animals = $\frac{\text{ED50 of mescaline}}{\text{ED50 of the compound evaluated}}$.

[b] Uyeno *et al.*, 1968.

[c] Potency ratio for humans = $\frac{\text{Effective dosage of mescaline}}{\text{Effective dosage of the compound evaluated}}$.

(Shulgin *et al.*, 1969).

only one-sixth of the value of ED 50 based on the starting latencies in the previous study. Therefore, the Lashley-III underwater maze seems to be more sensitive than the straight channel underwater alley in detecting the subtle behavioral effects of LSD-25.

Shulgin (1964) reported that TMA-2 was significantly more effective than TMA in producing hallucinations in man, whereas TMA-3 was hallucinogenic. The present study also shows that TMA-2 is significantly more effective than TMA and TMA-3. Table 5 shows that the rank order of effectiveness of the trimethoxyamphetamines in the present study is, in general, similar to that derived from the previous data on Squirrel monkeys (Uyeno *et al.*, 1968) and on man (Shulgin *et al.*, 1969).

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