THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 159, No. 1
Printed in U.S.A.

HALLUCINOGENIC COMPOUNDS AND SWIMMING RESPONSE[1]

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Accepted for publication September 15, 1967

ABSTRACT

UYENO, EDWARD T.: Hallucinogenic compounds and swimming responses. J. Pharmacol. Exp. Therap. **159**: 216-221, 1968. The effects of several hallucinogenic compounds and their nonhallucinogenic analogs were evaluated in an underwater swim alley. Rats were submerged and forced to swim underwater through plastic tubes 3.47 m long in order to escape at the other end of the tubes. All of the compounds tested significantly increased the starting latency but only *d*-amphetamine significantly increased swimming time. The peak effect time for *d*-lysergic acid diethylamide (LSD-25) and three amphetamine derivatives on latency was 20 min after the i.p. injection; that for 2-brom-lysergic acid diethylamide (BOL-148) was 40 min. Dose-response curves indicated that the increase in latency was a function of dose. The ED50's for LSD-25, BOL-148, 2,4,5-trimethoxyamphetamine (TMA-2) hydrochloride, 3,4,5-trimethoxyamphetamine (TMA) hydrochloride, *d*-amphetamine hydrochloride and 4,5,6-trimethoxyamphetamine (TMA-3) hydrochloride were 1.9, 11.0, 15.0, 31.0, 36.5 and 77.0 μmol/kg, respectively. An analysis of relative effectiveness indicated that LSD-25, TMA-2 and TMA (in descending order) were significantly more effective than their respective nonhallucinogenic analogs.

Lysergic acid diethylamide (LSD-25) has been reported to disrupt behavior at far lower dose levels than its analog, 2-brom-lysergic acid diethylamide (BOL-148), which has questionable hallucinogenic effects in man (Cerletti and Rothlin, 1955; Richards *et al.*, 1958). Appel and Freedman (1965) found that LSD-25 was approximately 10 times as effective on a dose-level basis as BOL-148 in disrupting the bar-pressing performance of food-deprived rats. Moreover, LSD-25 is significantly more effective than BOL-148 in reducing the aggressive behavior of mice in an isolation-induced aggression test (Uyeno and Benson, 1965; Uyeno, 1966a) and of rats in a food competition situation (Uyeno, 1966b).

The present experiment was designed to study the behavior of underwater swimming and its alteration by the hallucinogenic compounds, LSD-25, 3,4,5-trimethoxyamphetamine hydrochloride (TMA) (Peretz *et al.*, 1955; Shulgin *et al.*, 1961) and 2,4,5-trimethoxyamphetamine hydrochloride (TMA-2) (Shulgin, 1964), and their nonhallucinogenic analogs, BOL-148 and 4,5,6-trimethoxyamphetamine hydrochloride (TMA-3) (Shulgin, 1964). In addition, *d*-amphetamine hydrochloride was used as a reference compound.

METHODS. The swim alley consisted of a sheet-metal tank (3.66 by 0.40 by 0.15 m) with four parallel channels. Each channel was 3.47 m long and consisted of a translucent red plastic tube. The diameter of each tube was 9 cm, which allowed the rat swimming in it to turn around. At one end of the channels a detachable 40- by 17- by 16-cm plastic start box with four compartments was placed into the tank. At the other end, the channels led to the goal box containing four compartments into which the animals escaped. The tank was filled with 23°C water to the height of 13 cm, submerging the channels completely.

One hundred ninety male rats of the Long-Evans strain weighing between 180 and 200 g were housed individually and fed *ad libitum*. They were adapted to swimming underwater by a series of pretraining trials. A trial was initiated by placing one animal in each of the four compartments of the start box, submerging the start box and lifting the common guillotine door to allow each animal to swim into its respective tube. Lifting the door automatically started a set of four latency timers. The latency of an animal's response was the time which elapsed from the moment the start door was

Received for publication August 1, 1966.
[1] This work was supported by contract no. PH-43-65-1061 between the National Institutes of Health, U.S. Public Health Service, and Stanford Research Institute.

opened until the animal swam past the first photocell, located 17 cm from the start door. Swimming time was recorded as the time to swim from the first photocell to the second photocell at the escape exit. On the first pretraining trial the rats were forced to swim underwater through tubes 1.32 m long in order to escape. On the second and third trials they swam a distance of 2.1 and 3.47 m, respectively. After the pretraining trials they were given a series of training trials (with an intertrial interval of approximately 1 hr) with the full-length channel. They were given eight trials per day until they reached a criterion of three successive latency scores of 1.5 sec or less. On the basis of the mean latency scores of the three criterion trials, the animals were assigned to a control (0.9% saline) group and 18 experimental groups (three experimental groups/compound), with 10 animals per group.

On the day after reaching criterion they were given the first swim test 15 min before an injection. The second, third, fourth, fifth and sixth tests were given 1, 10, 20, 40 and 60 min respectively after an i.p. injection. The compounds and doses (in micromoles per kilogram) were: LSD-25, 1.5, 2.3 and 3.1; BOL-148, 6.2, 12.4 and 24.9; TMA hydrochloride, 12.0, 23.9 and 47.8; TMA-2 hydrochloride, 6.0, 12.0 and 23.9; TMA-3 hydrochloride, 23.9, 47.8 and 95.6; *d*-amphetamine hydrochloride, 11.7, 23.3 and 46.6. A standard volume of 2.5 ml/kg of saline or drug–saline solution was administered to each control or experimental animal, respectively.

TABLE 1

A comparison between the pre- and postinjection mean latencies

Compound	Mean Latencies		*F*	dF_1/dF_2	Significance
	Preinjection	Postinjection			
	sec				%
LSD-25	1.01	2.15	28.2	1/29	1
BOL-148	1.04	2.03	24.8	1/29	1
TMA	0.99	1.79	14.5	1/29	1
TMA-2	1.02	1.87	14.7	1/29	1
TMA-3	0.96	1.83	11.1	1/29	1
d-Amphetamine	0.98	1.91	10.8	1/29	1
Saline	0.97	0.98	0.1	1/9	N.S.[a]

[a] Not significant.

TABLE 2

A comparison between the mean latency of each dose group with that of the saline group

Compound	Low Dose		Medium Dose		High Dose	
	$\bar{X}_L$	$\bar{X}_L$ *vs.* saline[a]	$\bar{X}_M$	$\bar{X}_M$ *vs.* saline	$\bar{X}_H$	$\bar{X}_H$ *vs.* saline
	sec		*sec*		*sec*	%
LSD-25	1.60	N.S.[b]	2.16	5%[c]	2.68	5
BOL-148	1.55	N.S.	1.94	5%	2.61	5
TMA	1.09	N.S.	1.79	5%	2.48	5
TMA-2	1.28	N.S.	1.69	N.S.	2.65	5
TMA-3	1.09	N.S.	1.44	N.S.	2.97	5
d-Amphetamine	1.20	N.S.	1.65	N.S.	2.87	5

[a] The mean latency for the saline group was 0.97 sec.

[b] The difference between the two means (LSD-25 (Low), 1.60, *vs.* saline, 0.97) is not significant.

[c] The difference between the two means (LSD-25 (Medium), 2.16, *vs.* saline, 0.97) is significant at the 5% level.

TABLE 3

A comparison between the pre- and postinjection swimming times

Compound	Mean Swimming Time		*F*	dF_1/dF_2	Significance
	Preinjection	Postinjection			
	sec				%
LSD-25	12.7	13.1	0.366	1/29	N.S.
BOL-148	12.5	13.6	3.843	1/29	N.S.
TMA	13.0	13.7	2.740	1/29	N.S.
TMA-2	13.5	14.3	2.899	1/29	N.S.
TMA-3	12.4	13.8	4.000	1/29	N.S.
d-Amphetamine	13.1	15.3	13.314	1/29	5
Saline	13.0	12.8	0.639	1/9	N.S.

RESULTS. In general, all compounds increased the mean latency. The animals given LSD-25, TMA, TMA-2, TMA-3 or *d*-amphetamine showed the greatest increase in the mean latency during the 20-min postinjection trial. Therefore, the time of peak effect of these compounds was considered to be 20 min after the injection; that of BOL-148 was considered to be 40 min. A statistical analysis showed that the postinjection mean latency of the three experimental groups for each drug at the time of peak effect was significantly longer than the respective preinjection mean latency, indicating the debilitating effect of the compounds (table 1). The control group, however, showed no significant change in the mean latency.

Further analysis showed that the postinjection mean latencies of all experimental groups given a high dose, and those of some groups given a medium dose, were significantly longer than the mean latency of the saline group (table 2). None of the mean latencies of the groups given a low dose was significantly different from that of the saline group.

Table 3 shows a comparison of the postinjec-

TABLE 4

A comparison between the mean swimming time of each dose group with that of the saline group

Compound	Low Dose		Medium Dose		High Dose	
	$\bar{X}_L$	$\bar{X}_L$ vs. saline[a]	$\bar{X}_M$	$\bar{X}_M$ vs. saline	$\bar{X}_H$	$\bar{X}_H$ vs. saline
	sec		*sec*		*sec*	
LSD-25	12.8	N.S	12.5	N.S.	14.1	N.S.
BOL-148	13.3	N.S.[b]	13.3	N.S.	14.1	N.S.
TMA	13.3	N.S.	13.5	N.S.	14.3	N.S.
TMA-2	13.9	N.S.	14.1	N.S.	14.9	N.S.
TMA-3	13.6	N.S.	13.4	N.S.	14.5	N.S.
d-Amphetamine	13.9	N.S.	14.4	N.S.	17.6	1%

[a] The mean swimming time for the saline group was 12.8 sec.
[b] The difference between the two means (13.3 vs. 12.8) is not significant.

tion mean swimming times at the time of peak effect with the respective preinjection mean swimming times. Only the group given *d*-amphetamine showed a significant increase in swimming time.

Postinjection mean swimming times (at the time of peak effect) of the experimental groups were compared with that of the control group (table 4). Only the mean swimming time of the group administered the high dose of *d*-amphetamine was significantly different from that of the saline group.

Since the latency was more sensitive than the swimming time, the former measurement was used to calculate (according to the method of Litchfield and Wilcoxon, 1949) the median effective dose (ED50) and relative potency (effectiveness) of each compound. For each compound and each dose the total number of animals that showed an increase in latency at the time of peak effect was obtained. In the control group, one animal's latency increased on the 20-min postinjection trial, and another's on the 40-min postinjection trial. The difference between the total number of experimental animals that showed an increase in latency on the peak time trial and the total of the control animals (*i.e.*, one) that showed an increase in latency on the 20-min postinjection trial was calculated for each drug at each dose level. These difference

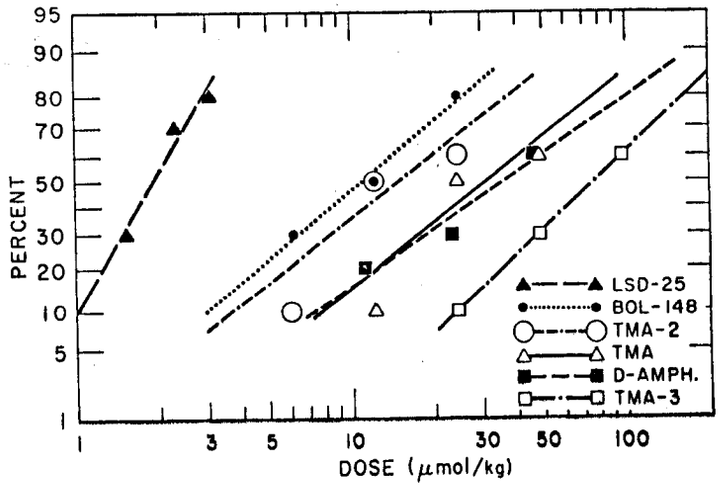


FIG. 1. Dose-response curves, giving effects of the compounds on latency. The ordinate shows the percentage of animals that showed an increase in latency.

TABLE 5

Analysis of latencies

Compound	ED50[a]	95% Confidence Limits
	μmol/kg	
LSD-25	1.9	1.5–2.4
BOL-148	11.0	6.7–18.2
TMA-2 HCl	15.0	8.6–26.3
TMA HCl	31.0	17.8–53.9
d-Amphetamine HCl	36.5	19.5–68.3
TMA-3 HCl	77.0	44.3–133.9

[a] The ED50 refers to the dose which produces an increase in latency in 50% of the animals.

TABLE 6

Relative effectiveness of compounds

Compound		BOL-148	TMA-2	TMA	*d*-Amphetamine	TMA-3
LSD-25	Potency ratio[a]	5.8	7.9	16.3	19.2	40.5
	Significance[b]	Yes	Yes	Yes	Yes	Yes
	Confidence limits	3.3–10.1	4.3–14.5	8.9–29.9	9.9–37.5	22.1–74.2
BOL-148	Potency ratio		1.4	2.8	3.3	7.0
	Significance		No	Yes	Yes	Yes
	Confidence limits		0.6–2.9	1.3–5.9	1.5–7.3	3.3–14.8
TMA-2	Potency ratio			2.1	2.4	5.1
	Significance			No	Yes	Yes
	Confidence limits			0.9–4.5	1.1–5.6	2.3–11.3
TMA	Potency ratio				1.1	2.5
	Significance				No	Yes
	Confidence limits				0.5–2.7	1.1–5.5
d-Amphetamine	Potency ratio					2.1
	Significance					No
	Confidence limits					0.9–4.9

[a] Potency ratio = (ED50 of a compound in the top row)/(ED50 of a compound in the first column).
[b] Significance at the 5% level.

scores, expressed as the percentage of the total number of experimental animals in each dose group, were taken as a measure of the effect of the drug on latency. Percent effect was plotted against dose (in micromoles per kilogram) on logarithmic probability paper (fig. 1). The dose-response curves in figure 1 illustrate that the effect of each compound was a function of dose. The ED50 and the 95% confidence limits are presented in table 5.

To compare the relative effectiveness of the compounds, the potency ratios and the 95% confidence limits were calculated. The potency ratio between two compounds was computed by dividing the ED50 of the less effective compound (*i.e.*, of the larger value) by the ED50 of the other. The compounds were arranged in descending order of effectiveness in the top row and in the first column of table 6. LSD-25 was significantly (at the 5% level) more effective than BOL-148, and TMA-2 and TMA were significantly more effective than TMA-3, indicating that the hallucinogens were significantly more effective than their respective "nonhallucinogenic" analogs. Comparison across classes of compounds showed, however, that BOL-148 tended to be more effective than TMA-2 and was significantly more effective than TMA.

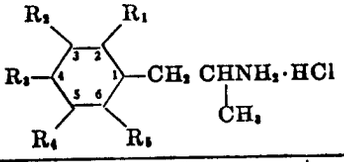
Table 7 illustrates how *d*-amphetamine and its derivatives retain the three carbon chains and differ structurally only in the location of their methoxy substituents. TMA-2, with a methoxy group at position 2, increases latency most and also appears to be a hallucinogen (Shulgin, 1964). Transposition of this methoxy group to position 6 to form TMA-3 results in a compound which increases latency least and is devoid of hallucinogenic properties (Shulgin, 1964).

DISCUSSION. The present results indicate that LSD-25 increases starting latency in an underwater swim test more than BOL-148. The present data are consistent with earlier studies which demonstrated that LSD-25 is more effective than BOL-148 in inhibiting aggression and dominance behavior of mice (Uyeno and Benson, 1965; Uyeno, 1966a) and rats (Uyeno, 1966b). In the experiment of Appel and Freedman (1965) and that of the author, the effective doses of LSD-25 were much lower than those of other compounds tested.

Shulgin (1964) determined the relative effectiveness of the TMA compounds in humans by comparing them with the effectiveness of mescaline. His results indicated that TMA-2 was significantly more effective than TMA as an hallucinogen, whereas TMA-3 was nonhallucinogenic. The results of the present experiment also showed that TMA-2 and TMA were significantly more effective than TMA-3 in increasing

TABLE 7

Activity of d-amphetamine HCl and its derivatives



Compound	R_1	R_2	R_3	R_4	R_5	Underwater Latency Test (ED_{50})	Hallucinogenic Properties
						μmol/kg	
d-Amphetamine HCl						36.5	No
TMA HCl		CH_3O	CH_3O	CH_3O		31.0	Yes[a]
TMA-2 HCl	CH_3O		CH_3O	CH_3O		15.0	Yes[a]
TMA-3 HCl			CH_3O	CH_3O	CH_3O	77.0	No[a]

[a] Shulgin, 1964.

starting latency in an underwater swim test. But TMA-2 was not significantly more effective than TMA.

All compounds significantly increased the starting latency, but only *d*-amphetamine significantly increased the swimming time. Other nonhallucinogenic compounds tended to increase the swimming time, but these increases were not statistically significant. Generally, the doses of the nonhallucinogenic compounds that increased the latency seemed to disrupt the neuromuscular coordination required to swim, as indicated by the increase in the swimming time. However, the doses of the hallucinogens that increased the latency were so low that they did not seem to affect the neuromuscular coordination needed for swimming.

The results of the experiments with the hallucinogens seem to be consistent with those of Appel *et al.* (1967), who reported that rats injected i.p. with 0.52 or 1.04 mg/kg of LSD-25 showed a significant increase in escape latency, but little change in the rate of wheel turning to avoid foot shock. These increases in latency without the change in the duration of swimming time or in the rate of wheel turning seem to indicate that the hallucinogens disrupt the perception of a starting stimulus (Schwartz and Cheney, 1965), interrupt the transmission of sensory impulses at the level of the lateral geniculate (Evarts and Marshall, 1955) and in the hippocampal system of the temporal lobe (Adey *et al.*, 1962) and produce cerebral functional disturbances (Revzin and Armstrong, 1966).

ACKNOWLEDGMENTS. The author wishes to thank Drs. C. Mitoma and L. S. Otis for their valuable criticism of the manuscript, and Dr. W. A. Skinner of Stanford Research Institute, Mr. H. Althouse of Sandoz Pharmaceuticals and Mr. A. Norton of Smith Kline & French Laboratories for the supplies of TMA compounds, LSD-25 and BOL-148, and *d*-amphetamine, respectively.

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