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ALTERATION OF A LEARNED RESPONSE OF THE SQUIRREL MONKEY BY HALLUCINOGENS

EDWARD T. UYENO
Stanford Research Institute, Menlo Park, California

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Summary—The relative effectiveness of N,N-dimethyltryptamine derivatives and amphetamine derivatives was evaluated in seven squirrel monkeys, trained to discriminate between two black discs of different sizes. After the animals learned the task, they were injected intraperitoneally with the test compounds and then retested. The dose-response curves showed that debilitating effects of the compounds were a function of dose. The median effective doses (ED_{50}) that disrupted their performance were calculated according to the graphic logarithmic probit method. The descending rank order of effectiveness of the compounds, according to the ED_{50} was: 2,5-dimethoxy-4-ethyl-amphetamine; 2,5-dimethoxy-4-methyl-amphetamine; psilocybin; 4-methoxy-N,N-dimethyltryptamine; N,N-dimethyltryptamine; and 6-hydroxy-N,N-dimethyltryptamine.

SEVERAL investigators have reported that N,N-dimethyltryptamine derivatives and amphetamine derivatives produced hallucination in man. For example, FABING and HAWKINS (1956) found that an intravenous injection of 4–16 mg of 5-hydroxy-N,N-dimethyltryptamine (bufotenine) produced hallucination. SZARA (1957) reported that several minutes after an intramuscular injection of 1·0 mg/kg of N,N-dimethyltryptamine, hallucination appeared and lasted about an hour. ISBELL (1959) found that *O*-phosphoryl-4-hydroxy-ω-N,N-dimethyltryptamine (psilocybin) elicited abnormal mental state characterized by alterations in visual perception and by difficulty in thinking. SNYDER *et al.* (1967) reported that 2,5-dimethoxy-4-methyl-amphetamine (DOM) is about 100 times more effective than mescaline in producing hallucination, and only one-thirtieth as effective as lysergic acid diethylamide (LSD-25). SHULGIN (1964) reported that trimethoxy-amphetamines were more effective than mescaline in producing hallucinations in man. Experiments with monkeys have also shown that the hallucinogenic trimethoxyamphetamines were more effective than mescaline but were significantly less effective than LSD-25 in disrupting the learned size discrimination performance (UYENO *et al.*, 1968).

The present experiment was designed to investigate the effects of N,N-dimethyltryptamine derivatives and amphetamine derivatives on the size discrimination performance of the squirrel monkey. More specifically, the relative effectiveness of N,N-dimethyltryptamine (DMT), 4-methoxy-dimethyltryptamine (4-methoxy-DMT) hydrochloride, 6-hydroxy-dimethyltryptamine (6-hydroxy-DMT) hydrochloride, psilocybin, DOM hydrochloride, and 2,5-dimethoxy-4-ethyl-amphetamine (DOET) hydrochloride in the disruption of previously learned visual discrimination performance was evaluated.

METHODS

The Wisconsin General Test Apparatus (WGTA) is a standard apparatus designed to test the visual discrimination ability of monkeys. The stimuli consist of round black plastic discs of different sizes ranging from 1·02 to 3·92 in². As a pair of discs was presented on the stimulus tray, four monkeys were trained to choose the larger one, the other three monkeys were trained to choose the smaller one, with both groups being given a reward. In an easy discrimination problem, the animals were required to discriminate a pair of discs in which the ratio of the area of the larger disc to that of the smaller one was 1·96 : 1. In a more difficult discrimination problem, the disc ratio was 1·24 : 1. The correct disc was placed either at the right or left position of the tray in a random manner according to a GELLERMAN Series (1933). During the training and drug testing stages, the animals were given thirty trials daily on each of the two problems. In each problem, the total number of correct responses of each monkey was expressed as a percent score. The performance on the easy problem stabilized at approximately 90% correct responses, that on the difficult problem at approximately 75%.

After the performance had stabilized, a coded test or control compound was administered twice each week to determine the effects of the compound on the animal's size discrimination ability. Trials without injection were given on Mondays, Tuesdays and Wednesdays to establish predrug baseline performance. On Thursdays the animals were injected intraperitoneally (1 mg/kg) with a predetermined dose of a test or control compound (Table 1).

TABLE 1. DOSES OF N,N-DIMETHYLTRYPTAMINE AND AMPHETAMINE DERIVATIVES EVALUATED

Compound	Dose (μmoles/kg)		
	Low	Medium	High
DMT	10·6	21·3	26·6
4-Methoxy-DMT HCl	13·8	18·4	22·9
6-Hydroxy-DMT HCl	25·4	50·7	—
Psilocybin	1·8	3·5	7·0
DOM HCl	1·0	2·0	4·1
DOET HCl	0·5	1·0	2·0

On Fridays the same doses of the same compounds administered on Thursday were reinjected and the animals were retested to assess any possible tolerance or cumulative effects. All the compounds except DMT were dissolved in saline solution; DMT was mixed in aqueous solution of 6% ethyl alcohol and 25% polyethylene glycol 200. Saline solution alone or aqueous solution of 6% ethyl alcohol and 25% polyethylene glycol 200 alone, was injected to check the stability of the discrimination performance under conditions of control injection. The animals were tested 30 min after the injection.

RESULTS

The performance on both the easy and difficult problems during "no-drug days" and during "placebo days" was relatively constant during 22 weeks of testing. However, the test compounds had a marked disrupting effect at high doses and some effect at almost all doses. Comparison of the responses on drug day 1 (Thursday) with those on drug day 2

(Friday) indicated drug tolerance occurred for DOM, but it was less evident for other compounds.

Statistical analyses were conducted to determine the median effective doses (ED_{50}) and relative effectiveness of the compounds. The analyses were based on either of two criteria of deterioration of performance during the drug trials: failure to respond, or a score less than 80% of the score obtained during the placebo trial. On the basis of these criteria, the number of the animals that showed a disruption or decrease in performance on the easy problem was obtained at each dose level of each compound and expressed as a percentage of the number tested. Similarly, the percentage of animals that showed a decrease in performance on the difficult problem was obtained. The percentage is plotted against dose (in μ-moles/kg) on logarithmic probability paper (Figs. 1 and 2). The dose–response curves for both problems show that percent disrupting effect of the compounds is a function of dose. The ED_{50} and the 95% confidence limits were computed according to the graphic method of LITCHFIELD and WILCOXON (1949) (Table 2).

The relative effectiveness of the compounds in the disruption of learned performance was computed by derivation of potency ratios according to the method of LITCHFIELD and WILCOXON (1949). The potency ratio between two compounds was calculated by dividing the ED_{50} of the less effective one (i.e. the one with the larger ED_{50}) by the ED_{50} of the other. The compounds are arranged in descending order of effectiveness in the first column of Tables 3 and 4, and in the top row of these tables from left to right. In general, the rank order of relative effectiveness determined from the data on the easy problem is similar to that on the difficult problem. The amphetamine derivatives (DOET and DOM) are significantly more effective than all the N,N,-dimethyltryptamine except psilocybin (the phosphoric acid ester of 4-hydroxy-dimethyltryptamine), which is as effective as DOM in disrupting the learned performance. 6-Hydroxy-DMT was significantly the least effective of all.

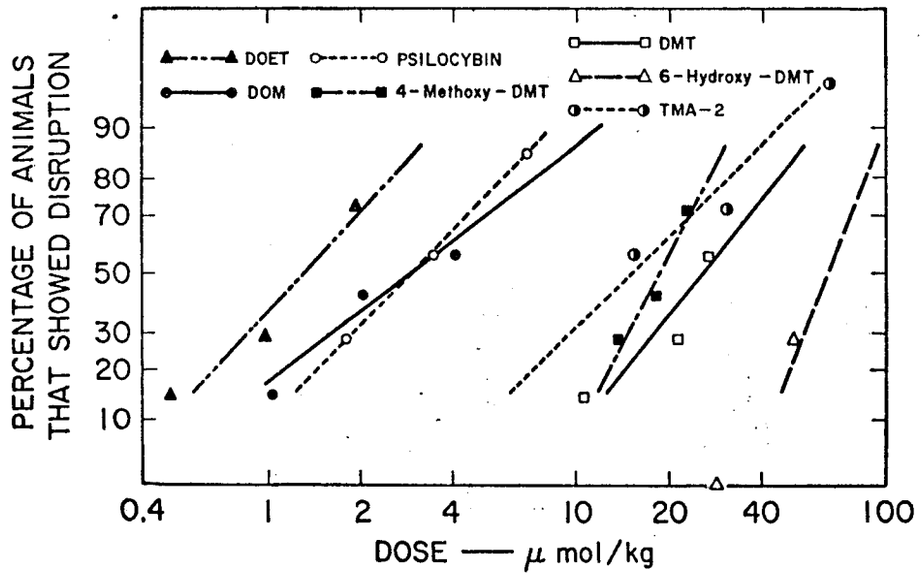


FIG. 1. Disruption of size-discrimination performance on the easy problem.

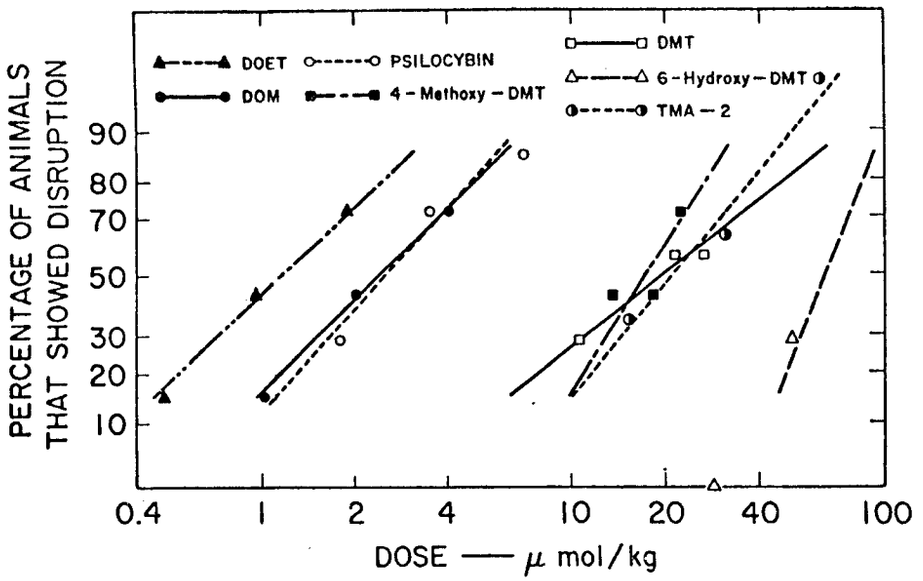


FIG. 2. Disruption of size-discrimination performance on the difficult problem.

TABLE 2. THE ED_{50} AND THE 95% CONFIDENCE LIMITS FOR DISRUPTING SIZE DISCRIMINATION PERFORMANCE IN THE WGTA

Compound	ED_{50} (μmoles/kg)	95% confidence limits (μmoles/kg)
	Easy problem	
DOET	1·3	(0·7 – 2·4)
DOM	2·9	(1·5 – 5·7)
Psilocybin	2·9	(1·6 – 5·4)
4-Methoxy-DMT	18·5	(13·2 – 25·9)
DMT	25·5	(15·4 – 42·3)
6-Hydroxy-DMT	64·4	(45·4 – 91·5)
LSD-25*	0·15	(0·09– 0·24)
Mescaline*	82·2	(62·8 –107·7)
	Difficult problem	
DOET	1·2	(0·7 – 2·1)
DOM	2·4	(1·4 – 4·0)
Psilocybin	2·5	(1·4 – 4·5)
4-Methoxy-DMT	17·2	(12·5 – 23·7)
DMT	19·4	(10·2 – 37·1)
6-Hydroxy-DMT	64·4	(45·4 – 91·5)
LSD-25*	0·13	(0·08– 0·22)
Mescaline*	71·0	(52·6 – 95·9)

*UYENO *et al.* (1968).

TABLE 3. RELATIVE EFFECTIVENESS OF EACH COMPOUND AS MEASURED BY A DISRUPTION OF DISCRIMINATION PERFORMANCE OF THE EASY PROBLEM

DOET	DOM	Psilocybin	TMA-2*	4-Methoxy-DMT	DMT	6-Hydroxy-DMT	Mescaline*

TABLE 3. RELATIVE EFFECTIVENESS OF EACH COMPOUND AS MEASURED BY A DISRUPTION OF DISCRIMINATION PERFORMANCE OF THE EASY PROBLEM

Compound		DOET	DOM	Psilocybin	TMA-2*	4-Methoxy-DMT	DMT	6-Hydroxy-DMT	Mescaline*
LSD-25*	P.R.†	9·1	19·9	19·9	100·7	127·6	175·9	444·1	548·0
	Sig.‡	yes	yes	yes	yes	yes	yes	yes	yes
	C.L.§	(4·2–19·9)	(8·5–46·7)	(8·9–44·1)	(45·9–236·4)	(69·0–236·0)	(85·8–360·5)	(238·8–826·1)	(318·5–1,009·1)
DOET	P.R.		2·2	2·2	11·4	14·0	19·3	48·8	62·3
	Sig.		no	no	yes	yes	yes	yes	yes
	C.L.				(4·8–27·2)	(7·1–27·6)	(8·9–42·0)	(24·6–96·6)	(32·4–119·6)
DOM	P.R.			1·0	5·2	6·4	8·8	22·2	28·4
	Sig.			no	yes	yes	yes	yes	yes
	C.L.				(2·1–13·4)	(3·0–13·7)	(3·8–20·6)	(10·4–48·3)	(13·7–58·7)
Psilocybin	P.R.				5·2	6·4	8·8	22·2	28·4
	Sig.				yes	yes	yes	yes	yes
	C.L.				(2·1–12·7)	(3·2–12·9)	(4·0–19·5)	(11·0–45·0)	(14·5–55·3)
TMA-2*	P.R.					1·2	1·7	4·3	5·4
	Sig.					no	no	yes	yes
	C.L.							(2·1–8·8)	(2·7–10·9)
4-Methoxy-DMT	P.R.						1·4	3·5	4·4
	Sig.						no	yes	yes
	C.L.							(2·1–5·7)	(2·9–6·8)
DMT	P.R.							2·5	3·2
	Sig.							yes	yes
	C.L.							(1·4–4·7)	(1·8–5·7)
6-Hydroxy-DMT	P.R.								1·3
	Sig.								no
	C.L.								

*UYENO *et al.* (1968).

†Potency ratio $= \frac{ED_{50} \text{ of compound in the top row}}{ED_{50} \text{ of compound in the first column.}}$

‡Significance at the 5% level.

§95% confidence level.

TABLE 4. RELATIVE EFFECTIVENESS OF EACH COMPOUND AS MEASURED BY A DISRUPTION OF DISCRIMINATION PERFORMANCE OF THE DIFFICULT PROBLEM

Compound		DOET	DOM	Psilocybin	4-Methoxy-DMT	DMT	TMA-2*	6-Hydroxy-DMT	Mescaline*
LSD-25*	P.R.†	9·1	18·8	19·7	134·4	151·6	159·2	503·1	546·2
	Sig.‡	yes	yes	yes	yes	yes	yes	yes	yes
	C.L.§	(4·2–19·8)	(9·0–39·0)	(9·0–42·9)	(72·3–250·0)	(65·9–348·6)	(93·4–392·4)	(267·6–945·9)	(301·5–1,020·6)
DOET	P.R.		2·1	2·2	14·8	16·7	17·8	55·5	61·2
	Sig.		no	no	yes	yes	yes	yes	yes
	C.L.				(7·7–28·5)	(7·1–39·6)	(8·2–38·9)	(28·3–108·8)	(32·1–116·9)
DOM	P.R.			1·1	7·2	8·1	8·6	26·8	29·6
	Sig.			no	yes	yes	yes	yes	yes
	C.L.				(3·9–13·1)	(3·6–18·4)	(4·2–17·9)	(1·4–5·0)	(16·3–53·5)
Psilocybin	P.R.				6·8	7·7	8·2	25·6	28·2
	Sig.				yes	yes	yes	yes	yes
	C.L.				(3·5–13·2)	(3·2–18·3)	(3·8–17·9)	(13·0–50·1)	(14·7–54·1)
4-Methoxy-DMT	P.R.					1·1	1·2	3·7	4·1
	Sig.					no	no	yes	yes
	C.L.							(2·3–6·0)	(2·7–6·4)
DMT	P.R.						1·0	3·3	3·7
	Sig.						no	yes	yes
	C.L.							(1·6–6·9)	(1·8–7·5)
TMA-2*	P.R.							3·1	3·4
	Sig.							yes	yes
	C.L.							(1·7–5·9)	(1·7–5·1)
6-Hydroxy-DMT	P.R.								1·1
	Sig.								no
	C.L.								

*UYENO *et al.* (1968).

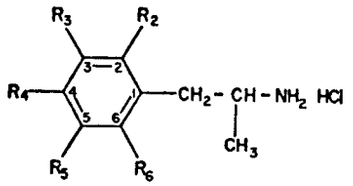
†Potency ratio $= \frac{ED_{50} \text{ of compound in the top row}}{ED_{50} \text{ of compound in the first column.}}$

‡Significance at the 5% level.

§95% confidence limits.

Table 5 illustrates the structure–activity relation of *d*-amphetamine and its derivatives. The three amphetamine derivatives retain the three carbon chains and the two methoxy substituents at position 2 and 5. They differ structurally only in the substituent located at position 4. DOET with the ethyl substituent and DOM with the methyl substituent in position 4 are significantly more effective in disrupting the visual discrimination performance than is TMA-2 with the methoxy substituent.

TABLE 5. STRUCTURE AND ACTIVITY OF AMPHETAMINE DERIVATIVES

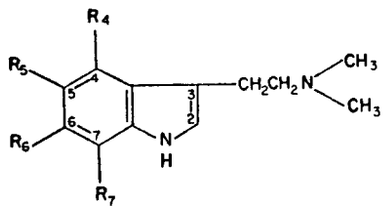


Compound	R_2	R_3	R_4	R_5	R_6	Discrimination test: Easy ED_{50}	Discrimination test: Difficult ED_{50}
						(μmoles/kg)	
d-Amphetamine*	H	H	H	H	H	2·6	2·6
DOET†	CH_3O	H	CH_3CH_2	CH_3O	H	1·3	1·2
DOM†	CH_3O	H	CH_3	CH_3O	H	2·9	2·4
TMA-2*	CH_3O	H	CH_3O	CH_3O	H	15·1	20·7

*UYENO *et al.* (1968).
†SNYDER and RICHELSON (1968).

Table 6 shows the structural formulae of the four N,N-dimethyltryptamine derivatives that retain the side chain and differ structurally only in the substituents located in position 4 and 6. Psilocybin, with the *O*-phosphoryl hydroxy group at position 4, is significantly

TABLE 6. STRUCTURE AND ACTIVITY OF N,N-DIMETHYLTRYPTAMINE AND ITS DERIVATIVES



Compound	R_4	R_5	R_6	R_7	Discrimination test: Easy ED_{50}	Discrimination test: Difficult ED_{50}
					(μmoles/kg)	
Psilocybin	H_2PO_3 | O	H	H	H	2·9	2·5
4-Methoxy-DMT	CH_3O	H	H	H	18·5	17·2
DMT	H	H	H	H	25·5	19·4
6-Hydroxy-DMT	H	H	OH	H	64·4	64·4

the most effective in the disruption of size discrimination performance. 6-Hydroxy-DMT with the hydroxy group at position 6 is the least effective.

DISCUSSION

The finding that the potency of DOM is significantly less than that of LSD-25, but more than that of mescaline, in disrupting previously learned visual discrimination performance, is consistent with the data of SNYDER *et al.* (1967). DOM and DOET are significantly more potent than 2,4,5-trimethoxyamphetamine (TMA-2), which is the most potent trimethoxyamphetamine (SHULGIN, 1964). Moreover, DOM and DOET are significantly more potent than DMT and its derivatives except psilocybin. The finding that psilocybin disrupts the learned performance of the squirrel monkey is in accord with the results of the human studies which show that this indole derivative produces disorder of sensory perception (ISBELL, 1959), difficulties in concentration (SERCL *et al.*, 1961; RINKEL *et al.*, 1960), and impairment of judgment (DELAY *et al.*, 1959).

The data showing that 6-hydroxylated DMT is less potent than DMT do not support the generalization of SZARA and HEARST (1962) who hypothesized that 6-hydroxylation confers greater psychotropic potency on N,N-dialkyltryptamines. Their hypothesis is based on their finding that 6-hydroxylated diethyltryptamine (6-hydroxy-DET) was more potent than the parent compound in decreasing the lever-pressing response of rats trained to depress a lever to avoid a foot shock and of other rats trained to press a bar for a food reward. In support of their hypothesis they reported a positive correlation between the amount of 6-hydroxy-DET excreted in the urine and the psychologic changes produced in man after administration of 1 mg/kg of diethyltryptamine (SZARA *et al.*, 1966).

The present results are congruous with those of ROSENBERG *et al.* (1963) who reported that 6-hydroxy-DMT is significantly less potent than DMT in producing hallucinations and with those of TABORSKY *et al.* (1966) who found 6-hydroxy-5-methoxy-DMT and 6-hydroxy-5-methoxy-tryptamine (TABORSKY *et al.*, 1965) are significantly less potent than non-hydroxylated 5-methoxy-N,N-DMT and 5-methoxy-tryptamine, respectively, in reducing the work rate of albino male rats trained to press a bar for a food reward in a Skinner box. The lower potency of 6-hydroxylated compounds as compared with their non-hydroxylated analogs may be attributable to the higher polarity of the 6-hydroxylated compounds, allowing lower concentration of 6-hydroxylated compounds to enter the brain. However, as suggested by TABORSKY *et al.* (1966), it is possible that a metabolite of tryptamine other than the 6-hydroxyl compound may be the active agent.

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