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Effects of Lysergic Acid Diethylamide upon Performance of Trained Rats. (22453)

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Although lysergic acid diethylamide (LSD) is known to produce symptoms in man resembling those of certain psychotic disorders, a quantitative measure of the effects of this drug in animals has been lacking. Woolley(1) has described a syndrome observed in mice injected with LSD, and Shore *et al.*(2,3) have shown that LSD antagonizes the potentiating effects of reserpine and serotonin upon hypnotic agents. In the present paper, we demonstrate that the effects of LSD upon trained rats bear a linear relationship to the log dose of the drug, and that this phenomenon can be used in the study of LSD-antagonists.

Methods. Holtzman male rats of 100 to 225 g were used. Preliminary evidence indicates that animals outside this range may not be suitable, but this has not been established with certainty. Animals, fasted for 24 hours or longer, were trained to climb a vertical rope of about 172 cm as described previously(4). Incentive for climbing was food cup placed on platform at top of the rope; in addition, there was an electrified wire grid at the bottom. Also at bottom of rope was a small "tweeter" type of loud speaker which emitted brief bursts of noise at intervals of about a half-second. Untreated animals needed neither grid nor noise, but these facilitated climbing in rats affected by drugs, and decreased variability of performance. Observations were made of behavior of animals while in their cages as well as while negotiating the climb. Time required for the climb was obtained to the nearest 0.1 second. Three control times were obtained before administration of drug, and the average value was used as base line for drug effect, after it was established that in saline-injected animals climbing time remained constant for 5 hours. Evaluation of effects of drugs on climbing per-

formance was made as follows: first, the action of LSD was very rapid after intraperitoneal injection; it was quite marked within 5 minutes, usually maximal in 10 minutes, and after 20 minutes declined rapidly for about 10 minutes, then more slowly until original base line was approached within 1 to 2 hours after injection. Drugs which modified the effects of LSD were found to affect both magnitude and duration of prolongation of climbing time produced by LSD; also, both parameters were affected by dose of LSD. Therefore, determinations of climbing time were made at 5, 10, 20, 30, 45, and 60 minutes after LSD, and thereafter at half-hour intervals until recovery had occurred. Climbing times found in this manner, when plotted against time, produced points which could be connected by straight lines to form a polygon whose area could readily be calculated. The effects of drugs could be evaluated by their effects on area of this polygon. Climbing time of well-trained rats was usually 4 to 5 seconds. At peak of drug effect, this would be markedly prolonged, and any animal failing to negotiate the climb within 60 seconds was marked "failure." For calculating the area of the polygon, an animal failing to climb within the cut-off time was arbitrarily assigned a climbing time of 60 seconds. Although this introduced some bias into the calculation, such strong effects usually lasted only a short time and did not seriously interfere with the evaluation. The unit of area under the climbing time curve has been designated the "minute-second," and may be defined as a delay in climbing time of one second over the base line, such delay lasting for one minute. Essentially, it is the product of the intensity of the effect and its duration. This concept has been more fully explained in a previous publication(5). Simple formulae were set up for rapidly calculating the areas; the total area of polygon was designated "climbing time delay" (C.T.D.) in min-sec. One example of such a formula: when readings are taken at time intervals listed in preceding paragraph, the maximal effect occurs at 20 minutes (taking injection time as 0 time), and C. T. nearly reaches the base line at 2 hours, it can be shown that C.T.D. in min-sec $= 5(b+1.5c+2d+2.5e+3f+4.5g+6h+3i-23.5a)$, where a = C.T. before injection, and $b,c,\ldots.i$ = C.T. at 5, 10, ... 120 min. after injection. This calculation is made quickly on calculating machine, and similar simple formulae can be determined for durations of drug action other than 2 hours.

Results. Symptoms of LSD intoxication in rats. When LSD is injected intraperitoneally in fasted rats, the first sign can be observed within 1 to 2 minutes, and consists of hyperactivity. The animal explores its cage more busily than usual. After 2 or 3 minutes, this activity is interrupted momentarily while the animal violently shakes his head; this shaking may be so pronounced that it involves not only the head but the entire body. After 3 to 5 minutes, the animals undergo occasional periods when all 4 legs are flexed so that the abdomen touches the floor of cage, and the animal will crawl around on the floor. There is none of the walking backward which Woolley(1) has described in mice. After about 5 to 10 minutes, hyperactivity subsides and the animals become unusually quiet; they usually withdraw to back of cage and remain nearly motionless for prolonged period. If the cage door is opened, the difference between these animals and untreated fasted rats is very striking; the normal rats crowd to the door and eagerly sniff at the observer, while the intoxicated rats remain at furthest possible point from the door. After about 15 minutes, the animals salivate profusely.

When the LSD-treated rat is picked up at height of drug effect, he may appear to be nearly normal as long as he is being held in the hand, but if he is placed at bottom of rope, he appears to be confused. The animal may stay on electrified grid for several seconds or more, uttering a "squeak" each time the shock is applied, before he jumps onto the rope to avoid the shocks. Once on the rope, his method of climbing is different from that of a normal rat. The normal animal advances both forepaws at once, then both hindpaws, so that he appears to be literally leap-

TABLE I. Dose-Response Relationship between Lysergic Acid Diethylamide (LSD) and Climbing Time Delay (C.T.D.) in Rats Trained to Climb a Rope.

Dose LSD, mg/kg	.175	.25	.35	.5
No. of animals	5	5	5	5
C.T.D., min.-sec.	165	484	1,047	1,316
± Stand. error	35.8	85.9	222.3	297.4

Analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	19	7,040,599		
Within groups (error)	16	2,931,284	180,205	1
Between groups	3	4,109,315	1,369,772	7.6*
Linear regression	1	3,672,129	3,672,129	20.2*
Deviations from linear regression	2	437,186	218,593	1.2†

* Highly significant $P < 0.01$. † Not significant.
Stand. dev. of single determination = 425.
Regression equation: $Y = 2032 + 2414 \log X$, in which Y = C.T.D. in minute-seconds, X = dose of LSD, mg/kg.
Index of precision (Lambda) = 425/2414 = 0.15.

ing up the rope; the intoxicated animal, on the other hand, slowly advances one paw at a time. The treated animal may climb a short distance, then stop and remain motionless as though staring into space. Often, he will become so confused that he will try to circle around the rope instead of climbing, or he may climb part way up, then turn around and come down again. If he does succeed in making the climb, he hangs his head over the food cup without eating.

The extreme effects just described are seen in the majority of animals at a dose of 0.5 mg/kg, and in an occasional rat at 0.25 mg/kg; at 1 mg/kg the effects are the same and last somewhat longer.

Dose-response curve for LSD in rats. As shown in Table I, the effect of LSD in producing delay in climbing time (C.T.D.) may be measured objectively and quantitatively, and is related to log dose in the range of 0.175 to 0.5 mg/kg. The statistical analysis shows that a fairly reliable assay can be achieved with 5 animals per dose, and that within the range used, there is no significant deviation from linearity. The experiments shown in Table I were all performed on the same day. In addition to these, nine other determinations of C.T.D. have been made at the dose of 0.5 mg/kg during a period of 3 months; the mean values obtained ranged from 832 to 1861 min-sec, with an overall mean of 1230 min-sec, a value not much different from that shown in the table.

Modification of the effect of LSD by other drugs. When either 5-hydroxy tryptamine* (serotonin) or 5-hydroxy tryptophane is injected intraperitoneally 30 minutes before LSD, the effects of the latter on climbing time are strongly antagonized (Table II). Serotonin alone, produces a characteristic syndrome which differs markedly from that of LSD. The eyes become half-closed, but open wide when animals are handled. Animals withdraw to back of cage, but instead of being flat on the floor as after LSD, they tend to elevate the fore quarters and stand erect. Strong contractions of abdominal musculature may be seen after intraperitoneal injection, with extension of hind legs from time to time. Animals appear apprehensive and fearful, and react strongly to noise. The tail is occasionally whipped back and forth in a horizontal plane. When climbing, they tend to advance one paw at a time instead of leaping up the rope, but they do this fairly rapidly so that climbing time is but little prolonged. When they reach the food cup, they usually eat readily. When LSD is injected into serotonin-treated rats, the LSD-syndrome is markedly attenuated. The hyperactivity, head shaking, and crawling movements still occur but at reduced intensity; the excessive salivation is abolished. When placed on the

* Serotonin was injected as creatinine sulfate, and dose is expressed as base.

TABLE II. Antagonism of 5-Hydroxy Tryptamine and 5-Hydroxy Tryptophane to Effects of Lysergic Acid Diethylamide (LSD) in Trained Rats. Antagonist injected 30 min. before LSD. Intraperitoneal administration.

LSD			LSD + antagonist			
Dose, mg/kg	No. animals	C.T.D., min.-sec.	Antagonist	Dose, mg/kg	No. animals	C.T.D., min.-sec.	P
.5	24	1045	5-hydroxy tryptamine	.9	26	221	*
.5	5	1616	" tryptophane	5	5	446	.04
.25	5	776	" "	2	5	258	.05

* Pooled results of 5 exp.; "P" for difference between rats treated with LSD alone and LSD + antagonist varied from .05 to <.001.

rope, there is little sign of confusion, and climbing movements are well coordinated.

5-hydroxy tryptophane in the doses used did not produce any signs in rats except some fur erection and considerable scratching at dose of 5 mg/kg. There was no effect on climbing performance or C.T. When these animals were given LSD, the results were identical with those receiving both LSD and serotonin.

The antagonism of LSD with intraperitoneally administered serotonin is of especial interest, since it has been claimed that peripherally administered serotonin does not enter the brain readily, and that the effects of LSD in mice can be antagonized by serotonin only if the latter is injected intracerebrally in combination with a cholinergic drug(1). Our results are more in accord with the findings of Shore *et al.*(3) that peripherally administered serotonin produces central effects.

Neither reserpine nor α-(4-piperidyl) benzhydrol antagonized the effects of LSD (Table III). Reserpine by itself produced considerable delay in climbing, as might be expected from the depressant properties of this drug. When LSD was injected into reserpine-treated rats, the C.T.D. was much greater than with either drug alone; indeed, the figure given in the table for the higher dose of reserpine is somewhat lower than the true figure, for the experiment was terminated 3 hours after LSD when the C.T. had not yet reached the base line. The LSD-syndrome was more intense and more prolonged in these animals than in any we have seen given this dose of LSD alone. In some of the rats, the effects of LSD were still maximal 45 minutes after injection; normally, maximal LSD effects are seldom seen beyond the 20 minute reading.

The LSD-syndrome in the rats pretreated with α-(4-piperidyl) benzhydrol was identical with that seen with LSD alone. An analog of this compound, α-(2-piperidyl) benzhydrol, has been reported to block the LSD psychosis

TABLE III. Effect of Reserpine and of α-(4-Piperidyl) Benzhydrol on Action of Lysergic Acid Diethylamide (LSD) in Trained Rats.

	Treatment	No. animals	C.T.D., min.-sec.	P*
1.	LSD 0.5 mg/kg	5	1236	
2.	Reserpine 5 mg/kg p.o. × 3 da.	5	1180	
	" 2.5 " " " "	5	470	
3.	Reserpine 4 daily doses prior to LSD†			
	" 5 mg/kg, LSD .5 mg/kg	5	3348	.005
	" 2.5 " , *Idem*	5	2335	>.05
4.	α-(4-Piperidyl) benzhydrol 25 mg/kg	5	217	
5.	α-(4-Piperidyl) benzhydrol 30 min. prior to 0.5 mg/kg of LSD:			
	α-(4-Piperidyl) benzhydrol 12.5 mg/kg	5	1120	>.05
	Idem 25 "	5	1223	>.05

* P = probability of difference between drug combinations and LSD alone.
† LSD inj. 30 min. after last dose of reserpine.

in man(6). We have but a single experiment on this analog in the climbing rats; 10 mg/kg failed to block the effect of 1 mg/kg of LSD. Further experiments of the effects of this drug in LSD-treated animals are indicated.

Summary. 1) A syndrome resulting from intraperitoneal injection of LSD in rats is described. In rats trained to climb a rope, LSD produces signs of confusion and markedly prolongs climbing time. This effect on climbing time can be objectively and quantitatively measured, and is linearly related to the log dose of LSD. 2) Effects of LSD could be partially antagonized by serotonin or by 5-hydroxy tryptophane administered intraperitoneally. α-4 (piperidyl) benzhydrol did not affect the action of LSD, while reserpine intensified and prolonged the LSD-syndrome.

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