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Effects of LSD-25 and Amphetamine on a Running Response in the Rat

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After injection of *d*-lysergic acid diethylamide (LSD-25) in the rat, the animal shows symptoms of autonomic effects, such as profuse salivation, urination, and defecation. There is some indication that the somatic nervous system is also involved, since the animal exhibits hyperactivity in the first few minutes after injection, while after three to five minutes there is a tendency for all four legs to flex and the abdomen touches the floor. At this point the animal may remain motionless for long periods if permitted to stay in its living cage undisturbed. These symptoms have been observed by Winter and Flataker [1] and also by me. Winter and Flataker, in the same study, also observed that for rats trained to climb a rope for food reinforcement, climbing times were increased as a function of dosage levels. At the height of the drug effect some of the rats did not climb and were assigned arbitrary climbing times. Those that did climb did not eat.

Freedman et al.[2] have extended the work of Winter and Flataker and, in addition to finding similar results, have discovered an apparent tolerance in the rat to daily injections of 0.13 mg. of LSD-25 per kilogram. Development of tolerance was rapid, evident in some rats after the first day and in all animals after four days. In a second group, given injections every 48 hours over 14 days, the evidence of tolerance was incomplete. When injections were given every 72 hours, no tolerance was encountered.

The present studies were initiated to determine the effects of LSD-25 on motor behavior in the rat in other than a food-reinforced situation. In view of the evidence from the Winter and Flataker study that at the height of the drug effect the rats failed to eat, it seemed logical to seek other means of motivating the animals.

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Method

A runway 9 ft. long, 6 in. high, and 2¼ in. wide was utilized. The floor of this runway consisted of two ¾-in. aluminum angles placed in a manner forcing the animals to straddle a ⅞ in. air space between the angles. These angles constituted the electrodes. Voltage was controlled by a Powerstat, through 47,000 ohms resistance in series with the electrodes and held constant at 90 throughout the run. The middle 7 ft. of the animal's run was timed to the nearest 0.01 second through the use of swinging doors and microswitches manipulated by the subjects. These doors operated relays, which, in turn, controlled a standard electric timer. Each trial consisted of placing the subject in the starting compartment of the runway, energizing the electrodes, and timing the run over the charged runway to the goal box. The only method available by which to escape the shock was to run to the goal box, since the swinging doors operated in only one direction.

Experiment 1.—The subjects of the first experiment were 26 male Sprague-Dawley rats, with a mean weight of 368±5 gm. on the first day of injection. Preliminary training trials were given until the mean running times reached an asymptote. Two runs, separated by at least three hours, were recorded each day. During the experimental phase, the first trial of each day was a control run without injection. LSD-25 was dissolved in Ringer's solution and injected intraperitoneally 10 minutes before second trials on alternate days. Every other day consisted of the control run, followed by a trial using an injection of Ringer's solution alone. Seven LSD-25 trials were given and two dosage levels investigated, viz., 0.50 and 1.0 mg/kg. Subjects were assigned to these groups in counterbalanced order on the basis of their speed of running during preliminary training.

These high-dosage levels and the 10-minute interval were used in this study because Winter and Flataker report maximal effects under these conditions.

Experiment 2.—In this study the duration of effects of LSD-25 were investigated. A group of 25 male rats of the Sprague-Dawley strain, with a mean weight of 377±2 gm. on the day of injection, were trained in the same apparatus and under the same conditions as the first group. After preliminary training, they were assigned to control and experimental groups in counterbalanced order on the basis of their running time on the last day of preliminary training. The experimental group, consisting of 13 animals, was injected intraperitoneally with 0.50 mg/kg. of LSD-25 dissolved in Ringer's solution and the control (12 animals) with Ringer's solution alone. Test runs were made at 6-minute, 30-minute, and 30-minute intervals after injection. The groups were then run until the mean running time of the LSD-25 group equaled or exceeded that of the controls.

Experiment 3.—This study investigated the tolerance effects of injections of 0.50 mg/kg. at 24-hour intervals. Twenty-three male rats of the strain used before, with a mean weight of 350±7 gm. on the first day of injection, were utilized. They were trained under the same conditions as the subjects of the first two studies, with one exception. The apparatus was modified by providing a photoelectric relay at the start end of the runway and a platform resting on a microswitch in the goal box. These operated the timer in place of the swinging doors and had the effect of increasing the runway length to 7 ft. 6 in. In addition, it appeared to facilitate running, since the animals no longer had to push aside the swinging doors.

After preliminary training, the first trial of each day was a control run without injection. LSD-25 was dissolved in Ringer's solution and injected intraperitoneally six minutes before second trials. This procedure was followed for nine consecutive days.

Experiment 4.—The tolerance phenomenon was investigated in this study with two smaller doses of LSD-25. Fifty-four male rats of the strain used before and weighing 324±3 gm. on the first day of injection were utilized. After preliminary training under the same conditions as existed in Experiment 3 the animals were assigned to three groups of 18 subjects each on the basis of their running times on the last training day. Two LSD-25 groups were used, one receiving an intraperitoneal injection of 0.13 mg/kg. and the other one of 0.26 mg/kg. LSD-25 was dissolved in Ringer's solution and injected intraperitoneally six minutes before the run. The control group was injected with Ringer's solution only. One trial a day was given for seven consecutive days.

Experiment 5.—The data of the first four experiments gave indications that the effects of LSD-25 were similar to what one would expect of an excitant. In this study amphetamine was chosen for comparison, and similarities between it and LSD-25 were investigated with the running response. Two dependent variables were used—running to escape shock and running to avoid shock. The first study (speed of escape) utilized 23 male rats with a mean weight of 359±5 gm. the day of injection. After preliminary training a control measure was recorded, and two hours later one group (12 animals) was injected intraperitoneally with 0.50 mg/kg. of LSD-25 per kilogram. The remainder of the animals were injected intraperitoneally with 2 mg. of amphetamine sulfate (racemic amphetamine sulfate) per kilogram. Both groups were run 6, 30, 60, 90, 120, and 150 minutes after injection.

The avoidance behavior was studied by using the shock runway with the photoelectric relay removed. A group of 40 male rats (mean weight on first day of injection 278±2 gm.) were run five trials a day with the following procedures. The animals were placed in the runway and a muffled buzzer sounded five seconds before the onset of shock. The shock levels were identical with those used in the escape work. Shock and buzzer remained on until the animal ran to the goal-box platform operating the microswitch. A conditioned response was defined as running to the platform after onset of buzzer, but before the onset of shock. Ninety such trials were given each subject. At the end of this period the animals were only partially trained, allowing for the effects of the drugs to show an increase in conditioned responses. After conditioning trials the animals were assigned to two experimental groups and one control group on the basis of the number of conditioned responses during conditioning. At this point experimental extinction training began. The buzzer was sounded as usual, but if the animal failed to traverse the runway in the five-second period, the buzzer was automatically turned off and no shock given. All animals were run five trials a day under these conditions and the number of conditioned responses recorded. Group I was run 8 minutes after IP injection of 0.50 mg/kg. of LSD-25; Group II, 60 minutes after IP injection of 2 mg/kg. of amphetamine sulfate, and Group III, 8 minutes after IP injection of Ringer's solution. The data were analyzed, with an analysis of variance of the number of conditioned responses during the first four days of extinction.

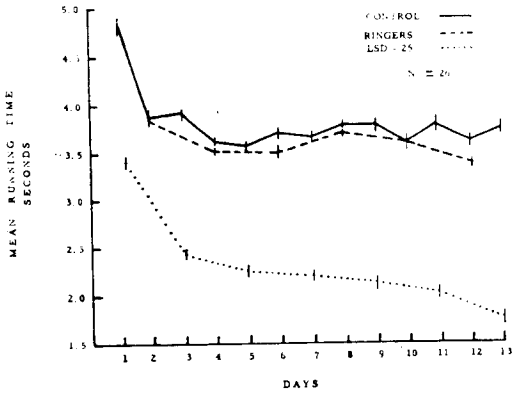

Fig. 1.—Mean running time to escape shock under three conditions with the same subjects.

Results

EXPERIMENT 1.—On completion of this work none of the test runs differentiated between dosage levels, since both groups showed a comparable drop in running times. For purposes of analysis the two groups were combined (Fig 1). All animals served as their own controls and *t*-tests were determined between control and injected trials. None of the Ringer's runs differed significantly from the control levels. All seven LSD-25 trials differed from the control runs beyond the 0.01 level.

EXPERIMENT 2.—Under the conditions of this study the maximum LSD-25 effects appeared at six minutes, and the subjects were back at the control level four hours after injection (Fig. 2). The mean difference between the two groups at the six-minute period was found to be significant beyond the 0.01 level with the *t*-test. This significance substantiates the findings of the first study on the effects of LSD-25 on escape from shock.

EXPERIMENT 3.—Data from this study are summarized in the Table. The value of these findings lies in the fact that the decrease in running time under LSD-25 was still highly significant statistically after nine injections. The mean difference for each of the nine days was significant beyond the 0.01 level with the *t*-test. In this test the animals were used as their own controls. For three days following the LSD-25 trials the rats were injected with Ringer's solution alone in place of the LSD-25. When these

Summary of Effects on the Running Responses of Daily Doses of 0.50 Mg. of LSD-25 per Kilogram of Body Weight

Day	Mean Running Time		Mean Difference	*t*	No. of Rats with Increased Speed Under LSD-25 N-23
	Control	LSD-25			
1	3.60	2.34	1.26	9.0 *	22
2	3.34	1.96	1.38		23
3	3.17	1.92	1.19		22
4	3.32	1.91	1.32		23
5	3.19	2.01	1.18		23
6	3.16	2.01	1.15		23
7	3.23	2.39	0.84		20
8	2.97	2.26	0.72		17
9	3.18	2.12	1.06	7.6 *	22

* Significant beyond the 0.01 level.

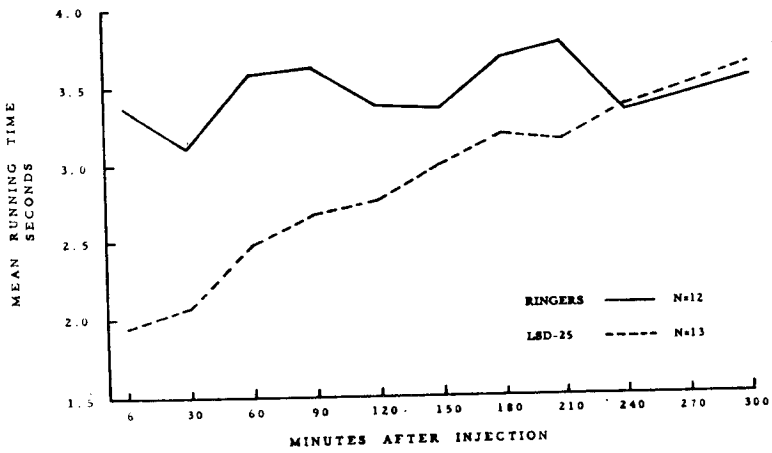

Fig. 2.—Duration of effect of LSD-25 on running time to escape shock.

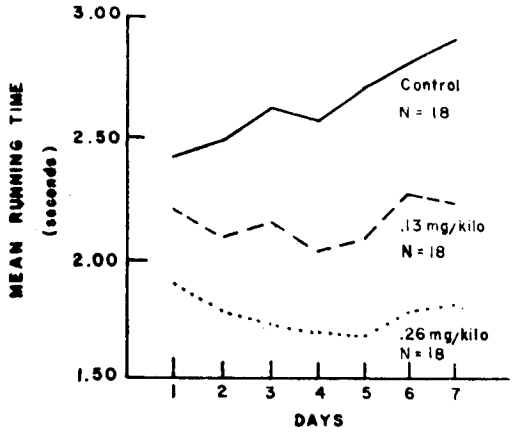

Fig. 3.—Effects on running time of two dosage levels of LSD-25 over seven consecutive days.

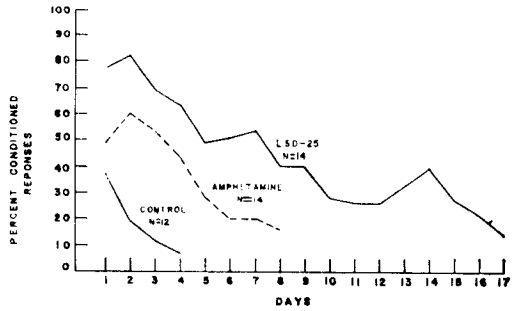

Fig. 4.—Group percentage of conditioned responses during extinction training. On the last day of conditioning the entire group (N-40) was at the 59% level. All animals were run and given injections at 24-hour intervals.

runs were compared with the control runs, there were no significant differences.

EXPERIMENT 4.—Figure 3 shows the results of this work. Since previous findings supported the hypothesis that LSD-25 results in a decrease in running time, the *t*-test of significance was applied directly to the three groups without first doing an analysis of variance. The difference between the control and the 0.13 mg/kg. group was not significant on the first injection day. However, on Day 7 the mean difference was significant at the 0.01 level with the *t*-test. The mean difference between the control and the 0.26 mg/kg. group was significant at the 0.05 level on Day 1 and at the 0.01 level on Day 7, with the *t*-test. The steady rise in running time for the control group over the test period is the usual picture with rats trained on the escape runway. After learning to escape and reaching an asymptote, the running times have a tendency to increase if the shock level remains constant. This bias has the effect of enhancing the probability of tolerance and operates against the observed results. It is believed that the slight rise in running time of the experimental groups after the low point reached in the middle portion of the seven-day period is better explained by the behavior of the control group than by an inference of tolerance.

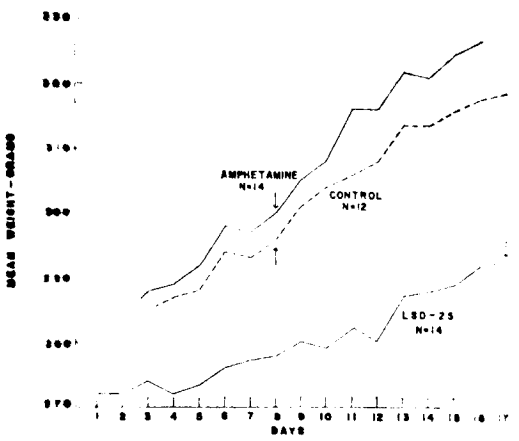

Fig. 5.—Effect of daily injections of LSD-25 on the weight curve of the rat. Arrows point to last injection day.

EXPERIMENT 5.—On the shock escape test the LSD-25 group attained its fastest speed at the six-minute interval, the same as in Experiment 2. The mean difference between the control and the LSD-25 run was 1.21 seconds, significant beyond the 0.01 level with the *t*-test. For the amphetamine group the fastest speed was attained at the 60-minute interval. The mean difference between control and amphetamine runs was 0.94 second, significant beyond the 0.01 level with the *t*-test. The direction of the effects of the two drugs was the same, differing only in time of maximal effect. Figure 4 shows the results of the avoidance data. The analysis of variance of the number of conditioned responses during the first four days of extinction resulted in an F of 9.3, significant beyond the 0.01 level, with

2 and 37 degrees of freedom. At this point it was decided to continue to run the experimental groups until the number of conditioned responses for each group fell below the 20% level. This point had already been reached by the control animals on Day 2, by the amphetamine group on Day 8, and by the LSD-25 group on Day 17. The sudden drop in number of conditioned responses of the control group was expected, since the entire group of 40 animals on the last conditioning day gave 118 conditioned responses out of a possible 200, for a percentage of 59. This permitted the effects of the drugs to go in either direction and allow for a more sensitive test.

Figure 5 shows the effects of LSD-25 on the weight curve of the rat. These weights were recorded from the first day of injection to the 17th or last day of injection for the LSD-25 group. Apparently, 2 mg/kg. of amphetamine sulfate per kilogram given daily is not sufficient to depress the food intake of the rat. At least it does not have an effect on the weight curve over an eight-day period. The rates of gain for the amphetamine and control groups are quite similar, while the rate of gain of the LSD-25 group is obviously depressed. Apparently, during the height of the drug effect the rats do not eat. Winter and Flataker [1] report that their LSD-25 rats that did climb the rope hung their head over the food cup but did not eat. In a personal communication, Freedman relates that human subjects report a decrease in appetite after ingestion of LSD-25. For the present study it is not believed that the decrease in running time is related to the depression of the weight curve. Rats have been run on the escape runway 24 hours deprived of food without a significant drop in running time. In addition, the effects of LSD-25 are noted six minutes after the first injection, hardly a long enough period to be related to food deprivation.

Comment

Three conclusions are supported by the data of these studies.

1. Rats run faster to escape shock and show more avoidance behavior after injection of LSD-25 than under control conditions.

2. This is so also for rats injected with 2 mg/kg. of amphetamine.

3. There is no evidence to support a hypothesis that tolerance to the above effects of LSD-25 is developed in the rat on repeated injections at 24-hour intervals over a seven-day period.

Behaviorally, these data support the hypothesis of Bradley and Key [3] that under certain conditions the effects of LSD-25 and amphetamine are similar. In recording alerting of electrical activity from the cortex in the conscious cat, they have observed similarities between LSD-25 and amphetamine. They believe these similarities to exist for different reasons. Both drugs may act as excitants on reticular formation, amphetamine through direct action and LSD-25 through its effects on afferent fibers to the brain stem. The decrease in running time found in the present study is what one would expect with an excitant. However, the excitability in the LSD-25 rat is not qualitatively the same as that in the rat with amphetamine. After injection with amphetamine the rat shows hyperactivity in his living cage and when picked up is alert and tense. With LSD-25 there is definite hypoactivity in the home cage, and when picked up, the animal does not appear alert. It is not until stimulated with shock or buzzer that the LSD-25 animal becomes hyperactive. This agrees with the prediction of Bradley and Key [3] that the behavioral effects noted after injection of LSD-25 are contingent upon the environmental conditions of the test situation.

The results of the avoidance test are interesting in light of the contradictory data obtained by Miller et al.[4] with chlorpromazine. However, caution must be exercised in interpreting these results. They are not believed to be the result of increased fear on the part of the drug groups. Instead, it is hypothesized that under the influence

of LSD-25 and amphetamine, the buzzer becomes a noxious stimulus for the rat whether or not it has been paired with the shock. This is another aspect of LSD-25 effect that needs to be studied. Sufficient at the present is the evidence that, using the traditional avoidance-conditioning paradigm, similar results are obtained with the two drugs.

The data related to the development of tolerance shown by the rats in the Freedman study are not supported by the present work. It is true that a slight rise in running time did occur; however, the large rise of the control group more than offset this. Furthermore, the animals of the present study were run three to five days after Freedman reported complete tolerance. It may be that with daily injections for longer periods (12-15 days) tolerance would develop. Even so, the tolerance picture for rope climbing and shock escape would show differences, if only from the standpoint of development time. Apparently, tolerance to LSD-25 does not develop in all systems. Freedman and his group [2] have shown that the bradycardia observed in the rat after LSD-25 injections shows no tendency toward the development of tolerance after 12 daily injections. This also held true for the respiratory arrest observed. Other studies [5,6] have shown that pyrexia, mydriasis, and piloerection are autonomic effects of LSD-25 which show the development of tolerance. In the present work (Experiment 5) note was made of the presence or absence of profuse salivation in the rat. This phenomenon is noticeable within the first eight minutes after LSD-25 injection. This response showed no evidence of tolerance, even after 17 daily injections. It appears that the differential tolerance observed at the autonomic level also exists in voluntary behavioral systems.

Summary

The effects of LSD-25 on the running speed of rats in an escape from shock situation were investigated. Over the dosage levels used (0.13 to 1.0 mg/kg.) an increased speed of running was consistently observed. There was no evidence to indicate the development of tolerance to these effects after seven daily injections. In addition, it was shown that, under the same testing conditions, amphetamine produced similar results. Studies of conditioned avoidance responses indicated higher percentages of avoidance in rats under LSD-25 and amphetamine than under control conditions.

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