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Pharmacological and Behavioral Components of Tolerance to LSD and Mescaline in Rats

T. F. MURRAY,[1] A. L. CRAIGMILL AND G. J. FISCHER

Washington State University, College of Pharmacy and Department of Psychology, Pullman, WA 99163

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MURRAY, T. F., A. L. CRAIGMILL AND G. J. FISCHER. *Pharmacological and behavioral components of tolerance to LSD and mescaline in rats.* PHARMAC. BIOCHEM. BEHAV. 7(3) 239–244, 1977. – A fixed-ratio schedule of water reinforcement (FR-10) was used to examine the relative contributions of pharmacological and behavioral mechanisms in the development of tolerance to the disruptive effects of LSD and mescaline in the rat. Rats treated daily with LSD or mescaline before operant testing developed tolerance to the impairment of responding, while rats treated daily after each session did not display tolerance when the drugs were administered before testing. These results indicate that behavioral compensatory mechanisms may be involved in the development of tolerance to the disruptive effects of LSD and mescaline on fixed-ratio (FR-10) performance.

Lysergic acid diethylamide Mescaline Pharmacological tolerance Behavioral tolerance
Schedule controlled behavior

DRUG tolerance may be defined as a state of decreased responsiveness to any pharmacologic effect of a drug as a consequence of prior exposure to the drug [7]. The development of tolerance to the behaviorally disruptive effects of d-lysergic acid diethylamide (LSD) and mescaline on operant responding in rats has been reported previously by several authors [1, 5, 15]. Freedman and coworkers [5] reported that all rats trained to respond for food on a fixed-ratio (FR) schedule developed complete tolerance to the effects of 130 μg/kg LSD within 7 to 8 days. Using the same paradigm, these authors also noted that only 2 of 10 rats developed tolerance to LSD when the dose was increased to 195 μg/kg. Appel and Freedman [1] and Tilson and Sparber [14] have reported a rapid development of tolerance to the rate depressant effects of mescaline SO_4 (10 mg/kg) using rats trained to respond for food on a FR-30 schedule. Winter [15] observed the development of tolerance and cross-tolerance to the disruptive effects of LSD tartrate (96 μg/kg) and mescaline HCl (9.9 mg/kg) on the performance of rats trained to respond for food on a FR-20 schedule. Winter also examined brain and liver concentrations of LSD in acutely and chronically treated animals and concluded that metabolic factors were not involved in the observed tolerance.

The purpose of the present investigation was to examine the relative contributions of pharmacological and behavioral mechanisms in the development of tolerance to the disruptive effects of LSD and mescaline in rats responding on a FR-10 schedule for water. Water reinforcements were used to control for the known anorexic effect of LSD in rats [9]. The experimental design that was employed to discriminate between the two possible mechanisms of tolerance was similar to the procedure developed by Chen [4]. In this design, one group of animals received daily treatments with the appropriate drug immediately prior to the operant session, while a second group received daily drug treatments following the operant session. Hence, both groups of animals receive identical pharmacological exposure to the drug, but only the first group has the opportunity to perform the operant task while experiencing the drug effect. After the presession treatment group displays tolerance to the behaviorally disruptive effects of the drug, both groups of animals receive the drug treatments prior to the operant session on a test day. If the observed tolerance is the result of true pharmacological mechanisms, then both groups should display it on the test day. However, if the tolerance observed in the presession treatment group is the result of behavioral compensatory mechanisms, then the postsession treatment group should not display tolerance on the test day.

METHOD

Animals

The animals used in this experiment were 8 experimentally naive, adult female hooded rats weighing 200–250 g. All animals were housed individually in the Washington State University Psychology Department colony with a 12 hr, day-night cycle. Throughout the experiment, all animals were maintained on a 23.5 hr per day water deprivation schedule with free access to lab chow in their home cage.

Apparatus

The experiments were conducted in two Grason-Stadler operant chambers equipped with liquid dippers which

[1] Send reprint requests to: T. F. Murray, Department of Pharmacology, School of Medicine, SJ-30, University of Washington, Seattle, WA 98195.

TABLE 1

DRUG TREATMENT SCHEDULE FOR 25 DAY TOLERANCE STUDY

Day	Drug Dose and Time of Treatment
1-3	Saline—all groups presession
4-10	LSD (250 μg/kg) or mescaline (100 mg/kg)—both pre- and postsession groups
11-17	Saline—all groups presession
18-23	LSD (100 μg/kg) or mescaline (10mg/kg)—both pre- and postsession groups
24	LSD (100 μg/kg) or mescaline(10 mg/kg)—all groups presession
25	LSD (250 μg/kg) or mescaline (100 mg/kg)—all groups pression

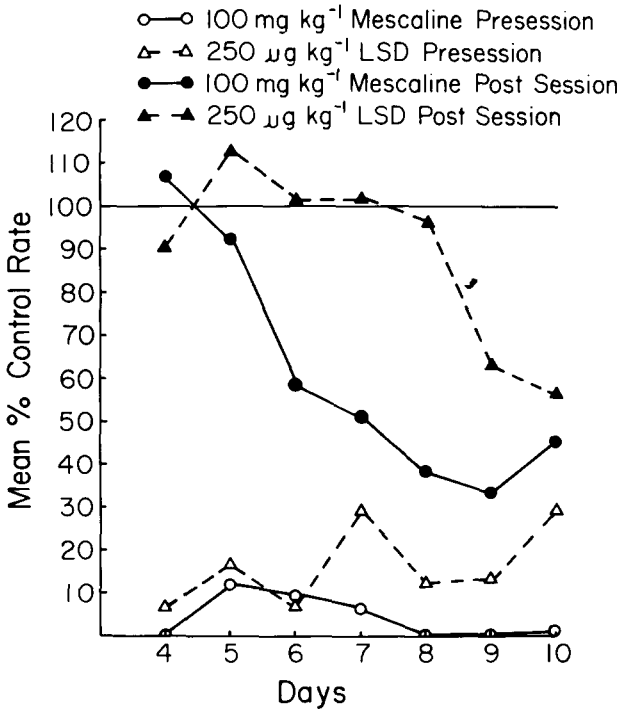

FIG. 1. Effects of daily presession or postsession treatment with LSD (250 μg/kg) or mescaline (100 mg/kg) on water reinforced bar pressing. The ordinate is the response rate for the 20 min session expressed as the mean percentage of the rate obtained from saline control sessions (Day 1–3). Each point is the mean of two rats. Presession animals were injected with the drug IP immediately prior to placement in the operant chamber, while postsession animals were treated after the session before return to their home cage.

dispensed 0.1 ml of tap water with each operation. Each box contained a single response lever projecting 5/8 in. into the chamber which required a force of 15 g to depress. External stimuli were masked by the noise of exhaust fans mounted on the sound attenuating cabinets. All experimental events were programmed by relay and timing circuits in an adjoining room and responses were recorded on electromagnetic counters and cumulative recorders.

Behavioral Procedure

The animals were first trained to respond on a continuous reinforcement (CRF) schedule with daily sessions lasting 20 min. All animals were run at the same time each day 7 days a week. After all rats were responding on the CRF schedule, a fixed ratio schedule was introduced and gradually increased to a FR-10. Training on the FR-10 schedule was continued for 21 days by which time stable rates of responding were obtained for all rats. To quantitate data for the various groups, response rates were calculated by dividing the total number of lever presses in a single session by 20 min. These response rates were calculated daily for each animal and group mean response rates were determined and expressed as the mean percentage of the appropriate control rate. Each animal served as his own control and Student's t tests were used to statistically evaluate the data.

Pharmacological Procedure

LSD tartrate was obtained from the National Institute of Mental Health, and mescaline HCl was purchased from Sigma Chemical Co. (St. Louis, MO). Doses of both compounds refer to their salts. Both drugs were dissolved in isotonic saline and injected IP in a volume of 2 ml/kg body weight.

The rats were randomly assigned to LSD and mescaline groups which were further subdivided into presession and postsession treatment groups (i.e., 4 experimental groups with 2 rats per group). Table 1 shows the drug treatment schedule employed in this study. The entire experiment was conducted in 25 consecutive days. On Days 1–3 and 11–17 (saline control sessions), all animals were injected IP with isotonic saline (2 ml/kg) immediately before placement in the operant chamber. On Days 4–10 and 18–23, animals in the presession treatment groups were injected with LSD or mescaline immediately before placement in the operant chamber, and with isotonic saline immediately following the session on Days 4–10 and 30 min after the session on Days 18–23. Animals in the postsession treatment groups were injected with isotonic saline immediately prior to placement in the operant chamber on Days 4–10 and 18–23, and with LSD or mescaline immediately following the session on Days 4–10 and 30 min after the session on Days 18–23. On Days 24 and 25, both the pre-and postsession groups were injected with LSD or mescaline immediately before placement in the operant chamber. On Days 4–10, the doses used were LSD (250 μg/kg) and mescaline (100 mg/kg), while on Days 18–24 the doses were reduced to 100 μg/kg for LSD and 10 mg/kg for mescaline. These doses were selected on the basis of pilot experiments in our laboratory and the previous studies mentioned in the introduction.

RESULTS

The mean response rates for all 8 animals during the

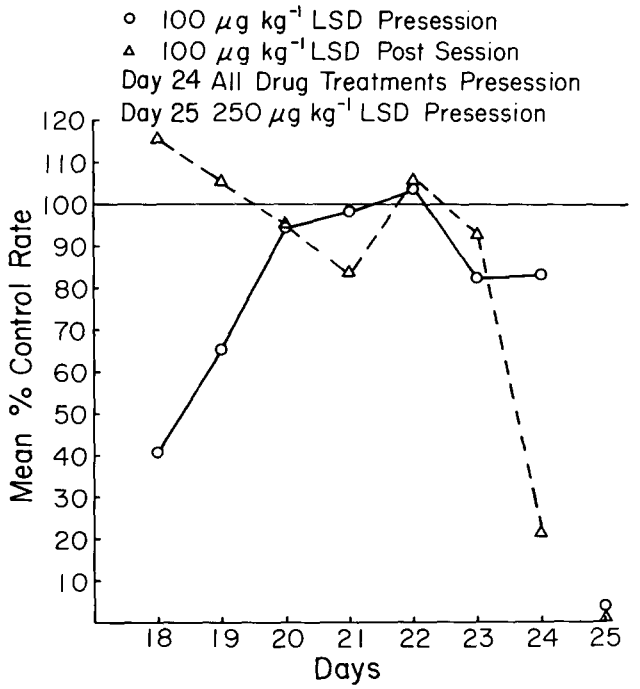

FIG. 2. Effects of daily presession or postsession treatment with LSD (100 μg/kg). The ordinate is the response rate for the 20 min session expressed as the mean percentage of the rate obtained from saline control sessions (Day 11–17). Each point is the mean of two rats. On Days 18–23 presession animals were injected IP with LSD (100 μg/kg) immediately prior to placement in the operant chamber, while postsession animals received the same treatment 30 min after the session. All animals were treated with LSD immediately before the session on Day 24 (100 μg/kg) and Day 25 (250 μg/kg). On Day 24 the mean rates of responding in the presession and postsession groups were significantly different ($p<0.05$).

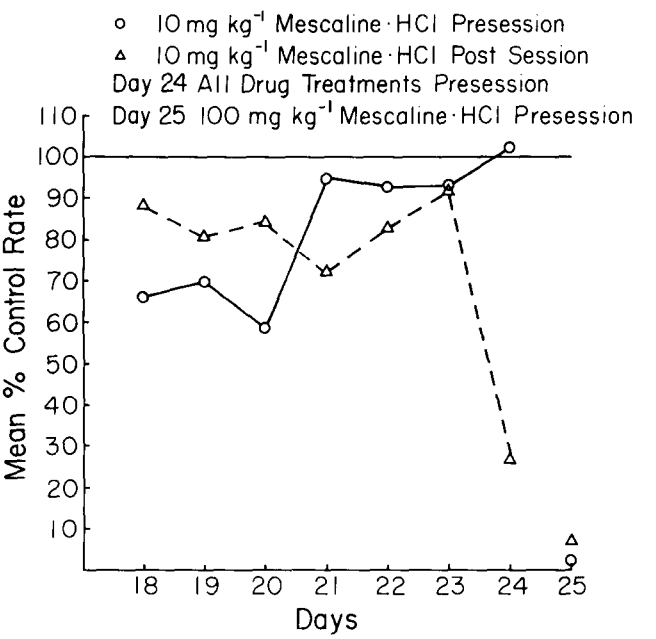

FIG. 3. Effects of daily presession or postsession treatment with mescaline (10 mg/kg). The ordinate is the response rate for the 20 min session expressed as the mean percentage of the rate obtained from saline control sessions (Day 11–17). Each point is the mean of two rats. On Days 18–23 presession animals were injected IP with mescaline (10 mg/kg) immediately prior to placement in the operant chamber, while postsession animals received the same treatment 30 min after the session. All animals were treated with mescaline immediately before the session on Day 24 (10 mg/kg) and Day 25 (100 mg/kg). On Day 24 the mean rates of responding in the presession and postsession groups were significantly different ($p<0.05$).

saline control sessions on Days 1–3 and Days 11–17 were, respectively, 40.2 and 42.7 responses per min and were not significantly different. Figure 1 shows the results of the initial 7 day tolerance schedule on Days 4–10. The animals in the presession treatment groups did not develop tolerance to LSD (250 μg/kg) or mescaline (100 mg/kg) during the 7 day treatment schedule. On Day 4 the animals in the mescaline presession group displayed a complete cessation of responding for the entire 20 min session, and after 7 consecutive days of treatment prior to the session, this group's mean performance on Day 10 was 1.2% of its control rate.

On Day 4, the animals in the LSD presession group responded normally for the first 3–5 min of the experimental session. This was followed by an abrupt cessation of responding for the remainder of the session. Both animals in this group did occasionally resume responding in the last few minutes of the session on subsequent days, and on Day 10 their mean rate of responding was 29.4% of their control rate. Both the LSD and mescaline presession animals exhibited the characteristic low extended posture and crawling behavior during periods of no responding. On Days 4–10 the mescaline and LSD postsession treatment groups responded at rates near or above their control rates for the first 2 and 5 drug days, respectively. Both groups then displayed a gradual decrease in response rates for the remainder of the tolerance schedule (Fig. 1). This decrease in response rate occurring prior to treatment with LSD (250 μg/kg) and mescaline (100 mg/kg) is similar to that observed by Cameron and Appel [3] with LSD (200 μg/kg) and may be a conditioned suppression effect associated with the aversive qualities of the high doses of LSD and mescaline used on Days 4–10.

Due to the failure of the presession treatment animals to develop tolerance to the rate depressant effects of LSD (250 μg/kg) and mescaline (100 mg/kg), a second drug treatment schedule (Days 18–24) using lower doses of LSD and mescaline was initiated subsequent to 7 days of saline treatment (Days 11–17). The experimental groups used in the second tolerance schedule were the same as those employed on Days 4–10. Figures 2 and 3 show the results obtained on Days 18–24 when the animals were treated with the low doses of LSD (100 μg/kg) and mescaline (10 mg/kg). On Day 18, LSD and mescaline presession treatments reduced the mean response rates, respectively, to 40.9 and 66.1% of the control values. On Day 23, tolerance had developed to the degree that the presession groups' mean response rates were 82.2 and 93.2% of control for the LSD and mescaline animals, respectively.

To minimize any conditioned suppression in the postsession treatment groups, the animals were not injected with the LSD or mescaline until 30 min after the operant session. This change in procedure combined with the lower, presumably less aversive, doses eliminated the gradual decrease in response rates observed in these groups on Days 4–10.

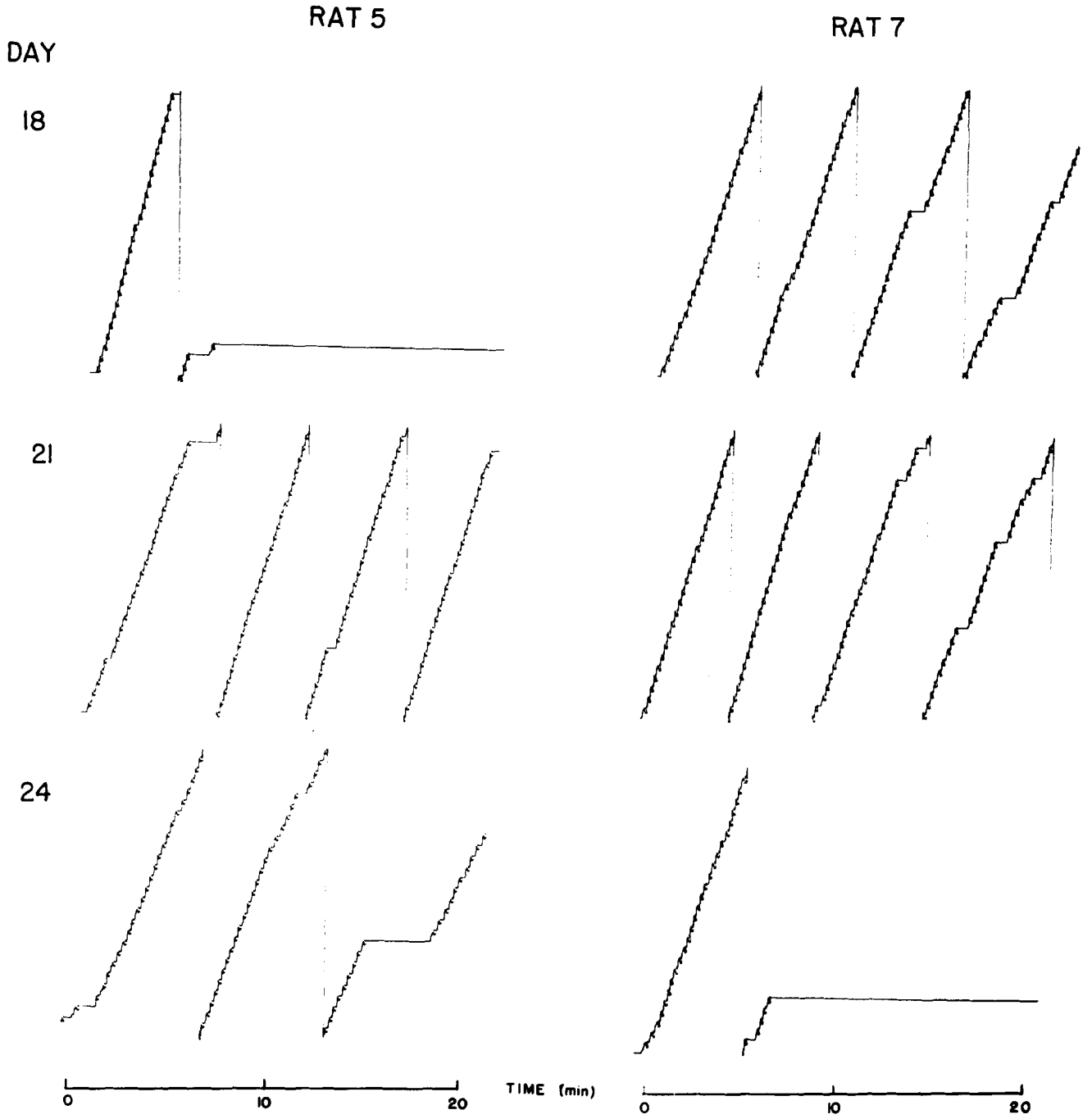

FIG. 4. Cumulative records showing the effects of daily presession or postsession treatment with LSD (100 μg/kg). The records on the left (Rat 5) were obtained from an animal treated with LSD (100 μg/kg) immediately before the operant session on Days 18–24. The records on the right were obtained from another animal (Rat 7) that was treated with LSD (100 μg/kg) 30 minutes after the session on Days 18–23, and immediately before the session on Day 24. Rat 7 did not display tolerance to the disruptive effects of LSD on Day 24 when drug treatment was prior to the session. Rat 5 did however, develop a tolerance to the disruptive effects of LSD after 3 consecutive daily presession treatments.

On Day 24, the presession administration of LSD (100 μg/kg) or mescaline (10 mg/kg) to animals in the postsession treatment groups reduced the mean response rates to 21.4 and 26.8% of their respective control rates. Conversely, on Day 24 the animals in the presession treatment groups maintained their response rates at levels comparable to those recorded on Day 23 (Figs. 2 and 3). The mean response rates for both the LSD and mescaline presession treatment groups were significantly different ($p<0.05$) from their corresponding postsession treatment groups on Day 24. Figure 4 shows typical cumulative records of 2 rats, one each in the LSD presession (left panel) and postsession (right panel) treatment groups, obtained on various days of the Day 18–24 tolerance

schedule. On Day 25, the presession administration of the high doses of LSD (250 μg/kg) and mescaline (100 mg/kg) again reduced the mean response rates of all groups to below 10% of their control values.

DISCUSSION

The possible mechanisms of drug tolerance may be divided into two general categories. The first is pharmacological tolerance, which includes changes in drug absorption, distribution, excretion, and metabolism that may reduce the concentration of drug at the receptor site. Also included under this category is tissue tolerance, which implies that the target tissue has become less sensitive to the effects of the drug. The second general category is behavioral tolerance, which is the result of the animal behaviorally compensating for drug-induced decrements in performance. This type of tolerance results from the interaction of chronic drug administration with repeated behavioral testing [6]. The development of true pharmacological tolerance is favored by the continuous presence of a drug in adequate concentration at the receptor site [7]. For example, Kalant and coworkers have reported that tolerance to ethanol in rats develops more rapidly with the administration of higher doses [10]. Similarly, Goldstein and Sheehan [8] found that for any given dosing interval, larger doses of levorphanol increased the rate of onset and the eventual extent of tolerance to the levorphanol-induced running-fit in mice. To optimize the conditions for the development of pharmacological tolerance in the present study, high doses of LSD (250 μg/kg) and mescaline (100 mg/kg) were administered for 7 consecutive days in the first tolerance schedule (Days 4–10). As shown in Fig. 1, neither of the presession treatment groups displayed tolerance to the rate depressant effects of these compounds. These results are in agreement with those of Freedman and coworkers [5] who reported that with increasing doses of LSD, tolerance to the effects on bar-pressing behavior was less likely to develop. Moreover, Rech *et al.* [11], using a FR-40 schedule, demonstrated that as the daily dose of LSD is increased, the number of days required for the development of tolerance to the drug-induced behavioral effects also increases. These results would seem to argue against an explanation of tolerance to the behaviorally disruptive effects of LSD and mescaline solely on the basis of pharmacological mechanisms.

In the second tolerance schedule (Days 18–24), the doses of LSD (100 μg/kg) and mescaline (10 mg/kg) used were not high enough to render practice impossible by the presession treatment animals in that there was a gradual shortening of the period of no responding. Again, the use of postsession treatment groups was to eliminate the possibility of these animals behaviorally compensating for the drug effects during the operant session. Thus, if a pharmacological mechanism was primarily responsible for the observed tolerance, no differences in performance would be expected between the pre- and postsession groups on test Day 24. While if a behavioral compensatory mechanism was involved in the development of the observed tolerance, the presession treatment group's performance would be expected to be superior to that of the postsession treatment group's on Day 24. Figures 2 and 3 show that both the LSD and mescaline presession groups performed significantly better than their corresponding postsession treatment groups on Day 24. Since animals in both pre- and postsession treatment groups received identical drug treatments, we conclude that pharmacological mechanisms alone cannot adequately account for the development of tolerance to the rate depressant effects of LSD and mescaline in rats responding on a FR-10 schedule of reinforcement. A reduction in the behaviorally disruptive effects of these hallucinogens occurred only when the animals had the opportunities to perform during the operant session while experiencing the drug effects. On Day 25, all groups received presession treatment with the high doses of LSD (250 μg/kg) and mescaline (100 mg/kg), and responding was virtually completely abolished in all animals. Again there was a conspicuous lack of tolerance to these high doses of LSD and mescaline.

The results of the present study conflict with those of a recent report by Rech and coworkers [11] which employed a similar experimental design. Using low doses of mescaline (10 mg/kg) and LSD (150 μg/kg), they reported that the development of tolerance to the repeated administration of these drugs was not dependent on the animal appreciating the drug effect at the time of exposure to the operant session. Although the explanation for the apparent discrepancies in our results is not obvious, there are several methodological differences in the two experiments. Rech and coworkers used a food reinforced FR-30 schedule with 40 minute sessions, while a water reinforced FR-10 schedule with 20 minute sessions was employed in the present study. A possible source of the different results is the food vs. water reinforcement, as it has been shown that increasing hunger attenuates the effects of LSD on a fixed ratio schedule of food reinforcement [2]. Hence the motivational condition of the animals is a critical variable. Also, experimentally naive female hooded rats were used in our experiment while Rech *et al.* do not state the sex, strain, or naivety of their rats. The importance of an animal's prior drug exposure when investigating the behavioral effects of drugs has been demonstrated [13], and strain-related differences in sensitivity to the behavioral effects of LSD are known to exist [2].

Whenever the response of an organism to a drug is capable of being attenuated by a learning process, the result may be misinterpreted as a pharmacological tolerance [7]. Although it is possible that the conditions for the development of pharmacological tolerance were not optimum in this investigation, these results stress the importance of behavioral compensatory mechanisms in the development of tolerance in rats to the disruptive effects of LSD and mescaline on fixed ratio 10 performance.

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