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TOLERANCE TO BEHAVIORAL EFFECTS OF LSD-25 IN RAT[1]

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It has been demonstrated (Abramson, 1956; Isbell *et al.*, 1956) that, in man, tolerance develops to the psychotomimetic and autonomic effects of *d*-lysergic acid diethylamide (LSD-25). In the rat, some autonomic effects of the drug show tolerance (Rothlin and Cerletti, 1956) as does the behavioral response of rope-climbing (Freedman *et al.*, 1958; Mahler and Humoller, 1959). The present experiments investigate the effects of LSD-25 and tolerance to these effects on bar-pressing maintained by food reinforcement. Fixed-ratio and variable-interval schedules, along with several pharmacological variables, were studied.

METHODS AND MATERIALS. *Behavioral procedures and apparatus.* Male albino rats of Sprague-Dawley strain were kept on a restricted diet until their body weights reached 70% of their expected normal weights. Animals were 60 days old at the beginning of the investigation and were experimentally naive. The experiments were run in light-shielded and sound-insulated boxes each of which contained a single lever. Approximately 15 grams of force activated the lever. Bar-pressing responses were recorded in an adjacent room on cumulative recorders and electromagnetic counters. Reinforcements consisted of 45 mg of Noyes rat pellets or a liquid diet of sweetened milk, eggs and vitamins. The weight of each animal was maintained at 70% by feeding supplementary pellets when necessary. No difficulty was encountered in maintaining constant body weights when drugs were administered. Daily experimental sessions were programmed automatically in the adjoining room by means of relay and timing circuits.

Two schedules of reinforcement were studied, fixed-ratio (FR) and variable-interval (VI). When animals were run on FR, sessions were 32 minutes in duration. The number of reinforcements obtained on any given day depended on the value of the ratio studied (FR 5, FR 15, FR 40) and on the rate of response. The animals usually received from 80 to 140 reinforcements during each FR session. The VI had a mean interval between reinforcements of 1.5 minutes. Sessions were 60 minutes long when this schedule was in effect so that rats received about 40 reinforcements each day.

Pharmacological procedures. LSD-25, in ampules containing 100 μg/ml, was supplied by Sandoz Pharmaceuticals. The drug was administered intraperitoneally immediately before the animal was placed in the box. Tolerance schedules consisted of a specified number of daily doses. In the study of acute tolerance, rats were (1) injected with 130 μg of LSD-25/kg at hourly intervals and, after their third injection, were placed in the box for 32 minutes on FR 15, (2) given a single injection of 390 μg of LSD-25/kg and run immediately thereafter, or, (3) given a single injection of 130 μg of LSD-25/kg and run on FR 15.

Methods of presenting drug effects. LSD-25 effects are shown on cumulative-response curves of representative animals drawn from the sessions following either saline control or drug injections, or as a percentage change in the number of responses normally emitted during a session. The percent for any given animal on any day is 100 times the total number of responses on that day, divided by the average number of responses during the four saline sessions preceding the first dose of LSD-25. The means of this percentage score are then calculated and plotted for the daily performances of all animals.

RESULTS. Within 4 to 8 minutes following a dose of 130 μg of LSD-25/kg to rats on FR schedules there was an abrupt onset of drug effect. This was ordinarily characterized by the complete cessation of responding which normally occurred for 20 minutes. The period of no responding sometimes, but by no means always, was observed immediately after reinforcement. The animal's normal ratio performance recovered by the last 4 minutes of the 32-minute experimental session (fig. 1).

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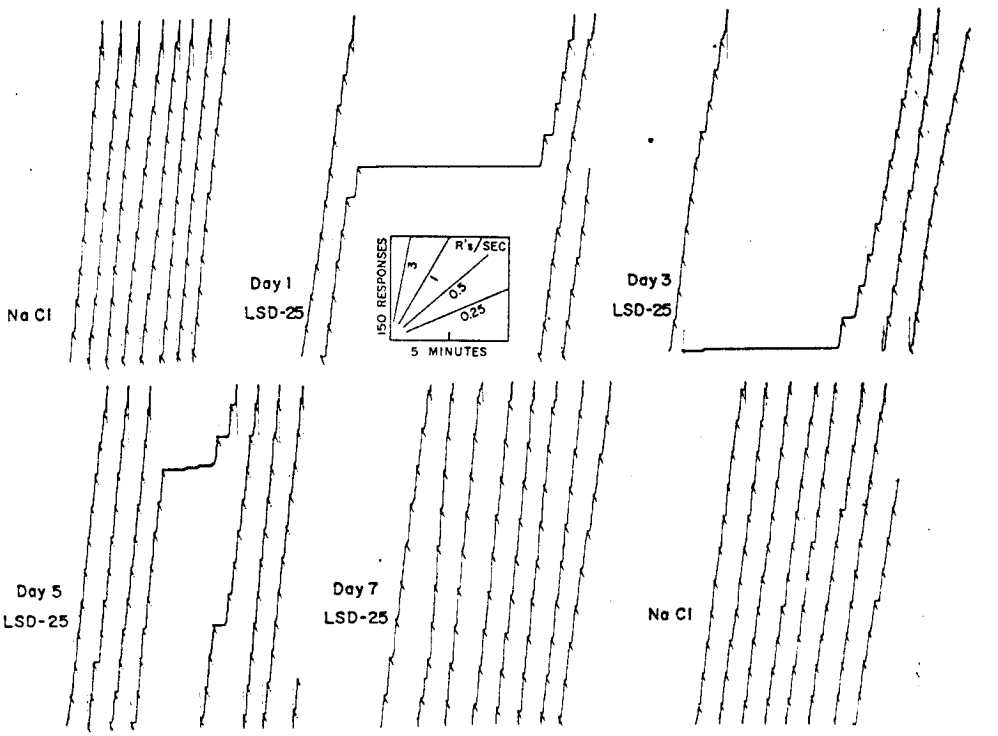

FIG. 1. Cumulative records of bar-pressing on an FR 40 (fixed-ratio 40) schedule of reinforcement for a single rat.

Control sessions before and after the tolerance run and the first, third, fifth and seventh days of treatment with 130 μg of LSD-25/kg are shown.

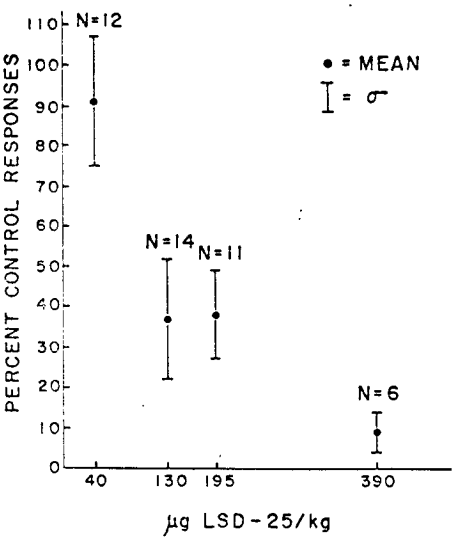

FIG. 2. Dose-response relationship for LSD-25 on FR schedules of reinforcement.

The data include the mean and ± the standard deviation (σ) during the first administration of the drug for each animal.

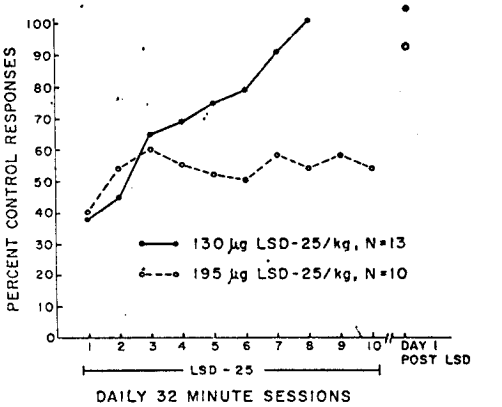

FIG. 3. The effects of daily injections of 130 and 195 μg of LSD-25/kg on FR behavior.

The solid line joins the average daily performances of all animals on all ratios (5, 15 and 40) which received 130 μg/kg and the broken line joins the averages for 195 μg/kg. Control performances are represented by the circles at the upper right.

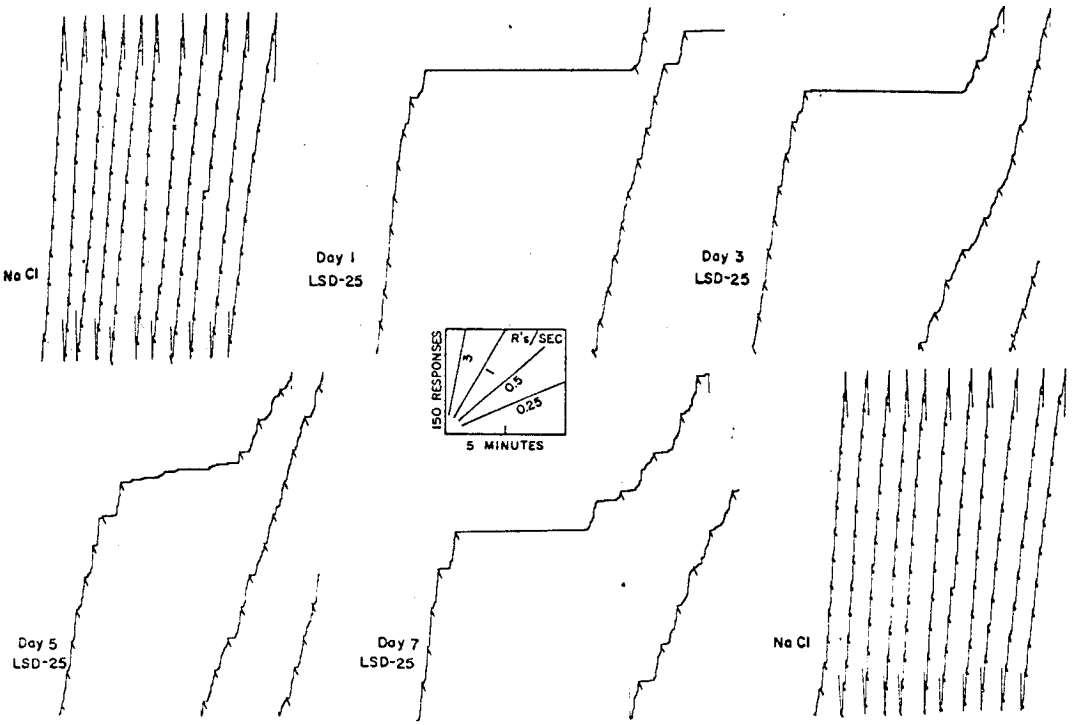

FIG. 4. Cumulative records of bar-pressing on an FR 40 schedule of reinforcement for a single rat. Control sessions before and after the tolerance run and the first, third, fifth and seventh days of treatment with 195 μg of LSD-25/kg are shown.

Dosage determined the response to LSD-25 on FR schedules (fig. 2). Forty μg of LSD-25/kg rarely induced an effect. All animals usually reacted to 130 and 195 μg of LSD-25/kg but neither intensity nor duration of effect differed with these doses. With doses of 390 μg/kg (and above) the period of no responding was considerably prolonged. The value of the FR (5, 15 or 40) did not influence the animal's response to LSD-25. Therefore, average data are pooled over ratios (fig. 3).

Tolerance to the drug can be demonstrated in all rats following a daily dose of 130 μg of LSD-25/kg for 7 to 8 days on each of the three ratio schedules; with 195 μg/kg, only two out of 10 rats developed tolerance (fig. 3). Thus, while no significant differences in the effects of the two doses are seen with an initial single treatment, a schedule of repeated injections demonstrates a difference between these doses.

When tolerance occurs, the decrease in drug effect is primarily a shortening of the period of no responding (fig. 1). Occasionally, repeated injections of LSD-25 also induce disruptions in local rates of response (FR straining). These disruptions usually occur at doses with which there is little or no tolerance (fig. 4).

Four animals reinforced on a VI 1.5 schedule were given doses of 130 and 195 μg of LSD-25/kg. A gradual lowering of the rate of bar-pressing rather than a period of no responding is observed on this schedule (fig. 5). There are no differences in the intensity or duration of effects following doses of 130 or 195 of μg/kg. Although the effects of LSD-25 on the interval schedule are qualitatively different from those observed on ratio schedules, onset and recovery times are similar.

With seven daily doses of 130 μg/kg, tolerance develops on the VI schedule, *i.e.*, there is a decrease in drug effect each day. In contrast to the findings with FR (fig. 4), there is some evidence that tolerance does occur with a dose of 195 μg/kg (fig. 5). However, this occurs more slowly (*i.e.*, requires more days) than the tolerance which develops to 130 μg/kg on VI or on FR (fig. 1).

In the study of acute tolerance the animals which received three injections of 130 μg of

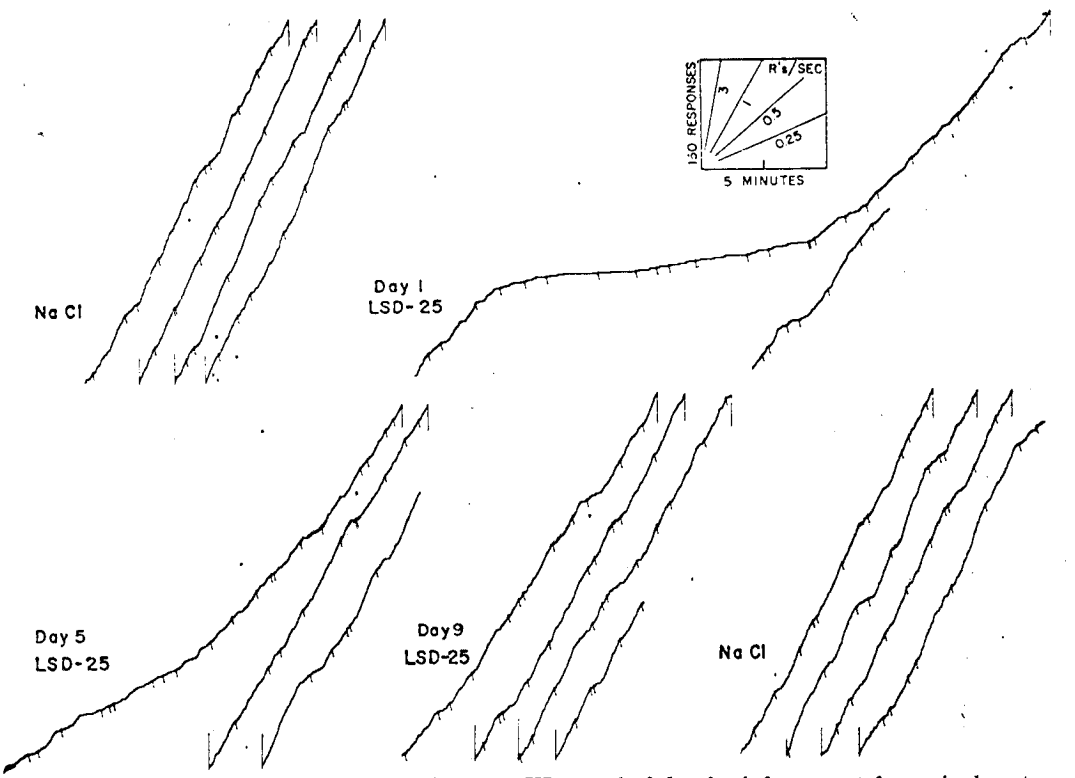

FIG. 5. Cumulative records of bar-pressing on a VI 1.5 schedule of reinforcement for a single rat. Control sessions and the first, fifth, and ninth days of treatment with 195 μg of LSD-25/kg are shown.

LSD-25/kg, group 1, were less impaired than those given the same total amount of drug in a single dose, group 3 ($P < .05$). They were also less impaired than rats given a single treatment of 130 μg of LSD-25/kg, group 2, but this difference was not significant. The means and standard deviations of drug effects for this experiment are given in table 1.

DISCUSSION. The ability of LSD-25 to disrupt behavior depends upon dose. In rat, the minimal dose which affects observed but nonmeasured behavior in all animals appears to be about 130 μg/kg. At this dose, grooming behavior ceases, and an ataxia of the hind limbs occurs.

Effective doses of LSD-25 decrease the frequency of food reinforced bar-pressing. On FR schedules, the drug effect is seen as a dose-dependent period of no responding. This failure to respond cannot be ascribed to a simple inhibition of motor function since bar-pressing occurs on the VI schedule and, with strong electric shock, an increase in running speed is observed after the drug (Hamilton, 1960).

The time of onset of drug effects is the same in a number of test situations. In studies of FR, rope-climbing (Winter and Flataker, 1956; Freedman *et al.*, 1958) and mating (Gillett, 1960), the period of no responding usually begins within 3 to 8 minutes after a dose of 130 μg of LSD-25/kg. A similar time of onset is seen on the VI schedule, with the EEG in rat (Hartman, 1962), for the occurrence of a number of autonomic effects in rat and rabbit (Rothlin and Cerletti, 1956; Rothlin, 1957), and for an increase in the level of brain serotonin (Freedman, 1961).

The duration of drug effect varies with dose and with the response being measured. The effect of 130 μg of LSD-25/kg on bar-pressing

TABLE 1

Acute tolerance to LSD-25 in rats

Dose of LSD-25 (μg/kg)	Percent Δ[a]
3 × 130	44.8 ± 19.2
390	15.2 ± 17.6
130	26.5 ± 9.3

[a] Mean and standard deviation of the percent change in the number of responses on fixed-ratio 15 (FR 15) following administration of LSD-25.

and rope-climbing is usually not seen after 40 minutes while the effects on mating behavior last approximately 90 minutes (Gillett, 1960). Bradycardia in the unrestrained rat can be observed for approximately 90 minutes (Freedman *et al.*, 1958), and the effects of 130 μg/kg on shock-induced escape behavior (Hamilton, 1960) last for approximately 4 hours.

Tolerance can be defined as a decrement of a specific drug effect contingent upon dosage schedule. Tolerance to the behavioral effects of LSD-25 in rope-climbing studies was shown to depend upon drug regimen rather than upon learning or practice. The development and loss of tolerance in bar-pressing situations is similarly contingent upon drug regimen. This is evident in the studies of acute tolerance in which the rat received three injections of drugs spaced at intervals of 1 hour, but was tested *only* after the final injection and showed partial tolerance. Studies of acute tolerance also show that tolerance can develop rapidly.

Tolerance does not develop to each and every behavioral effect of LSD-25. The period of no responding measured in the rope-climbing, mating, and bar-pressing tests do show tolerance to 130 μg/kg, but the ratio of copulations to ejaculations in mating studies and the running time in the escape from shock situation do not show tolerance to the same dose. In the rope-climbing situation, tolerance was observed after 4 daily doses of 130 μg/kg, while on the FR and the VI, 7 to 8 days were required to observe a complete return of performance to base line. The autonomic effects of LSD-25 also differ with respect to the development of tolerance. The drug-induced bradycardia (Freedman *et al.*, 1958) and the respiratory arrest which occurs with toxic dosage (Rothlin and Cerletti, 1956) do not show tolerance, whereas pyrexia (Gogerty and Dille, 1956), piloerection and mydriasis do (Rothlin and Cerletti, 1956). Thus, the identification of tolerance and the pattern of its development depend upon the particular behavioral or autonomic response being studied.

For any given response the development of tolerance is related to the quantity of drug administered. With relatively small daily doses, tolerance occurs on the FR and VI schedules. Tolerance does not develop with repeated large daily doses. Within the range of low dosages (130 to 195 μg/kg) tolerance to 195 μg/kg does not normally develop on FR with 32-minute sessions and the rate of its development on VI is slower than that observed with 130 μg/kg. Apparently, patterns of tolerance to LSD-25 depend upon the interaction of a number of factors such as the particular response measured, multiple behavioral variables, the quantity of drug administered and the schedule of dosage.

SUMMARY

The effects of LSD-25 on bar-pressing maintained by food reward, and tolerance to these effects, were investigated in the rat with two schedules of reinforcement, fixed-ratio (FR) and variable-interval (VI). With FR, the drug effect was defined by a period of no-responding; a gradual decline in response rate occurred on the interval schedule. The onset of behavioral disturbances began 4 to 8 minutes after i.p. injection and the duration depended upon dosage.

Tolerance was observed on both FR and VI within 7 to 8 days with daily doses at or below 130 μg of LSD-25/kg. It was characterized by a shortening of the time required for recovery from the maximal drug effect. The study of acute tolerance shows that partial tolerance develops rapidly (within 3 hours). With increased doses of LSD-25 tolerance to these effects on bar-pressing behavior is less likely to develop.

The importance of both behavioral and pharmacological variables in affecting both the response to LSD-25 and the development of tolerance was stressed.

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