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LSD Serotonin

# Behaviorally Induced Sensitivity to the Discriminable Properties of LSD

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*Abstract.* Rats were initially trained to discriminate LSD from saline in two-lever operant chambers by reinforcing responses only on one lever following intraperitoneal injections of 80 µg/kg of LSD and only on the other lever following saline injections. Choice responding during extinction periods (no water reinforcement for either response) indicated a high level of discriminability (95% correct) following either LSD or saline. A dose-response curve for LSD, obtained by tests for lever choice after injections of 10, 20, 30, 40, 50, and 60 µg/kg, indicated that 10 µg/kg produced only 30% responding on the LSD lever. This percentage was increased (to 83%) by reinforcing responding on the LSD lever following injections of 10 µg/kg. Subsequent tests indicated that doses of 5.0 and 2.5 µg/kg produced a majority of responses on the LSD lever. Since at these low doses LSD has few measurable biochemical or behavioral effects, we hypothesize that the discriminable cue of LSD is related to direct stimulation of central serotonergic receptors.

*Key words:* LSD – Discriminable Properties of Drugs – Serotonin Receptors – Rats.

It is well known that rats can discriminate a large number of drugs from both the nondrug (saline) state and from other drug states (Overton, 1971, 1974). Animals soon learn to make discriminated choice responses solely on the basis of drug-induced cues by reinforcement for responding in one manner after drug injections (*e.g.*, pressing lever A or turning left in a T-maze) and for responding in the opposite manner after saline injections (*e.g.*, pressing lever B or turning right in a T-maze). Although it is not known which effect of a drug forms the basis for discrimination, it has been possible to classify drugs according to their discriminable effects (Barry, 1974).

The hallucinogens LSD (Cameron and Appel, 1973; Hirschhorn and Winter, 1971; Hirschhorn and Rosecrans, 1974; Schechter and Rosecrans, 1972), mescaline (Winter, 1973, 1974; Browne *et al.*, 1974; Hirschhorn and Winter, 1971) and to some degree psilocybin (Harris and Balster, 1971) are all discriminable from saline and are similar enough to each other so that mescaline and psilocybin produce the drug response in rats trained to discriminate LSD from saline. It is not known whether these similarities are qualitative or quantitative (Cameron and Appel, 1973) but assuming that the similarity is qualitative, transfer tests among these hallucinogens represent a special case of a dose-response effect.

It has been demonstrated that on drug *vs.* no-drug discrimination tasks, test trials with lower doses of the training drug produce orderly decreases in drug-related responding (Overton, 1974; Waters *et al.*, 1972; Kuhn *et al.*, 1974; Cameron and Appel, 1973). Overton (1974) has pointed out that dose-response effects for drug discriminability are determined by the training dose used to establish discriminability. Therefore, tests with other doses of the training drug are not dose-response curves for drug discriminability, in a pharmacological sense, because this variable should not be influenced by the training dose. Thus, the relationship between training dose and the shape of transfer test gradients is partly a behavioral phenomenon.

If this hypothesis is correct, it should be possible to train rats to discriminate sub-maximally discriminable doses of training drug from saline by "fading" them to a much lower dose.

The purpose of the present study was to test this hypothesis with LSD, a drug which has strong discriminative stimulus properties.

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## Methods

*Subjects.* Six male albino Sprague-Dawley rats were used. They were approximately 120 days old at the beginning of the experiment and weighed 250–300 g. They were maintained at 80% *ad libitum* weight by restricting water intake. Food was always available *ad libitum*. The rats were housed individually in a room of constant temperature (22°C) and humidity (40–50%), and were exposed to a 12-hr day-night cycle. Sessions were conducted at the same time each day, 5 days a week. Additional water was given after each session and on weekends to maintain body weight at 80%.

*Apparatus.* Four commercially available experimental chambers (Lehigh Valley Electronics, Model No. 1316) contained in sound-proof, ventilated enclosures were used for training and testing. Each box contained two levers on the front panel separated by a liquid feeder. Each reinforcement consisted of 0.01 ml of tap water; sessions were 30 min long. Electromechanical programming and recording equipment was located in an adjacent room.

*Procedure.* Rats were trained to discriminate LSD from saline in the same manner as in previous studies (Kuhn *et al.*, 1974). Initially, all rats were shaped to bar-press on a continuous reinforcement schedule on one lever and then on the other, with the first lever removed. On any given day, only one lever was available to avoid presenting the rats with a choice before differential cues (drug injections) were presented. The schedule of reinforcement was gradually increased from CRF to FR 10, usually on Day 1 of training. After 2 days of FR 10 on each lever, a variable interval component averaging 1 min was attached to the FR component so that 10 responses at the end of each variable interval (Tand VI 1 FR 10) resulted in 0.01 ml of water reinforcement. The rats were given two additional days training on each lever with the Tandem VI 1 FR 10 schedule of reinforcement before injections were begun.

After completion of this preliminary training, either 80 µg/kg of LSD or an equivalent volume of saline (2 ml/kg) was given intraperitoneally one-half hour before each session. For half of the animals, responding on the left lever after LSD was reinforced whereas responding on the right lever after LSD was reinforced for the remaining half. Responding on the opposite lever was reinforced after saline injections for each group, respectively. During this phase, only responding on the appropriate lever produced reinforcement. Responding on the incorrect lever was recorded but had no programmed consequences unless it occurred in the ratio component. If a rat was responding in the ratio sequence on the correct lever and then responded incorrectly on the opposite lever, the ratio requirement was reset so that only 10 *consecutive* responses on the appropriate lever produced reinforcement. This punishment occurred only early in drug discrimination training; it effectively prevented chaining from the incorrect to the correct lever.

LSD and saline injections were given randomly with the restriction that the same treatment not be given more than 3 days consecutively. Discrimination training continued for approximately 3 months. Accuracy of discrimination was based strictly on data gathered in short extinction periods run each day. To accomplish this, variable extinction periods were run for an initial interval between 10 sec and 5 min before the first reinforcement. This procedure produces much pre-reinforcement responding and provides a reliable index of discriminability.

At the end of discrimination training, various novel doses of LSD were randomly substituted for the training dose and, following each dose, the rats were tested for lever choice during 5 min extinction periods. No training followed extinction periods with novel doses. The doses tested were 10, 20, 30, 40, 50, and 60 µg/kg. Each dose was replicated three times; as before all injections were given i.p., 30 min before testing. All test days were preceded by a saline day to ensure that any residual drug would not influence test dose results. The training dose of LSD was also given at least once weekly to maintain the discrimination.

Since we found that 10 µg/kg of LSD produced the smallest amount of responding on the LSD lever (of the doses we tested), other than saline (below), we chose to substitute 10 µg/kg for 80 µg/kg as the new "training" dose to determine if the rats could learn to detect the presence of this sub-maximal dose of LSD. The training procedure was the same already described for the original discrimination. At the completion of this phase the rats were returned to the original discrimination (80 µg/kg of LSD *vs.* saline) for 2 weeks. The dose-response curve was then re-determined. However, in addition to one injection of each of the previous doses (10–80 µg/kg) 3 injections of 2.5 µg/kg of LSD and 2 injections of 5.0 µg/kg of LSD were given. As before, 80 µg/kg of LSD and saline days were interspersed between test days. The injection route and time parameters remained the same.

*Drugs.* LSD tartrate powder was obtained from NIMH, Center for Narcotic and Drug Abuse. It was dissolved in a solution of 0.9% sodium chloride to a concentration of 40 µg/ml.

## Results

All data were obtained during extinction periods and are expressed as per cent of total responding on the LSD lever. Since acquisition of the 80 µg/kg *vs.* saline discrimination was no different from numerous other published drug discrimination acquisition curves, these data are not presented. The two dose-response curves (before and after training at 10 µg/kg of LSD) are presented in Fig. 1 to facilitate comparison. It can be seen that the rats learned to discriminate 80 µg/kg of LSD from saline with a high degree of accuracy. The average per cent total responding on the LSD lever was 96% after 80 µg/kg of LSD and 5% after saline. As the dose of LSD decreased, proportionately less responding occurred on the LSD lever (Fig. 1).

After reinforcing the rats for responding on the LSD lever following training with 10 µg/kg of LSD, a high degree of accuracy was again obtained. After only eight injections of 10 µg/kg of LSD, total LSD responding increased to 83.3%. However, the per cent responding on the LSD lever following saline injections also increased (to 18%) when the discrimination problem was made more difficult. Nonetheless, the rats rapidly learned to discriminate 10 µg/kg of LSD from saline. Redetermination of the dose-response curve indicated that over 90% of all responses occurred on the LSD lever after 10 µg/kg of LSD and less than 10% of the responses occurred on the LSD side after

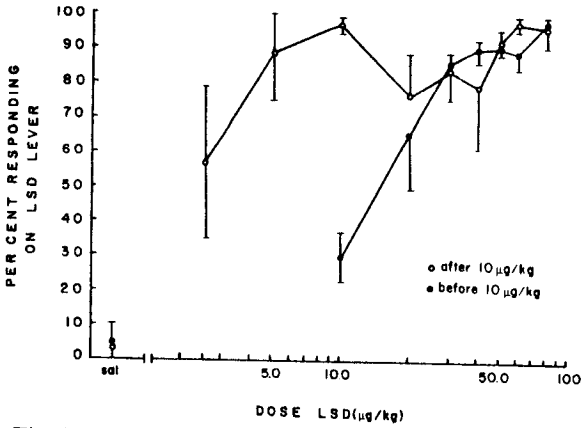


Fig. 1. Per cent of total responding on the LSD lever after various doses of LSD (log scale). Solid circles represent lever choice before the rats were taught to discriminate 10 µg/kg of LSD; open circles represent redetermination of the dose response curve after reinforcing the rats for LSD-like responding after 10 µg/kg of LSD. Verticale lines represent ± S.D. All injections were administered i.p. 30 min before testing in extinction for lever choice. See text for the number of replications of each dose. Each data point represents the average of 6 animals

saline. Test injections of 5.0 µg/kg of LSD produced 89.0% responding on the LSD lever and 2.5 µg/kg of LSD produced 57.9% responding on the LSD lever.

## *Discussion*

Our results confirm others showing that LSD has strong discriminative stimulus effects. Furthermore, our results agree with Overton (1974) demonstrating the importance of the training dose in determining subsequent test dose gradients. This is demonstrated in Fig. 1. After acquisition of the 80 µg/kg of LSD-saline discrimination, 10 µg/kg of LSD produced 30% responding on the LSD lever. The same dose of LSD produced 83% responding on the drug lever after the animals received additional discriminative training with LSD (10 µg/kg) and saline. It is doubtful that rats would have learned to discriminate 10 µg/kg of LSD from saline had previous training not been carried out at a larger dose. Cameron and Appel (1973) report that 20–40 µg/kg of LSD is at about the threshold for establishing an LSD-saline discrimination and other reports demonstrating LSD discriminability have done so at 120 µg/kg (Hirschhorn and Winter, 1971), 80 µg/kg (Cameron and Appel, 1973), 72 µg/kg (Hirschhorn and Rosecrans, 1974), and 48 µg/kg of LSD (Schechter and Rosecrans, 1972). Thus, there appears to be a minimal dose which can be used to establish initial discriminability. In general, discriminability is proportional to dose for most drugs tested thus far (Overton, 1973).

The biochemical (Freedman, 1961; Freedman and Boggan, 1974) and behavioral (Appel *et al.*, 1970; Appel and Freedman, 1964) effects of LSD have been linked to its action on serotonergic neurons. However, these effects are seen at much larger doses than presently used. The most sensitive technique thus far developed to measure LSD effects is electrophysiological recording of serotonin-containing neurons (Aghajanian *et al.*, 1968; Aghajanian *et al.*, 1970; Aghajanian *et al.*, 1972; Haigler and Aghajanian, 1974a), and indicates that doses as low as 10 µg/kg have direct effects on both presynaptic (raphé) and postsynaptic (optic-tectum; amygdala) cells of a putative serotonergic system in the brain.

If, indeed, drug cues are based on the drug's interaction with certain neurochemical systems as some data suggest (Rosecrans *et al.*, 1973; Kuhn *et al.*, 1974; Hirschhorn and Rosecrans, 1974), it is interesting that rats in the present study could detect doses as low as 10 µg/kg of LSD.

Rats depleted of 5-HT with PCPA continue to discriminate LSD (Cameron and Appel, 1973) which would suggest that if the serotonergic system is involved, the cueing effects of LSD are produced by direct stimulation of 5-HT receptors. The putative serotonin antagonists cyproheptadine and methysergide did not block the LSD cue (Hirschhorn and Rosecrans, 1974) which is not compatible with a receptor stimulation hypothesis for LSD discrimination. However, this may be explained by the recent work of Haigler and Aghajanian (1974b) which indicates that several peripheral serotonin antagonists (including cyproheptadine and methysergide) failed to block 5-HT effects centrally of raphé cells and other brain areas receiving heavy serotonergic input. These data strongly suggest that the putative serotonin antagonists are not central 5-HT blockers.

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