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A Behavioral and Pharmacological Analysis of Some Discriminable Properties of d-LSD in Rats*

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Abstract. d-LSD was employed as a discriminative stimulus in a two-lever, free-choice procedure involving water reinforcement with rats. The results indicated that 1. doses of 0.02—0.04 mg/kg of d-LSD were at the "threshold" level of discriminability from no drug (saline), 2. 0.08 mg/kg was clearly discriminable from no drug, 3. the same dose of d-LSD (0.08 mg/kg) was also discriminable from d-amphetamine, and 4. subjects could probably discriminate, albeit weakly, between d-LSD and two other hallucinogens, mescaline and psilocybin.

When a variety of drugs which were not used during training were tested during subsequent extinction sessions, it was found that most non-hallucinogenic drugs elicited responding predominantly on the non-drug lever; when hallucinogenic drugs were tested, preferences in general depended on dosage of the test drugs. It was also found that administration of PCPA, a drug which sensitizes rats to certain disruptive effects of LSD also sensitizes them to discriminative effects of the drug. Manipulations which might be expected to desensitize subjects (e.g., pretreatment with chlorpromazine, or with d-LSD itself in an "acute-tolerance" regimen), however, had no effect.

Whether the discriminations of different drugs are due to qualitative or to quantitative (dosage) differences between drug states has proved to be a problem in the interpretation of drug discrimination experiments. It may, for example, contribute to the discrepancy observed between the present results and those of previous investigators who attempted to equate doses of LSD and mescaline.

Key words: LSD — Discrimination — State-Dependent Learning — "Dissociation" — Hallucinogenic Drugs.

Numerous studies have been reported in which behaviors which were acquired under some drug state are not observed under similar circumstances when the drug state is changed (e.g., John, 1967; Overton, 1968, 1971a, b). This phenomenon has been called "state-dependent" or "dissociated" learning. A variety of drugs seem capable of producing dissociation including barbiturates, alcohol, and "minor transquilizers"

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(strong control); antimuscarinic and nicotinic drugs, narcotics, convulsants, amphetamines, and mescaline (moderate control); and scopolamine methyl nitrate, physostigmine and phenothiazines (weak control) (see Overton, 1968, 1971a, b). Indeed, the effect is sufficiently important that Grossman and Miller (1961) have argued that the results of numerous studies which supposedly demonstrate other kinds of drug effects (e.g., facilitation of learning) cannot be unequivocally interpreted because the possibility that dissociation may have occurred has not been ruled out by using appropriate controls.

Drugs have also been employed in a variety of operant discrimination procedures as discriminative stimuli (S^Ds), including an extensive series of experiments by Harris and Balster (1971). [While Overton, 1968, has discussed dissociated learning and the use of drugs an S^Ds separately, he has admitted that these two paradigms may, in fact, be the same—"nonetheless, the results obtained with these two procedures probably reflect the same properties of drugs" (p. 918). In any case, no conclusive evidence has yet been reported that state-dependent or dissociated learning, and the use of drugs as S^Ds, are different phenomena.]

Several hallucinogenic drugs have been found to have state-dependent effects. Overton (1971a, b) has classified mescaline (10 mg/kg) as a moderately effective S^D. Harris and Balster (1971) found that 0.4 mg/kg of psilocybin showed only weak control of lever choice when compared to saline and 0.2 mg/kg of psilocybin apparently could not be discriminated from 0.025 mg/kg of LSD. In another series of experiments, Hirschhorn and Winter (1971) reported that rats could discriminate both LSD and mescaline from saline; ability to discriminate (indicated by percent choice on the correct lever) improved with increasing doses (up to approximately 90% correct responses with 19.8 mg/kg of mescaline in one group and 0.12 mg/kg of LSD in another group). However, subjects could not discriminate these two drugs from each other at doses which produced approximately equal control at 70% correct responses (mescaline at 9.9 mg/kg and LSD at 0.028 mg/kg). And Schechter and Rosecrans (1972) found that rats trained in a T-maze with 0.048 mg/kg of LSD and saline chose the LSD side when given psilocybin (2.62 mg/kg) or mescaline (approximately 22—30 mg/kg), but chose the saline side when given 2—4 mg/kg of amphetamine. These data suggest that hallucinogenic drugs such as LSD, mescaline, and psilocybin produce states which are different from no drug, but are similar to each other.

The experiments to be reported below analyze the discriminative properties of d-LSD more extensively than has been previously reported. The state induced by this compound is compared with that induced by a variety of other agents, both hallucinogenic and non-hallucinogenic. Various doses of different drugs are employed during both training and testing (extinction) sessions involving a two-lever free-choice procedure.

General Methods

Subjects. Twenty-four naive male albino rats of Sprague-Dawley strain were used. They were approximately 120 days old at the start of each experiment and weighed between 250 and 300 g. Food was always available in separate home cages in a room maintained at constant temperature (24—26°C) and humidity (40—50%). Animals were run six days per week and were given free access to water for 24 h after the last session of the week. No other water was available except that which was obtained in the experimental chamber. All animals remained healthy under this regimen.

Apparatus. A commercially available operant chamber (Lehigh Valley Electronics, Model No. 143-24) equipped with two levers was used to train and test the drug discriminations. The levers were mounted at opposite ends of one wall of the chamber. A single dipper which delivered 0.05 ml of tap water was positioned between the levers. The chamber was illuminated by a 28-v white house light and was placed in a sound- and light-attenuating outer shell (Lehigh Valley Electronics, Model No. 132-04). All programming and recording were done electromechanically in an adjoining room.

Drugs. The fluid used for drug dilutions and saline injections was 0.9% sodium chloride and 0.9% benzyl alcohol in distilled water. d-LSD was prepared from LSD-25 (Delysid) powder manufactured by Sandoz Pharmaceuticals, Hanover, New Jersey, and obtained from the National Institute of Mental Health (NIMH), Center for the Study of Narcotic and Drug abuse. Delta-9-tetrahydrocannabinol (Δ^9THC), psilocybin and L-LSD were also obtained from NIMH. Mescaline sulfate and PCPA (para-chlorophenylalanine-methyl-ester) were obtained from Regis Chemicals, Chicago, Illinois. Scopolamine was injected from ampules of scopolamine hydrobromide, obtained from Burroughs-Wellcome, Tuckahoe, New York. Chlorpromazine HCl (CPZ) was obtained from Smith, Kline, and French, Philadelphia, Pa. Methyl atropine nitrate was from Sigma Chemical, Chicago, Illinois. d-Amphetamine sulfate (administered by weight as the salt; all others were given as weight of pure drug) came from K and K Laboratories, Plainview, New York. All injections were administered intra-peritoneally (i.p.); volume in milliliters equalled weight in kilograms.

General Procedure. After two days of water deprivation, all animals were trained for two days to bar-press for water on a continuous reinforcement schedule (crf) on the right lever. The animals were then shaped to press on the left lever (the right lever was present but had no programmed consequences). After two days on the left, subjects were shifted back to the right lever. On the second day on the right, the schedule was changed from crf to drl 2 sec and each subject was given the appropriate drug S^D (below) before the start of each subsequent session (correct sides for training drugs were different for different subjects). From this point on, the correct side was reversed randomly between sessions with the restriction that the same side was never correct more than three successive days. Each day, the appropriate drug was administered 15 min before each animal's 30 min session and the drl was gradually raised to drl 20 (this usually required 6—8 sessions). On this schedule, subjects learned the drug discriminations in about 20 sessions, 10 with each of the two training drugs (or drug and no drug). In all situations in which a new training drug was introduced. at least eight further training sessions were given with the new drug(s). Thus, the schedule could be considered to be a multiple drl 20 drl 20 with drug states as the S^Ds and stimuli changing randomly between, but never within, sessions.

Outlines of the specific procedures of each of the four experiments are presented in the text, and the results are reported both in the text, and in Figs. 1 to 4, below, as percentages (%). The side (lever, drug) on which the percentage is based is

indicated on each figure. For subjects which distributed their responses fairly equally between both levers the number of responses on one lever before one response, and before five responses, on the other lever are reported in the text. These data indicate the side (lever) of the animal's initial tendency to respond. Less than 10 total responses has been arbitrarily designated as the cut-off point below which the data are presumed to be unreliable; nevertheless, these data are reported (and are noted in the figures with asterisks) as *suggestive* of preferences. Situations in which the number of responses on the favored side did not exceed 60% are arbitrarily designated "chance" responding (no systematic choice of one side or the other). In cases where differences between groups are suggested, but are not clearly identified, statistical tests (one-tail F tests) were performed. The validity of these tests perhaps can be questioned, however, because of the small number of subjects.

Experiment I

Procedure. Four animals were trained with d-LSD (0.02 mg/kg) and saline according to the above procedure, and then tested, two with d-LSD and two with saline. These subjects were than retrained with 0.04 mg/kg of d-LSD and saline for eight trials (sessions) and tested, again two with d-LSD and two with saline. Testing always consisted of administering the test drug and running the subject for one complete 30-min session of extinction (no water available). No subject in any of the four experiments was re-tested until the discrimination was re-established; this usually required 2—4 training sessions. Finally, the same four subjects were tested with a discrimination "reversal" in which saline was administered, but the operative lever (reinforcement available) was the one which had been associated previously with d-LSD. This was done in order to assess how much control was exerted by dipper "cues" (reinforcement) and how much by the actual drug state. After one "reversal day", subjects were returned to the original discrimination for one session.

Results. After nine initial training trials with each stimulus, subjects were emitting, on the average, 80% correct responses during training (Fig. 1 at A and B). However, when a test day was programmed after a total of 20 training trials (at C) subjects given LSD emitted fewer responses on the LSD side (31% and 45%) than those given saline (both at 66%) indicating that no control was exerted by the drugs. In addition, no early reponse preference was evident (not shown).

Following four more training trials (two with LSD and two with saline, this last training day shown at D), on a subsequent test day (at E) one subject given LSD responded above "chance" (75%) on the LSD side while the other did not (58%). One subject which was given saline responded more on the saline side (76%) but the other saline subject emitted 71% of its responses on the LSD side. These two groups were not significantly different from each other ($F = 1.0$, $P > 0.05$. $df = 1,2$) on this test day. However, the early response data on the same test day did suggest that the drugs exerted some control over choice behavior, i.e., three of the four subjects emitted their first response on the correct side and, in addition, emitted five correct responses before giving five incorrect. These data suggest that a dose of d-LSD at or near 0.02 mg/

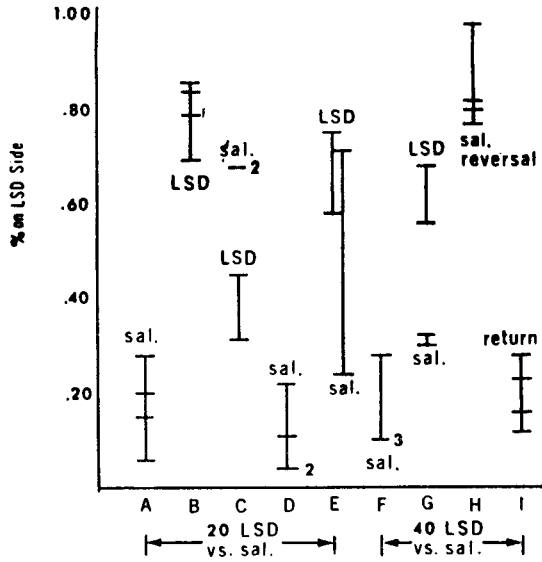

Fig. 1. Short horizontal lines designate a datum for one subject (unless labelled as 2 or 3 subjects); vertical lines connect all subjects which were given the same drug. Each datum indicated the percent of the total responses which were emitted by that subject on the designated (on the ordinate) side. The drugs which were administered before the trial being represented are indicated for each group. (This format is employed in all figures.) In Experiment I, four subjects were trained with d-LSD *vs.* no drug (saline) at different doses of LSD (in this figure, and in Fig. 2 *LSD dose* is designated in micrograms per kilogram). *A* to *E* per 0.02 mg/kg of d-LSD *vs.* saline, training days (*A*, *B*, *D*), on which the correct lever was operative, and extinction tests days (*C*, *E*). *F* and *G* training (*F*) and extinction test (*G*) days with 0.04 mg/kg *vs.* saline. *H* saline "reversal", in which the lever which had been paired with LSD was operative, but saline was administered, to test for control exerted by the drug discrimination *vs.* control exerted by dipper operation cues (reinforcement); *I* saline given, but now the originally correct lever (side) was again functioning ("return"). (In *H* and *I* the conditioned discriminations were with 0.04 mg/kg of d-LSD *vs.* saline.) All data in Experiment I are presented as the percent of total responses which were emitted on the LSD side

kg is about at the "threshold" level of discriminability from no-drug under the conditions of the experiment.

When the LSD dose was increased to 0.04 mg/kg and the subjects were given eight additional training days, (last are at F) more control was apparent; on the first extinction test day (G), the two rats which were given LSD emitted 56% and 68% responses on the LSD side, respectively, while saline subjects emitted only 30% and 32% on the LSD side. Moreover, on this test day, the groups were statistically different ($F = 24.64$, $P < 0.05$, $df = 1,2$), even with this very small sample,

The same subjects were then given saline but the operative bar was reversed such that the bar which had been functional when d-LSD was given was now functional, and the other was not (at H). At the last dose of d-LSD used to train these subjects (0.04 mg/kg), more control was exerted by the dipper cues than by the drug (saline), i.e., all subjects responded primarily on the operative bar (77%, 80%, 82%, and 98% respectively), even though this was the lever which had been associated with LSD during training. In addition, subjects responded primarily on the operative bar (72%, 77%, 84%, and 88%) when the lever which was originally correct when saline was administered was again made functional and saline was given again (at I).

Experiment II

Procedure. In Experiment II, two subjects were trained with 0.08 mg/kg of d-LSD and 8.0 mg/kg of mescaline, and two with 0.08 mg/kg of LSD and 0.8 mg/kg of psilocybin. These doses of LSD, mescaline, and psilocybin have been shown to have approximately equal potency with respect to their ability to disrupt appetitive behavior (Appel and Freedman, 1965). Following training and testing with these doses, the dose of LSD in both groups was reduced to 0.04 mg/kg for eight additional training sessions; doses of mescaline or psilocybin remained the same. These subjects were then tested with the lower dose of LSD, and mescaline or psilocybin.

Eight additional subjects were run—four with 1.0 mg/kg of d-amphetamine and saline, and four with 1.0 mg/kg of d-amphetamine and 0.08 mg/kg of d-LSD. These animals served as controls in order to compare the ability to discriminate hallucinogenic drugs (d-LSD, mescaline, psilocybin) from each other with the ability to discriminate an hallucinogen from a drug which is presumably not hallucinogenic at the dose tested, i.e., d-amphetamine (1.0 mg/kg).

Results. In this study (Fig. 2) two subjects were trained initially with LSD and mescaline, and another two were given LSD and psilocybin (at A, B, C, D, and E). Good control was evident on training days. This was true after a total of 17—18 training trials (at A and B) and also after a total of 22 trials (at D). However, on test days (C and E) the control was only fair. While the subjects tested with LSD never fell below 50% responses on the LSD side, they never exceeded 70% (59%, 64%, and 67%). While the subjects given mescaline or psilocybin never emitted more than 40% of their responses on the LSD side (mescaline—23%, 38%, 13%; psilocybin—30%, 39%), only one mescaline subject emitted more than 80% correct responses. Early responses on test days also indicated only fair control. Statistically, the subjects given LSD were different from those given the other drugs (both groups considered as one); $F = 25.81$, $P < 0.005$, $df = 1,6$. (Note that the eight percentages obtained are from only four subjects—two mescaline and LSD, two psilocybin and LSD—each tested twice.)

When the LSD dose was reduced to 0.04 mg/kg, the control observed during training was maintained (at F), but little improvement was

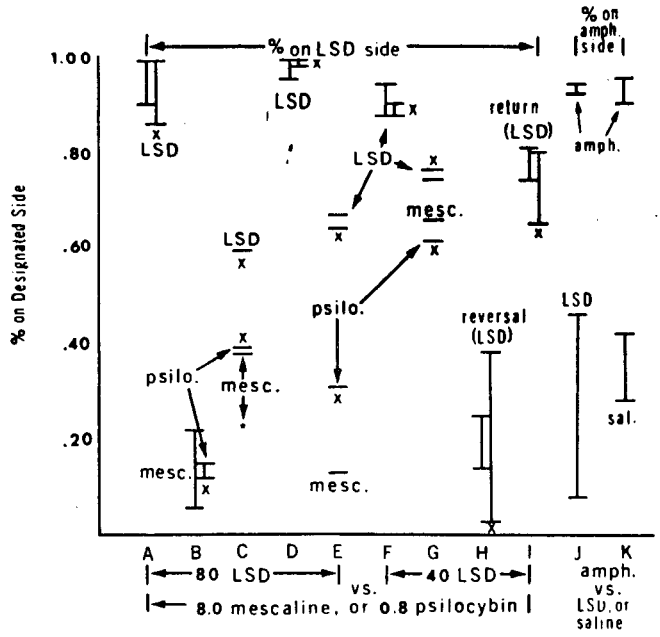

Fig. 2. Two subjects trained with d-LSD *vs.* mescaline, and two with d-LSD *vs.* psilocybin. *A* to *E* training (*A*, *B*, *D*) and testing (*C*, *E*) with 0.08 mg/kg of LSD (LSD dose again designated in figure in *micrograms*) *vs.* 8.0 mg/kg of mescaline, or 0.8 mg/kg of psilocybin. (*x* marks the animals *trained* with psilocybin.) *F* and *G* training and testing with 0.04 mg/kg of LSD *vs.* the same mescaline or psilocybin doses. *H* "reversal" (0.04 mg/kg of LSD given, but other lever operative-see legend, Fig. 1). *I* "return" (0.04 mg/kg LSD given again, "correct" bar again operative—see legend, Fig. 1). Data *A* to *I* presented as percent responses on the LSD side. Note again that *x* marks animals *trained* with psilocybin; drug designations on figure indicate *test* drug. *J* a test day for four other subjects (Group 3), trained with d-LSD (0.08 mg/kg) *vs.* *d*-amphetamine (1.0 mg/kg). *K* a test day for four more subjects (Group 4), trained with 1.0 mg/kg of *d*-amphetamine *vs.* saline. For *J* and *K*, the designated percent of total responses was on the amphetamine side. Asterisks mark days on which a total of less than 10 responses was emitted by the designated subject (also done in Figs. 3 and 4)

apparent during testing after 10 training days at the decreased LSD dose (at G); indeed, control was poorer here than with 0.08 mg/kg of LSD. Note that, in the situations where some control was suggested, mescaline seemed to be slightly more discriminable from LSD than psilocybin. This, however, may be a quantitative difference caused by the particular doses used.

After re-establishment of the d-LSD and mescaline or psilocybin discriminations, a reversal trial was given (at H). The results again indicated that more control was being exerted by dipper cues than by the drugs.

The "return" trial (at I) shows that when the "correct" lever was again operative, subjects pressed it more often that the "incorrect" lever, but control was still only moderately good (81, 80, 74, and 66% on the LSD side). Thus, at the doses used, some control was exerted by the training drugs, but the amount of control was not as great as with LSD and no drug.

Four different subjects in this experiment were trained with LSD and d-amphetamine. As can be seen, the control demonstrated in the LSD-amphetamine subjects on the extinction test day (J) was excellent (with the exception of the one LSD animal unexplained) and, hence, considerably better than that obtained when two hallucinogens were used (above).

The last four subjects in Experiment 2 which were trained with d-amphetamine and saline (K), demonstrate that d-amphetamine is not being discriminated from LSD simply on the basis of its inability to produce a characteristic state. That is, d-amphetamine also produces a characteristic state which is discriminable from no drug, a finding which confirms those of Harris and Balster (1971). The data of Experiment 2 suggest that hallucinogenic drugs may produce qualitatively different stimulus characteristics (contrary to the findings of Hirschhorn and Winter, 1971, and Schechter and Rosecrans, 1972), but that at least one non-hallucinogenic drug, d-amphetamine, is clearly more distinctively different from d-LSD than are the two hallucinogens tested. It is possible, however, that quantitative, i.e., dosage, differences may have been at least partially responsible for the observed effects.

Experiment III

Procedure. Four rats were trained with 0.08 mg/kg of d-LSD and saline, and were tested with the same agents. After re-establishing the training discrimination two of these subjects were tested in extinction with amphetamine (1.0 mg/kg), and the other two were tested with an "acute tolerance" regimen of LSD (0.08 mg/kg at −2 h, −1 h, and 0 h). This regimen has been shown to produce tolerance to the behaviorally disruptive effects of 0.08 mg/kg of LSD on at least one appetitive schedule (Freedman *et al.*, 1964). Following these tests, all four animals were tested with 1. CPZ (0.5 mg/kg), or CPZ (0.5 mg/kg) plus d-LSD (0.08 mg/kg), 2. L-LSD (0.08 mg/kg), 3. PCPA (50 or 100 mg/kg two subjects each), and 4. lower doses of d-LSD (i.e., 0.04, 0.02 or 0.01 mg/kg) given to the PCPA—pretreated animals (see below). During this testing regimen saline was also given to two of the PCPA pretreated animals during extinction to determine if the initial training discrimination was maintained. At least 2—4 training sessions were always interspersed between test sessions (extinction) in order to re-establish the discrimination.

All drugs given during testing were administered approximately 15 min before test sessions except at the end of the study (last two test sessions "4" above) on which days LSD or saline was given at −15 min but was given to PCPA-pretreated animals. The pretreatment regimen was three consecutive daily doses of 100 mg/kg (to the subjects tested with 100 mg/kg of PCPA), or of 50 mg/kg, 150 mg/kg, and 100 mg/kg in three consecutive doses (to the subjects tested with 50 mg/kg), for a total of 300 mg/kg in both groups, on days seven, six, and five previous to the first

of the two test days; the second of these tests followed three days after the first with no interspersed training trials. This PCPA regimen (300 mg/kg over 33 days) has been shown to deplete 5-HT (serotonin) in the brain at seven to ten days post administration (Koe and Weissman, 1966; Appel *et al.*, 1970a). Therefore, the last two test sessions must be considered not as simply the test drugs at 15 min, but as the drug given to "abnormal" (i.e., serotonin-depleted) subjects.

Results. All subjects in Experiment III were able to discriminate between 0.08 mg/kg of LSD and saline during training (Fig. 3). With the exception of one animal (at C), no subject emitted less than 80% correct responses on any of the last three days of the initial training regimen (at A, B, and C); in fact, on none of the days during which LSD was given did the percent correct of any subject fall below 90%. (Note that less control of choice behavior was exerted by saline than by LSD).

On the first extinction test day, control was not as good as on the previous training days (probably because there were no dipper cues to "guide" the animals). However, some control was evident; no subject emitted less than 50% correct responses and only one of the four emitted less than 60% correct (D). The F-ratio was "marginally significant" ($F = 11.07$, $0.10 > P > 0.05$, $df = 1,2$). Records of the number of

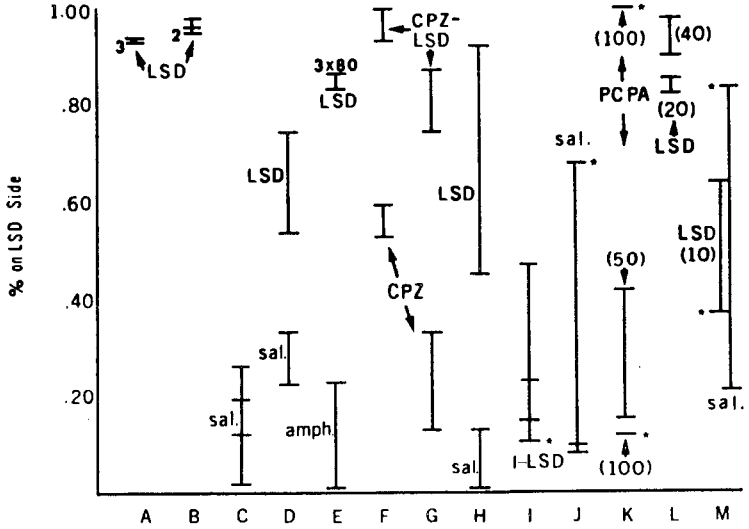

Fig. 3. Percent responses on the LSD side for four subjects trained throughout to discriminate d-LSD (0.08 mg/kg) from saline. *A*, *B* and *C* initial training days. *D* a training drug test day. *E* testing with d-amphetamine (1.0 mg/kg), or the LSD "acute tolerance" regimen (see text). *F* and *G* testing with CPZ (0.5 mg/kg), or CPZ plus LSD (0.5 mg/kg and 0.08 mg/kg, respectively). *H* another training drug test day. *I* L-LSD test (0.08 mg/kg). *J* a saline test. *K* PCPA test (50.0 mg/kg, or 100.0 mg/kg). *L* and *M* testing with lower doses of d-LSD and saline on days five (*L*) a nd eight (*M*) following the end of the 300 mg/kg PCPA pretreatment regimen (see text)

incorrect responses before either one correct or before five correct also indicated that, on both training and extinction days, subjects could discriminate which compound they had received (thus, they were not relying solely on dipper cues); even on extinction days subjects emitted most of their early responses on the correct lever.

Following re-establishment of the discrimination, two subjects were tested with the "acute tolerance" regimen of LSD (3×0.08 mg/kg of LSD, at -2 h, -1 h, and 0 h), and the remaining two subjects were given a single dose of 1.0 mg/kg of d-amphetamine 15 min before testing (at E). The "tolerance" regimen did not produce tolerance to the discriminative properties of the drug; that is, both subjects were "correct" (more than 80% of their responses were on the LSD side) even though extinction was in effect. The two subjects which were given d-amphetamine, a "novel" test drug, both responded as if d-amphetamine were more similar to saline (87% and 99% responses on the saline side), than to 0.08 mg/kg of LSD.

Following re-establishment of the original discrimination two subjects were tested with CPZ (0.5 mg/kg) and the other two with CPZ (0.5 mg/kg) plus LSD (0.08 mg/kg) administered at the same time, 15 min before the start of the session (at F). On the next test trial (at G), the two rats which were tested with CPZ were given CPZ and LSD while those originally given both CPZ and LSD were tested with CPZ.

On all test trials in which subjects were given LSD plus CPZ, responding was primarily on the LSD side (74%, 87%, 93%, and 100%); thus, at this dose, CPZ did not disrupt the discriminability of LSD from no-drug. When given CPZ alone, two subjects responded at "chance" levels, although early responses were predominantly on the LSD side. The other two animals responded primarily on the saline side (13% and 33% on the LSD side). Hence, subjects could discriminate the LSD state "through" the combination of CPZ and LSD. The F-ratio for all eight trials was 18.13, $P < 0.01$, $df = 1,6$. These results indicate that CPZ does not antagonize rats' ability to discriminate LSD, in contrast to the findings of Appel and Freedman (1964), Ray and Marrazzi (1961), and Bradley and Elkes (1964) who found that CPZ would antagonize LSD under a variety of circumstances. And these results were probably not due to the animals' discrimination of CPZ as being similar to LSD because (a) CPZ alone produced saline-lever responding predominantly and (b) CPZ apparently does not induce any distinctive state (see Harris and Balster, 1971; Overton, 1968).

Following re-establishment of the original discrimination, all subjects were retested in extinction with the original training drugs (at H). With the exception of one animal which was given LSD, animals could still make the LSD-saline discrimination, and all rats emitted early responses primarily on the correct side. However, the control which the

LSD-saline discrimination was exerting over choice behavior was not as strong at this point as it had been originally. While the total number of responses emitted on this test day was larger than on previous test days (when "novel" drugs were administered), the number fell to less than half the number observed during initial training (50 to 100 responses *vs.* 200 to 300).

Next, the discriminability of L-LSD (0.08 mg/kg) was tested (at I). At the dose used, L-LSD produced responding similar to saline; only one subject emitted more than 40% responses (47%) an the drug side. However, the control was not as good as with saline itself (at D and H). Note that while L-LSD is not hallucinogenic in man and has few biological effects in other species (Snyder and Richelson, 1968), the possibility remains that L-LSD is qualitatively similar to d-LSD in the task being employed here; the observed difference may be due to the particular dosages used (see Experiment IV, below).

Finally, all animals were tested with either 50 mg/kg or 100 mg/kg of PCPA (at K). Little control was evident either on the basis of percent responses or on early response choices. The results of the two subjects given 100 mg/kg were unreliable; a total of less than 10 responses were emitted by each of these animals.

Following the first PCPA day, all subjects were administered two more daily doses of PCPA for a total of 300 mg/kg. Then, after five days of not running, subjects were tested with either 0.04 mg/kg or 0.02 mg/kg of d-LSD, followed five days later by another test day with 0.01 mg/kg of LSD or saline, two subjects tested with each (at L and M). This PCPA regimen has not only been demonstrated to selectively deplete brain levels of serotonin (Koe and Weissman, 1966; Appel *et al.*, 1970a) but also to increase the sensibility of rats to the disruptive effects of LSD (Appel *et al.*, 1970a). Would this PCPA pretreatment regimen also increase subjects ability to discriminate the LSD state (hence, increase the tendency to choose the LSD side, even at lower LSD doses)? To answer this question, the results obtained here (L and M) must be compared to those of animals which were given similar low doses of LSD but which had not been pretreated; this was done in Experiment IV (below).

Experiment IV

Procedure. Four animals were trained and tested with 0.08 mg/kg of d-LSD and saline in the same manner as those in Experiment III. The regimen of testing during extinction consisted of 1. two tests with the training drugs, 2. lower doses of d-LSD (0.04 mg/kg or 0.02 mg/kg), 3. mescaline (4, 6, or 8 mg/kg), 4. psilocybin (0.8 mg/kg or 0.4 mg/kg), 5. scopolamine (4 mg/kg), 6. THC (2.5, 5.0 or 10.0 mg/kg), 7. L-LSD (0.25 mg/kg), and 8. d-LSD (given at 1 or 6 h before testing). In addition, a "reversal day" (see Experiment I) was given between 4 and 5. to assess the control the drug was exerting.

Results. The four subjects of Experiment IV showed control on both of the last two training days and on a subsequent test day (Fig. 4, A, B, and C) although control was not as strong on the test day ($F = 3.74$, $df = 1,2$, $P > 0.05$) and one subject given LSD emitted more responses on the saline side (57%). However, following two more training days (a total of 12 LSD trials), control sharpened with LSD. On an intervening saline training day (D), control was about as good as on the previously reported saline training day (A). On the next test day (at E), on which control was not as apparent in the animals given saline as it previously had been (C), the two rats given LSD emitted 94% and 96% correct responses on the LSD side. Moreover, the two groups were significantly different ($F = 110.11$, $P < 0.01$, $df = 1,2$). The early response data support the finding that control was present on this day; three subjects emitted five correct responses before any incorrect and all animals emitted five correct responses before five incorrect.

Following the second test day, and re-establishment of the original discrimination, two subjects were tested with 0.04 mg/kg of LSD and two

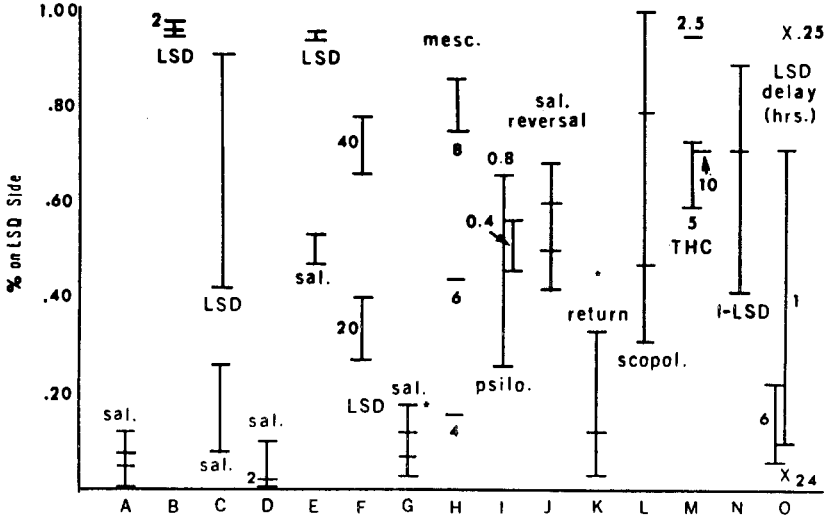

Fig. 4. Subjects trained throughout with 0.08 mg/kg of d-LSD *vs.* saline. *A* and *B* training days. *C* extinction test with training drugs. *D* and *E* further training (*D*) and testing (*E*) with the training drugs. *F* test with two lower doses of d-LSD (0.04 mg/kg and 0.02 mg/kg). *G* a saline test day. *H* and *I* testing with different doses of mescaline (*H*) or psilocybin (*I*) (designated in figure in mg/kg). *J* saline "reversal" (see legend, Fig. 1). *K* "return" (see legend, Fig. 1). *L*, *M*, and *N* testing, respectively, with scopolamine (4.0 mg/kg), several doses of THC (designated in mg/kg), and 0.25 mg/kg of L-LSD. *O* testing with 0.08 mg/kg of d-LSD after two temporal delays, 1 h and 6 h (*X* marks approximate levels usually observed with 0.25 and 24 h delays)

with 0.02 mg/kg (at F). The subjects given 0.04 mg/kg of LSD emitted 66% and 78% responses respectively on the LSD side while those given 0.02 mg/kg emitted only 42% and 28% on the LSD side. These data are in contrast to those obtained following the same doses of LSD (0.04 mg/kg and 0.02 mg/kg) after pretreatment with PCPA (Fig. 3) in that animals given low doses of LSD after pretreatment with PCPA emitted a larger percentage of their responses on the LSD side than did non-pretreated animals which were given the same doses of LSD. The results of these experiments suggest that, indeed, PCPA treatment did "sensitize" the rats to the discriminative properties of the LSD state; in fact, animals pretreated with PCPA gave a higher percentage of responses of the LSD side after 0.01 mg/kg of LSD (Fig. 3M) than did non-pretreated animals that were given 0.02 mg/kg of LSD (Fig. 4). However, this result is not as clear as might be desired because animals in the PCPA group had emitted so few responses (above); nevertheless, it does seem to be valid. After testing with these intermediate doses of LSD, all four subjects were retrained, and then tested with saline; clearly, the discrimination was still intact (G).

Testing the four animals with mescaline (8 mg/kg, 6 mg/kg, and 4 mg/kg) appeared to produce orderly control (H). Of the rats tested at 8.0 mg/kg, one emitted 75% responses on the LSD side and the other emitted 86%. In the one animal which was given 6.0 mg/kg of mescaline, 44% responses were observed on the LSD side; with 4.0 mg/kg, only 16% were on the LSD side.

The effects of another hallucinogen, psilocybin (0.8 mg/kg and 0.4 mg/kg), were also examined in Experiment IV. This drug produced little control at either dose. Thus, as in our earlier results (above) one hallucinogen (mescaline) did seem to produce some control in LSD-saline animals, but another (psilocybin) was not as strong.

Next, saline was given to all four subjects but the lever which was operative (reinforced) was switched for one trial (session). The conditions of the original discrimination were then reinstated during the following session (another "reversal" test). Both drug and dipper cues apparently controlled behavior in this group. When the operative lever was reversed and saline was given, subjects emitted about an equal number of responses on each lever (Fig. 4). When the original side was again operative and saline was again given (K), subjects emitted many more responses on the originally-correct side (97%, 88%, 67% and 56% respectively). Hence, if these results are compared with those of Experiments I and II when 0.04 mg/kg of LSD was given it can be concluded that when the drug dose is sufficiently large, (i.e., the "state" is strong enough) the drug S^D can control behavior even when dipper cues are available.

All subjects in this group were then tested with several "novel" drugs—scopolamine (4.0 mg/kg) (at L), THC (10.0 mg/kg, 5.0 mg/kg, or

2.5 mg/kg) (at M) and L-LSD (0.25 mg/kg) (at 0); the original discrimination was, of course, re-established between each treatment. Scopolamine exerted little control over choice behavior at the dose given; the results were quite variable (100%, 79%, 47%, and 31% on the LSD side). Early responses suggested a result opposite to that suggested by the percentage data, i.e., three of the four rats initially responded on the saline side.

THC, at all three doses (at M), produced fairly good control; subjects responded primarily on the LSD side following THC (2.5 mg/kg—95%, 5.0 mg/kg—73%, and 59%, and 10.0 mg/kg—71%), although the percentage of responding on the LSD side for some reason was not clearly related to dose. These results are in contrast to those of Kubena and Barry (1972) who found that THC and LSD were not clearly discriminable. These authors used Δ^1-THC instead of Δ^9-THC. Finally, L-LSD at 0.25 mg/kg (approximately 3 times the dose of d-LSD used in training or in testing with LSD in Experiment III) produced some control—a shift towards the LSD side. Since 0.08 mg/kg of L-LSD produced responding primarily on the saline side (above), differences in effects of d-LSD and L-LSD may be quantitative rather than qualitative—at least as far as state discriminations are concerned.

The last procedure to which this group was exposed involved allowing different periods of time (1 h or 6 h) to elapse between injections of d-LSD and measuring choice behavior (O). At 1 h, one subject responded primarily on the LSD side (71%), and one on the saline side (89%). At 6 h both subjects responded on the saline side (78% and 94%). Thus, the data indicate that the discriminable properties of the state induced by 0.08 mg/kg of LSD dissipates between 30 min and 6 h, probably more precisely at 1—2 h after i.p. injection; this parallels the results of neurochemical research, which indicate that LSD virtually disappears from the brain by 40—60 min post injection (Rosecrans *et al.*, 1967).

Discussion

The data of Experiment I indicate that a dose of about 0.02—0.04 mg. kg of d-LSD is at the "threshold" level of discriminability. This finding agrees with those of Hirschhorn and Winter (1971), who employed a similar procedure in studying a discrimination task, and Frredman, *et al.* (1964) and Appel *et al.* (1968), who found a dose of about 0.04 mg/kg to be at the threshold level of disruption of an appetitive task (bar-pressing on an FR schedule of food reinforcement). The fact that these doses did not control behavior when dipper cues were available support the finding that these doses are not discriminated strongly.

The results of Experiment II suggest that subjects may be able to discriminate at least some hallucinogenic drugs (d-LSD, mescaline, and

perhaps psilocybin) from each other (in contrast to Hirschhorn and Winter, 1971, whose findings indicate that they could not, when the doses were adjusted), although this ability is not as marked as when an hallucinogen (d-LSD) is compared to a non-hallucinogen (d-amphetamine), and is at least partially dependent on the doses used. It should be noted that the doses of d-LSD and mescaline found to be most equivalent in Experiment II and III do not agree completely with the findings of Hirschhorn and Winter. These investigators found a dose of 9.9 mg/kg of mescaline to be equivalent to about 0.03 mg/kg of LSD whereas, in the present experiments, 8.0 mg/kg of mescaline produced responding more like 0.08 mg/kg of LSD than like saline; 6.0 mg/kg of mescaline seemed to be at threshold (i.e., about "midway" between 0.08 mg/kg of LSD and saline). But if 9.9 mg/kg of mescaline is equivalent to 0.03 mg/kg of LSD, as Hirschhorn and Winter report, then 8.0 mg/kg of mescaline should probably be more like saline than like 0.08 mg/kg of LSD (almost 3 times the 0.03 dose) but it wasn't. In other words, Hirschhorn and Winter found a larger "spread" between the equivalent doses of d-LSD and mescaline than were found in this study. The reason for this discrepancy in dose effect is not clear; it may be related to the difference in LSD training dose (0.08 mg/kg) vs. approximately 0.120 mg/kg in the Hirschhorn and Winter study, or to the method Hirschhorn and Winter employed to equate dosages. Schechter and Rosecrans (1972) found an even larger "spread"—0.048 mg/kg of LSD equal to approximately 25 mg/kg of mescaline. But they did not test a range of doses. Their doses may not be "most equivalent".

The d form of LSD was found to be clearly discriminable from no drug (saline) if the dose was high enough (0.08 mg/kg). It was also clearly discriminable from 1.0 mg/kg of d-amphetamine, a non-hallucinogenic drug, as was noted. In addition, d-amphetamine was found to be easily discriminable from saline—a finding which confirms those of Harris and Balster (1971). While it cannot be stated unequivocally that the LSD-amphetamine discrimination is based on qualitative differences between the induced states, i.e., that the observed differences are not due to the particular doses chosen, the latter explanation seems unlikely. If there is only a quantitative difference between the states induced by d-LSD and by d-amphetamine, then, either 1.0 mg/kg of d-amphetamine should be discriminated as, quantitatively, between 0.08 mg/kg of d-LSD and saline (no drug), or the d-LSD dose should fall between the amphetamine dose and saline; however, the data of Experiments II, III, and IV suggest that this is not the case. Not only did all three combinations of training drugs produce good control, but during testing, it appeared that, if anything, saline (no drug) fell between LSD and amphetamine! Thus, our data agree with those of Overton (1966) who argued that several different classes of drugs do produce qualitatively different states. It

should be noted, however, that the only conclusive way to determine if there are indeed qualitative differences between the two drugs is to test if some dose A of drug X (for example, 0.08 mg/kg of LSD) is discriminable from all doses of drug Y (e.g., amphetamine).

Harris and Balster (1971) report the results of extinction tests with "novel" drugs similar to those that were employed in Experiments III and IV. Using a one-lever schedule choice with an FR associated with 1.0 mg/kg of amphetamine and a drl with saline, these investigators found that 4.5 mg/kg of methylphenidate (another "stimulant" similar to amphetamine) produced FR-like responding. In these animals, atropine (10.0 mg/kg), on the other hand, produced saline-like drl responding. In another experiment, with methyl atropine nitrate and saline as the training drugs, several interseting effects were observed. First, methyl atropine nitrate (10 mg/kg), a primarily peripherally—acting drug (see Longo, 1966), did seem to exert some control over choice behavior. When tested with atropine (7.0 mg/kg) animals responded as if methyl atropine nitrate had been given; when tested with amphetamine (1.5 mg/kg), subjects chose the saline side. However, subjects trained with atropine vs. saline, when given methyl atropine nitrate, chose the saline side.

These observations agree with the results of the present research; when animals which were trained with drug and no drug are given drugs from classes which are different from the training drug, they usually chose the saline side. These "novel" drugs included d-amphetamine, CPZ, scopolamine, PCPA and low doses of d-LSD, mescaline, and L-LSD. While these effects were certainly based upon dose (i.e., were quantitative) in the case of d-LSD and probably also with L-LSD and mescaline, they probably were not with d-amphetamine or the other drugs. In contrast to this, higher doses of L-LSD and mescaline, as well as several doses of THC, were discriminated as similar to d-LSD at the 0.08 mg/kg training dose; all these drugs may be considered to be related to d-LSD, either structure or in effect (i.e., they are all hallucinogenic). But another hallucinogen, psilocybin, did not produce orderly control at either of the doses tested; the reason for this is not clear.

When two subjects trained with d-LSD (0.08 mg/kg) and saline were exposed to an "acute tolerance" regimen (Freedman *et al.*, 1964), no effect on discrimination was observed: both subjects went to the LSD side. This suggests that subjects could still discriminate doses of LSD which no longer would disrupt behavior [note, however, that the "acute tolerance" regimen has been demonstrated only with a high-rate (FR) schedule, and a low-rate (drl) schedule was employed here]. However, pretreatment with another compound, PCPA, demonstrated that there are some pharmacological manipulations which do affect drug discriminations. In contrast to "tolerance", however, PCPA pretreatment made

subjects more sensitive to the LSD state. It would be very interesting to examine whether other neurological or pharmacological manipulations which have been shown to alter sensitivity to LSD—e.g., lesions in the brain stem Raphe system (Appel *et al.*, 1970b)—would affect the discriminability of LSD from other compounds or from other "abnormal" physiological states in similar ways.

While PCPA did seem to have an effect on the discriminability of d-LSD from saline, CPZ did not. It may be that PCPA affects the pharmacological "system" which mediates such a dicsrimination while CPZ does not (there are apparently different "systems" which mediate the development of tolerance and there may be a specific "system" which similarly controls the development of discriminations). However, another explanation is possible. Theoretically, PCPA should "sensitize" an organism to LSD, while CPZ should antagonize the LSD effect (discriminability). Given that these animals 1. have been very well conditioned to make the LSD-saline discrimination and 2. must maintain the ability to make the discrimination effectively in order to obtain their reinforcers, it would be "instrumental" to "react" to PCPA (thus to become "sensitized" to the LSD state), but not "instrumental" to "react" to CPZ (and thus, to lose the ability to discriminate). In this context, however, it must be noted that at least one investigator (Gardner, 1961, cited in Overton, 1971a) apparently has observed specific pharmacological antagonisms of the discriminability of other drug states.

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