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Increasing the Selectivity of Drug Discrimination Procedures

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APPEL, J. B., W. B. WEST, W. G. ROLANDI, T. ALICI AND K. PECHERSKY. *Increasing the selectivity of drug discrimination procedures.* PHARMACOL BIOCHEM BEHAV **64**(2) 353–358, 1999.—In an attempt to increase the selectivity of drug discrimination, rats were trained to discriminate LSD (0.08 mg/kg) from a group of "other" compounds consisting of cocaine (10 mg/kg), pentobarbital (5 mg/kg), and saline. Acquisition of this LSD-other discrimination was rapid (31 days) in chambers equipped with retractable levers and did not differ significantly from that of a group of animals trained to discriminate LSD from saline (26 days). In substitution (generalization) tests, hallucinogens such as LSD, DMT, and DOM mimicked LSD in a dose-dependent manner in both groups. The designer drug (±)MDMA substituted for LSD in the LSD-other group (ED_{50} = 1.38) but did not substitute for the training drug in the LSD-ND group; neither (+) MDMA nor PCP mimicked LSD in either group. Most importantly, lisuride, quipazine, and yohimbine, drugs that have been described as "false positives," substituted for LSD in animals trained to discriminate LSD from saline (ED_{50}s = 0.012, 1.662, 2.344, respectively), but not in animals trained to discriminate LSD from other drugs. Thus, the LSD-other training procedure can be described as more selective than the standard drug-ND procedure. © 1999 Elsevier Science Inc.

Drug discrimination (DD) D-other, LSD (*d*-lysergic acid diethylamide) DMT (5-methoxy-*N,N*-dimethyltryptamine) DOM(±)2,5 dimethoxy-4-methylamphetamine) lisuride, MDMA (methylenedioxy-methamphetamine) quipazine, PCP (phencyclidine) yohimbine

Drug discrimination (DD) has proven to be a reliable, robust, selective, and sensitive animal model of the behavioral effects of psychoactive drugs as well as a useful in vivo assay of their underlying neuronal and receptor mechanisms (11,14). For example, by using the most common DD procedure (drug vs. no-drug; D-ND) the effects of LSD (*d*-lysergic acid diethylamide) have been characterized as being similar to those of indole- and phenylethyl-amine hallucinogens as well as other 5-HT_{2A}/5-HT_{2C} agonists (1,8,12,19). This is because subjects respond in a manner appropriate to the no-drug (ND) condition following treatment with compounds that are pharmacologically distinct from the training drug (16). However, there are problems with the D-ND procedure that limit its validity as an animal model of the effects of drugs that are normally classified as "hallucinogens" (on the basis or their reported effects in humans); these include the occurrence of so-called "false positives" (6,9). Thus, when LSD-trained animals are given substitution (generalization) tests with compounds such as lisuride (18), quipazine (3,5) or, perhaps, yohimbine (4,9), which are *not* known to be hallucinogenic, responding may occur on the drug-, rather than the ND-appropriate lever.

It has been shown that different training procedures can reduce both the occurrence of false positives and the more general problem of incomplete or partial substitution (13). These include, but are not limited to, procedures in which animals are trained to discriminate between two or more pharmacologically active substances (13) or different doses of the same drug (6), and various behavioral manipulations (5); however, such training is time consuming and, therefore, costly (2,18). In the present article, we report that another procedure, involving the discrimination of a drug from a group of other substances (15), can be almost as efficient and considerably more selective than D-ND in that it appears to eliminate the problem of false positives.

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TABLE 1

RESULTS OF SUBSTITUTION TESTS IN ANIMALS TRAINED TO DISCRIMINATE LSD (0.08 mg/kg) FROM SALINE OR FROM A GROUP OF OTHER COMPOUNDS CONSISTING OF SALINE, PENTOBARBITAL (5 mg/kg) OR COCAINE (10 mg/kg)

Drug	Doses Tested (mg/kg)	*n**	LSD-Saline		Sub.	*n*	LSD-Other		Sub.
			ED_{50}	95% CI			ED_{50}	95% CI	
LSD	0.02, 0.04, 0.08	10	0.033	0.02–0.05	++†	9	0.036	0.03–0.05	++
DMT	0.063, 0.09, 1.3	9	0.953	0.81–1.12	++	8	0.877	0.65–1.18	++
DOM	0.05, 1, 2, 3	8	1.117	0.54–2.30	++	6	0.828	0.64–1.07	++
(±) MDMA	0.38, 0.75, 1.5	9	—		0§	6	1.382	0.82–2.31	+‡
(+) MDMA	0.75, 1.5, 3	9	—		0§	9	—		0§
PCP	1, 2, 4	9	—		0§	7	—		0§
Lisuride	0.01, 0.02, 0.04	8	0.012	0.01–0.02	++	7	—		0§
Quipazine	1, 2, 4	9	1.662	0.99–2.8	+	7	—		0§
Yohimbine	0.5, 0.75, 1.5	7	2.344	1.82–3.02	+	9	—		0§

* Number of animals completing the test.
† ≥80% responding on LSD-appropriate lever.
‡ 50% ≥79% responding on LSD-appropriate lever.
§ No substitution: ≥49% responding on LSD-appropriate lever.

METHOD

Subjects

Experimentally naive male, Sprague–Dawley rats ($n = 22$), 60 days old at the beginning of experimentation, were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were housed individually in a colony maintained on a 12 L:12D schedule, with lights on from 0700–1900 h. Temperature and relative humidity were held constant at 20–22°C and 40–50%, respectively. Initially, animals had free access to both food and water. Five days before training, access to water was restricted for 23 h. Access to water was then restricted to the amounts obtained during test sessions (about 20 ml), the amount consumed on weekends (Friday evening to Sunday morning), and during a 10-min period following test sessions.

Apparatus

Eight commercially available experimental chambers (MED Associates ENV 018) housed in light- and sound-attenuating shells (MED Associates ENV 008) were used. Each chamber contained two retractable levers and a dipper that was programmed to deliver 0.1 ml of water for 0.3 sec whenever a reinforcer was scheduled.

Training Procedure

Animals were assigned randomly to either a control group (LSD-Saline; $n = 10$) that was trained to discriminate LSD bitartrate (0.08 mg/kg) from saline (0.9% NaCl) in a manner described in detail elsewhere (7). All drugs were given intraperitoneally (IP), 15 min before daily (Monday–Friday) experimental sessions. Rats in a second group (LSD-Other; $n = 9$) were trained to discriminate the same dose of LSD from a set of compounds that included saline, pentobarbital sodium (5 mg/kg), and cocaine HCl (10 mg/kg). Animals were given LSD on 50% of the sessions and one of the three other drugs (saline, pentobarbital, or cocaine) during the remainder of the training sessions; thus, on any given session, animals had an equal chance of receiving LSD or one of the other drugs.

During the first stage of experiment, only the condition-appropriate lever was present—LSD, saline, or other drug

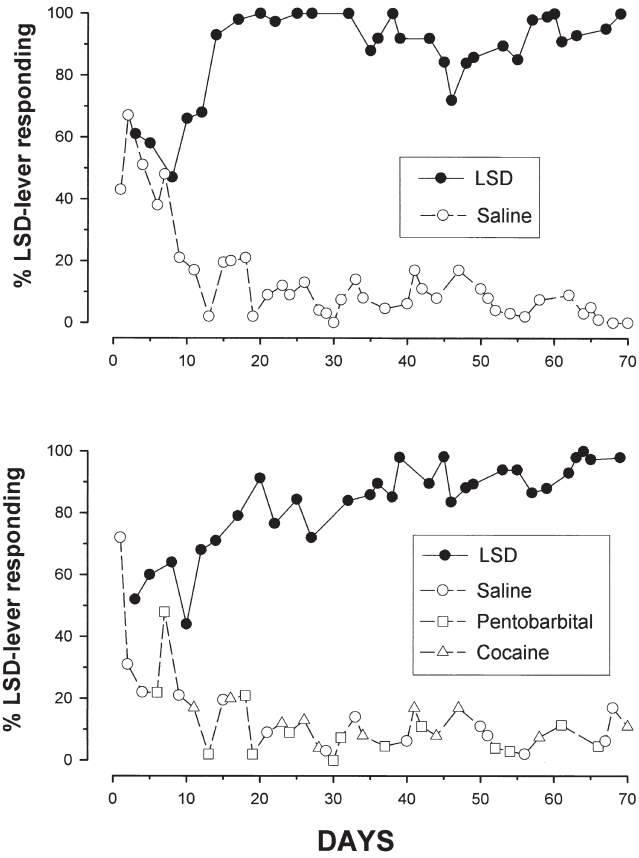

FIG. 1. (Top) Acquisition of a discrimination between LSD (0.08 mg/kg, IP) and saline (0.9% NaCl) using a "standard" drug-no drug (D-ND) procedure. Criterion (at least 80% of the responses occurring before the first FR was completed for seven consecutive sessions) was attained in 26 days. (Bottom) Acquisition of a discrimination between LSD (0.08 mg/kg, IP) and a set of other compounds consisting of saline, cocaine (10 mg/kg, IP), and pentobarbital (5 mg/kg, IP). Criterion was attained in 31 days.

(cocaine, pentobarbital, or saline). The position of the lever (left or right) was assigned randomly within and between groups of animals to control for lever bias. The order of stimulus (drug) presentation in both groups of rats was also assigned randomly, with the restriction that neither drug was administered more than three consecutive sessions. Conditioning of lever pressing began under a fixed-ratio (FR 1) schedule of reinforcement; as response rates stabilized, the ratio was raised gradually to FR 20.

Discrimination Training

After all animals were responding reliably under the FR 20 schedule, both levers were presented simultaneously. Responses on the correct lever (the LSD-appropriate lever following an injection of LSD or the other lever following an injection of saline or of saline, pentobarbital, or cocaine) continued to be reinforced under the FR 20 schedule. Responses on the incorrect lever were recorded, but had no additional consequences. Training continued until all animals in each group reached a criterion of 80% of the first 20 responses occurring on the condition-appropriate lever for seven consecutive sessions.

Substitution Testing

Substitution tests were given with hallucinogens [LSD; *d*-lysergic acid diethylamide bitartrate, DOM; (±)2,5 dimethoxy-4-methylamphetamine, and DMT; [5-methoxy-*N,N*-dimethyltryptamine hydrogen oxlyate] substituted amphetamines (+) MDMA; methylenedioxymethamphetamine hydrochloride, and (±)MDMA), PCP (phencyclidine hydrochloride), and the false positives (lisuride hydrogen maleate, quipazine dimaleate, and yohimbine hydrochloride (see Table 1, above). These tests, which terminated as soon as the first 20 responses on either lever were completed, were conducted under extinction conditions, one to two times a week.

Drugs

All drugs were dissolved in 0.9% saline and were given IP in a volume of 1 ml/kg. LSD was obtained from the National Institute on Drug Abuse (NIDA, Rockville, MD); all other drugs were purchased from Research Biochemicals, Inc. (Natick, MA). Doses were calculated as salts.

Data Analysis

The datum of major interest during both acquisition and substitution testing was the proportion of responses that occurred on the LSD-appropriate lever prior to the completion of 20 responses. Individual ED_{50}s and 95% confidence intervals (CIs) were calculated for each animal that made at least 50% of its responses on the LSD-appropriate lever (17) during test sessions.

Rates of responding prior to completion of the first 20 responses on one lever were analyzed for each test drug with repeated-measures ANOVAS. When *F*-values were significant ($p < 0.05$), post hoc tests were performed using the Bonferroni All Pairwise method of comparison (10).

RESULTS

The acquisition of the LSD-saline and LSD-other discriminations are shown in Fig. 1. Criterion was attained rapidly in both groups of animals, 24 sessions in the LSD-saline group (top), and 31 sessions in the LSD-other group (bottom), and did not differ significantly as a function of training condition, $t(20) = 1.22$, $p = 0.2$. In addition, rates of responding were relatively high, at least when compared with those reported

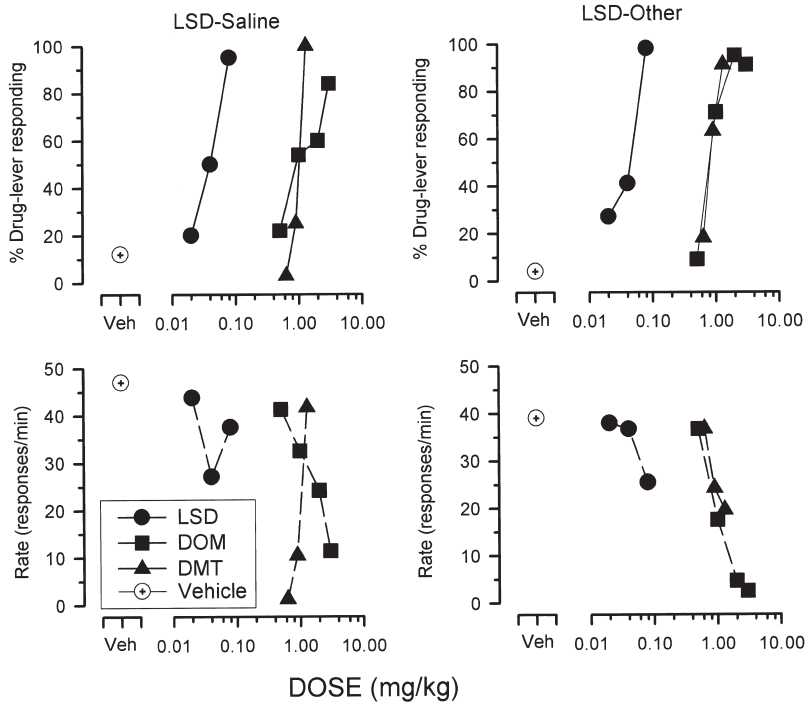

FIG. 2. Results of substitution tests with three hallucinogens in animals trained to discriminate LSD (0.08 mg/kg, IP) from either saline (left panels) or a group of other drugs (right panels).

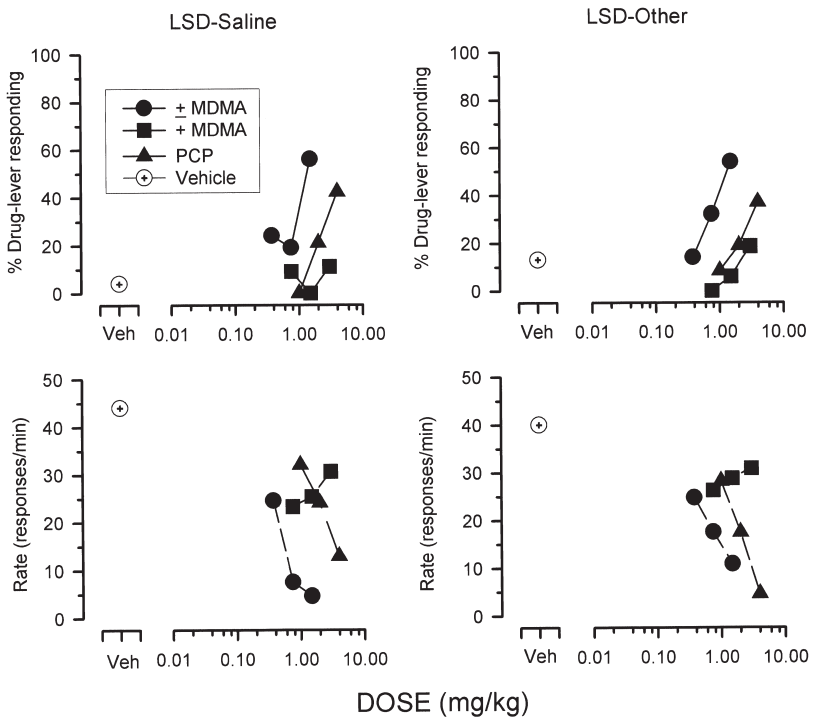

FIG. 3. Results of substitution tests with two isomers of MDMA and PCP in animals trained to discriminate LSD (0.08 mg/kg, IP) from either saline (left panels) or a group of other drugs (right panels).

previously in this laboratory (7). The average rate for each animal in the LSD-Saline group during its last 7 days before testing was begun was 48.2 responses/min; the rate in the LSD-other group was 44.9 responses/min (data not shown).

The results of substitution tests are shown in Figs. 2–4 and are summarized in Table 1. The three indole amine hallucinogens (LSD, DOM, and DMT) substituted for LSD under both training conditions (Fig. 2); LSD was considerably more potent than either DOM or DMT (Table 1). In addition, DOM and DMT appeared to disrupt responding whereas LSD did not (Fig. 2); however, only the effects of the two highest doses of DOM on the rates of LSD-other trained animals were statistically significant, $F(5, 20) = 5.89$, $p < 0.001$.

The results of substitution tests with PCP and the isomers of MDMA are shown in Fig. 3and Table 1. Only (±)MDMA mimicked LSD (60%) in the LSD-other group. PCP disrupted responding significantly in animals trained to discriminate LSD from either saline, $F(8, 24) = 4.312$, $p < 0.005$, or from other compounds, $F(5, 18) = 4.63$, $p < 0.005$; (±)MDMA also disrupted rate in the LSD-other group, $F(5, 15) = 2.82$, $p < 0.05$.

In the LSD-saline group, all three of the putative "false positives" substituted for LSD (Fig. 4); none of these drugs substituted for LSD in the LSD-other group. Lisuride was considerably more potent than either quipazine or yohimbine (Table 1). Lisuride also disrupted responding significantly in rats trained to discriminate LSD from both saline, $F(7, 21) = 3.62$, $p < 0.01$, and other compounds, $F(6, 18) = 2.91$, $p < 0.05$, at the relatively high dose of 0.04 mg/kg. Yohimbine significantly disrupted the rates of LSD-other, $F(8, 24) = 2.79$, $p < 0.05$, but not LSD-other trained animals.

DISCUSSION

The results of this experiment indicate that rats can be trained to discriminate LSD from a group of "other" drugs consisting of a CNS depressant, pentobarbital, a CNS stimulant, cocaine, and saline. Thus, they confirm and extend the work of Overton (15) to a different class of compounds (hallucinogens rather than depressants). Thus far, however, the limits of the LSD discrimination have not been explored by varying either drugs or doses in the "other" group. This task should be undertaken forthwith, because it is known that these variables alter the efficiency and, hence, the usability of the procedure (15).

Under the conditions of the present experiment, it was possible to train rats to discriminate LSD from the set of other drugs rapidly. Indeed, such training required little more time than the "standard" LSD-saline procedure. This could have been caused by the high rates of responding that were observed under both training conditions, the use of new conditioning chambers equipped with retractable levers, or by restricting the availability of reinforcement to a limited duration (3 s). All experiments conducted previously in this laboratory involved chambers in which liquid dippers remained in the "up" position unless a reinforcer was programmed (7).

The drug-other procedure appears to be more sensitive than the drug-ND procedure in that, in general, it is more susceptible to the rate-disruptive effects of test compounds. In the present experiment, for example, the LSD-other rate data generated five significant *F*-values [(±)MDMA, DOM PCP, lisuride and yohimbine]; while the LSD-saline data generated only two (lisuride and PCP).

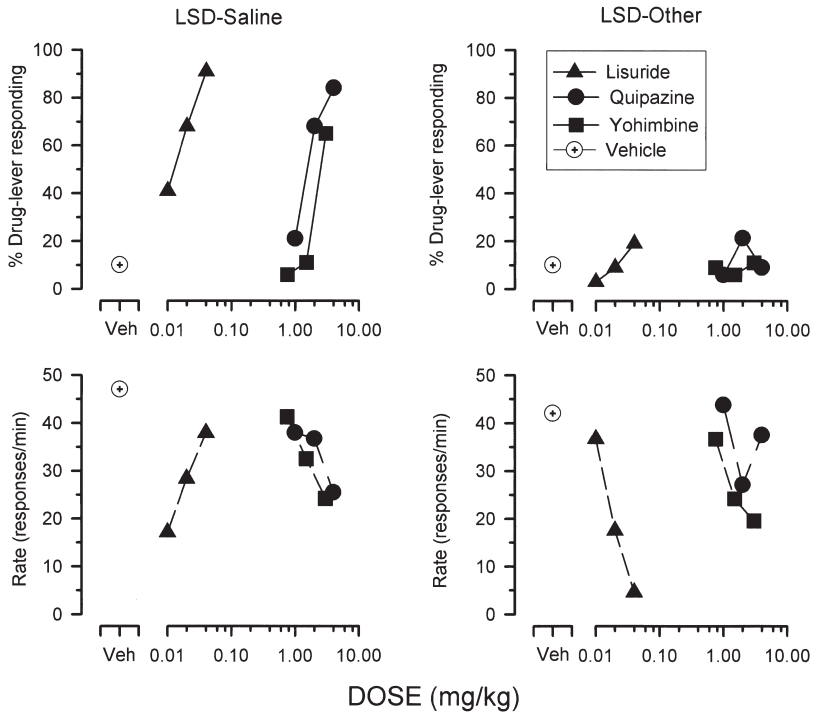

FIG. 4. Results of substitution tests with three "false positives" in animals trained to discriminate LSD (0.08 mg/kg, IP) from either saline (left panels) or a group of other drugs (right panels).

More importantly the LSD-other procedure was more selective in that false positives did not occur. Thus, under conditions in which animals are required to "attend" to specific LSD-related states rather than to the presence or absence of a drug, compounds that are sometimes "recognized" as being LSD-like but are not known to be hallucinogenic, are not characterized as being "similar" to LSD. In this sense, the drug-other assay may be a more valid animal model of hallucinosis (in humans) than the drug-ND assay.

Although the D-other procedure seems to be both more selective and more sensitive than the standard D-ND procedure, at least with hallucinogens, it should be noted that at least two procedures in addition to D-D discrimination (13) have also been reported to increase selectivity. These involve dose–dose discriminations (6) and the manipulation of reinforcement contingencies (5). It is not known, however, if either of these procedures can be implemented successfully with hallucinogens or that, if so, they would be as efficient as D-other training. Indeed, our laboratory has gathered preliminary evidence that relatively low and high doses of LSD cannot be discriminated reliably from each other.

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