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Drug-Drug Discrimination: Stimulus Properties of Drugs of Abuse Upon a Serotonergic-Dopaminergic Continuum

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SCHECHTER, M. D. *Drug-drug discrimination: Stimulus properties of drugs of abuse upon a serotonergic-dopaminergic continuum.* PHARMACOL BIOCHEM BEHAV **56**(1) 89–96, 1997.—Ten male N/Nih rats were trained to discriminate between the interoceptive cues produced by the purportedly dopaminergically-mediated drug *d*-amphetamine at 0.4 mg/kg intraperitoneally administered 20 min prior to training and those produced by the purportedly serotonergically-active agent norfenfluramine at 0.7 mg/kg. Once this discrimination was successfully acquired, the rats were tested with saline and with both drugs administered simultaneously and these manipulations were seen to produce random responding; indicating roughly equivalent cueing strength. Subsequently, various drugs thought to act upon serotonergic neurons, i.e., LSD and MDMA, were tested and shown to generalize in a dose-responsive manner to the norfenfluramine-appropriate lever. In contrast, the dopaminergically-active agent methcathinone and the D$_3$ agonist 7-OH-DPAT produced generalization on the amphetamine-appropriate lever. Results are discussed in light of the increased specificity of behavioral testing available in a drug vs. drug discriminative paradigm using two drugs with different mechanisms of action. **Copyright © 1997 Elsevier Science Inc.**

Stimulus properties of drugs   Amphetamine   Norfenfluramine   Time-course   LSD   MDMA
7-OH-DPAT   Methcathinone   Rats

THE ever-growing utilization of the stimulus discriminative learning procedures (e.g., 42) has allowed for its being adopted as a specific in vivo assay of centrally-activated drugs. In effect, the vast majority of these drug discrimination studies involve training a drug vs. its vehicle, a non-drug condition, where the discriminative interoceptive cues function to signal the presence or absence of the drug, respectively. In contrast, fewer studies have investigated the drug-drug discriminative procedure in which animals are trained to discriminate between the interoceptive cues produced by two different drugs; this allows for a generalization gradient to be established for both drugs. The drugs used to train differential responding have been reviewed (38) and, as early as 1979 (3), it was suggested that the strength, or distinctiveness, of a drug-produced interoceptive cue not only depends on the dose of the drug used, but upon the characteristics of the drug. Thus, two drugs, at roughly equivalent potencies, may be different in their distinctiveness or discriminability than the difference existing between the respective drug and its nondrug training condition. Indeed, this has been shown to be the case in that increased sensitivity to lower doses of the training drug have been reported in animals trained in drug vs. drug discrimination (6,16,22,39).

One of these drug-drug discrimination studies employed the central stimulant amphetamine vs. the depressant pentobarbital to test other drugs, fenfluramine and nicotine, upon this "upper-downer" continuum of discriminative cues (34). Another study employed the discriminative stimulus properties of 0.8 mg/kg amphetamine, a drug thought to act by dopamine release (20), to train rats to discriminate it from 1.4 mg/kg norfenfluramine, a drug thought to act by virtue of its ability to release pre-synaptic serotonin (7). These two drugs had been previously shown not to generalize to one another as norfenfluramine will not generalize to amphetamine (5) nor will, the related drug, fenfluramine generalize to amphetamine (49). The lack of generalization of these drugs suggests a distinct pharmacological difference in the mechanism of action between these two anorectic drugs; the norfenfluramine having mostly serotonergic activity, whereas the amphetamine having dopaminergic activity. Therefore, the drug-drug discriminative effects between these two agents would allow for testing of other drugs along a "continuum" that may, in fact, be mediated at its extremes by each of these two distinct neurotransmitters. The purpose of the present study, therefore, was to replicate a previous experiment in which norfenfluramine vs. amphetamine was trained in rats but to, in addi-

tion, halve the training dose of each drug. In most cases, decreasing the training dose employed in producing discriminative stimulus effects reduces the subsequent ED_{50} value, suggesting a greater degree of sensitivity in the animal to the lower drug dose's interoceptive cue (9). Thus, 0.7 mg/kg norfenfluramine was trained in rats on one of two levers, whereas 0.4 mg/kg amphetamine was trained on the second of these two levers. Subsequently, saline and coadministration of the training drugs/doses, as well as novel drugs of abuse, with known or suspected serotonergic-dopaminergic activity, were tested to ascertain their discriminative properties along a serotonergic (norfenfluramine)-dopaminergic (amphetamine) continuum. In addition, the co-administration of the training dose(s) of norfenfluramine and amphetamine was tested at various post-administration times in an endeavor to evaluate if the offset of discriminative effect, i.e., the behavioral half-life, of each drug was different from the other.

METHODS

Subjects

The rats used in the present experimentation were 10 male N/Nih rats delivered to this site from the Small Animal Section of the National Center for Research Resources of the National Institutes of Health. These animals comprise one of the few new outbred stocks to be developed in the last fifteen years (19). The animals were approximately 90 day-old at the initiation of training and were individually housed in galvanized cages with free access to water except during experimental sessions. They were maintained at $85 \pm 5\%$ of their free-feeding body weights as determined by weighing an ad libitum fed rat. The rats were trained five days per week, at the same time of day (1200–1400 h).

Apparatus

Twelve standard rodent operant test cages (Lafayette Instrument Company, Lafayette, IN) were equipped with two levers mounted 7 cm above the metal grid floor and 7 cm apart. The greater number of experimental chambers than subjects allowed for random assignment of a rat to any chamber on any given day to preclude the possibility of inter-animal olfactory cues (11). Equidistant between the two levers and 2 cm above the floor was located a food-pellet receptacle capable of receiving the delivery of a 45 mg Noyes (Lancaster, NH) food pellet. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9W house light.

Shaping and Discriminative Training

The training procedure was similar to that previously described (6). Briefly, the rats were placed into the operant chamber 20 min after intraperitoneal (IP) injection and autoshaped to press one of the two levers in order to receive a food reinforcement on a fixed-ratio 1 (FR1) schedule. For half the rats in each group, the right lever was assigned as the "amphetamine-correct" lever, whereas the other half of this group was assigned to the left lever. This was done to counterbalance any possible position preferences. Training continued as the FR schedule was gradually increased to an FR10 schedule of reinforcement over a period of 6 days. For all animals, the first drug trained was 0.4 mg/kg amphetamine (sulfate) and the FR10 schedule was maintained for an additional 2 days. On the following training session, the rats received the alternative drug condition, 0.7 mg/kg norfenfluramine, and

were required to respond upon the opposite lever starting with an FR1 schedule. The schedule was gradually incremented over a period of five days until stable FR10 reinforcement responding was attained and this schedule was maintained for one additional day. Following these initial lever-press training sessions, the bi-weekly, repeating injection schedule of A-NF-NF-A-A; NF-A-A-NF-NF, where A= 0.4 mg/kg amphetamine and NF=0.7 mg/kg norfenfluramine, was used. This schedule of administration was continued on a daily schedule until all rats selected (pressed 10 times first) the appropriate lever based on the condition imposed on 8 out of 10 consecutive sessions. This 80% discriminative performance was required to occur for two 10-consecutive session durations; this often occurred in two subsequent 10 consecutive session blocks such that the rat correctly selected the appropriate lever in 16 (or more) sessions of the twenty consecutive sessions conducted. In a few cases, the second 10 consecutive sessions did not immediately follow the first 8 of 10 correct sessions.

Dose-Response Testing

After all ten rats had met the 80% discriminative criterion on two occasions, they each received various doses of *d*-amphetamine (A) or norfenfluramine (NF) according to the two-week, repeating schedule: A-DR^1-NF-DR^1-A, DR^2-NF-DR^2-A-DR^3, NF-DR^3-A-DR^4-NF, DR^4-A-DR^5-NF-DR^5, A-DR^6-NF-DR^6-A, etc., where DR^n = novel doses of either norfenfluramine (at 0.0875, 0.175, 0.35 and the training dose of 0.7 mg/kg) or amphetamine (0.05, 0.1, 0.2, as well as the training dose of 0.4 mg/kg). Thus, each novel dose of either norfenfluramine or amphetamine was preceded by a maintenance session with both the 0.4 mg/kg amphetamine, as well as one 0.7 mg/kg norfenfluramine maintenance session. During these maintenance sessions, the first 10 responses accumulated on one of the two levers was considered the "selected" lever yet animals were allowed to respond in order to receive 40 reinforcements (and, thus, required to make 400 responses on the FR10 schedule) only upon the state-appropriate lever; this allowed a test of discriminability of one of the two training drug/doses and a continuing training session to help ensure that rats maintained discrimination between amphetamine and norfenfluramine. During interspersed test sessions, the animals were allowed to lever press until a total of 10 responses had accumulated on either lever. The rats were immediately removed from the operant chamber, without receiving reinforcement, so as to preclude reinforcement/training after administration of a dose differing from their training dose of either amphetamine or norfenfluramine. The "selected" lever (pressed 10 times first) was used for the quantal measurement and the number of presses on both levers at the time that one was selected was the quantitative measure for that session (see Measurements and Statistics, below).

Administration of Saline Vehicle or Norfenfluramine Plus Amphetamine Simultaneously

The administration of the vehicle used to dissolve both the drugs (i.e., 0.9% NaCl in distilled water) was tested in the drug-drug trained rats to indicate the possibility of equal responses on both levers occurring (i.e., random responding). If, in fact, there was unequal cue strength inherent in the two trained drugs, the responses may be indicated by a greater number of responses on the lever appropriate for the training drug that provides the weaker cue (3,23). In addition, the

simultaneous co-administration of the training doses of each of amphetamine and norfenfluramine were injected immediately after each other on contralateral sides intraperitoneally and tested twenty min later in an effort to indicate if equal responses on both levers would occur. If the cue strengths were unequal, the animals would select the lever responding to the drug producing the stronger discriminative cue with a higher frequency than that representing the drug with the weaker discriminative cue (23, 43). The co-administration of 0.7 mg/kg norfenfluramine and 0.4 mg/kg d-amphetamine was performed twice at the onset of generalization experiments (below) in $n = 9$ rats and in two sessions immediately following the generalization tests with the remaining rats ($n = 8$). In addition, the two drugs were co-administered, on two sessions at each post-injection time of 10, 40, 60, 90 and 120 min, to investigate how the rats perceived the two "states" over this time period.

Tests for Generalization with Doses of Drugs of Abuse

In an effort to determine the norfenfluramine-like effects which may, indeed, be serotonergically-mediated vs. the amphetamine-like discriminative effects, which may, in fact, be dopaminergically-mediated, various doses of three drugs of abuse were administered to the norfenfluramine-amphetamine trained animals at 20 min post-injection. Starting doses for each of these drugs came from the voluminous research which has detailed how each of them has been employed as drugs capable of controlling discriminative behavior (42). Thus, the starting dose for each of these was the dose previously shown to produce discriminative behavior and lower doses were then also tested. When a drug, at any particular dose, was observed to produce 80% of responding on the amphetamine-appropriate lever, it could be thought to generalize from amphetamine since this 80% criterion was originally employed to ascertain criterion-level discriminative performance with amphetamine in the drug-drug trained animals. Likewise, if 20% of the responses were made on the amphetamine-appropriate lever, this would indicate that 80% of the responses were made on the other, or norfenfluramine-appropriate, lever and generalization to norfenfluramine could be adjudged. The drugs of abuse that were employed were MDMA (0.125–2.0 mg/kg), methcathinone (0.125–1.0 mg/kg) and LSD (at 0.015–0.12 mg/kg). In addition to these known drugs of abuse, another agent, the D_3 agonist 7-OH-DPAT (0.063–0.5 mg/kg), was tested. In each case, each dose of the novel test drug was randomly tested once following a maintenance session with 0.7 mg/kg norfenfluramine and once after a maintenance session with 0.4 mg/kg amphetamine. Each drug was administered in at least four doses by the IP route in an equal volume (1 ml/kg), tested 20 min post-administration, and the animal immediately removed upon accumulating 10 responses upon either lever.

Measurements and Statistics

The first lever on which 10 presses accumulated was designated the "selected" lever. The percentage of rats selecting the lever appropriate for amphetamine was employed as the quantal measurement of discrimination and this is graphically represented as the percent first choices on the amphetamine-appropriate lever. The number of lever presses on the amphetamine-appropriate lever divided by the total number of responses on both levers prior to the accumulation of 10 responses on either lever, constitutes the quantitative measure-

ment. Both measurements were utilized as has been previously suggested (41). The midpoint of 50% correct responding was chosen as the point of comparison. These ED_{50} values, where quantal data are compared by the method of Litchfield and Wilcoxon (29) using probit vs. log-dose effects, not only allows for generation of ED_{50} values but also tests for parallelism and potency ratios as a computerized program (44). Quantitative data, as well as sessions-to-criterion (STC) 1 and 2 (i.e., the first of 10 consecutive sessions in which 8 of the 10 correct levers were chosen for the first time-STC1, and for the second time-STC2) were analyzed by an unpaired Students' t-test (two-tailed;44) and the level of statistical significance was chosen at $p < 0.05$. Drug discriminability may be examined by acquisition rate for the drug discrimination, as well as by determining the dose of drug which produces drug-appropriate responding 50% of the time, the ED_{50} value (37).

Drugs

The drugs (abbreviation; supplier) used in this study were: d, l-norfenfluramine HCl (NF; A.H. Robins, Richmond, VA), d-amphetamine sulfate (AMPH; Sigma Chemical, St. Louis, MO), d-3,4-methylenedioxymethamphetamine hydrochloride (MDMA; National Institute on Drug Abuse or NIDA, Rockville, MD), d,l-methcathinone hydrochloride (NIDA), d-lysergic acid diethylamide tartrate (LSD; NIDA) and d, l-7-hydroxy-dipropylaminotetralin hydrobromide (7-OH-DPAT; Research Biochemical International, Natick, MA). All drugs were dissolved in 0.9% sodium chloride solution (in distilled water), administered intraperitoneally at a constant volume of 1 ml/kg and tested 20 min post-injection except during the time-course study. Doses were all calculated as base.

RESULTS

Rate of Drug-Drug Discriminative Learning

The rats trained to discriminate between 0.4 mg/kg d-amphetamine and 0.7 mg/kg norfenfluramine rapidly learned to discriminate between these two drug states. The mean ($\pm$S.D.) number of sessions needed to reach the first of the 10 consecutive sessions in which 8 out of 10 lever selections were drug-appropriate, the STC1, was 6.2 (3.34) with a range of 2 to 11 sessions. Thus, by the 21st session, all 10 N/Nih male rats had learned to discriminate between amphetamine and norfenfluramine. The second session-to-criterion (STC 2) occurred in a mean ($\pm$S.D.) of 17.0 (4.81) sessions with a range of 12-26. Thus, even the slowest rat to learn the drug-drug discrimination required only 36 sessions (18 with each drug condition) to learn to discriminate between amphetamine and norfenfluramine.

Dose-Response and Time-Course Effects of Amphetamine and Norfenfluramine

Doses lower than the training dose of d-amphetamine, 0.2, 0.1, 0.05 mg/kg, produced decreased quantal responding, i.e., decreasing percentage of animals selecting (pressing 10 times first) the amphetamine-lever, with a general decrease in the quantitative measurement as well (Fig. 1). In addition, the administration of doses of norfenfluramine lower than the 0.7 mg/kg dose used in training, 0.35, 0.2 and 0.1 mg/kg, produced increasing responding upon the amphetamine-appropriate lever and, thus, decreased responding upon the norfenfluramine-appropriate lever. Both quantal and quantitative measurements in the norfenfluramine-tested animals were seen to
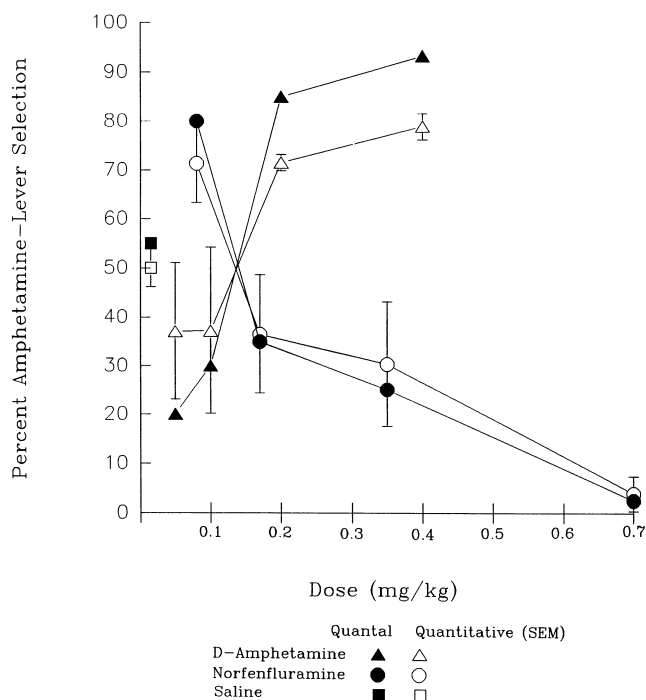
FIG. 1. Dose-response of 0.0 (saline), 0.1, 0.2, 0.35 mg/kg norfenfluramine, as well as 0.05, 0.1, 0.2 *d*-amphetamine, in rats trained to discriminate between 0.4 mg/kg amphetamine vs. 0.7 mg/kg fenfluramine. Abscissa: dose in mg/kg body weight with each point representing ten rats; Ordinate: percent of rats pressing the amphetamine-appropriate lever ten times first, i.e. selecting this lever at 20 min post-administration. Each point represents two trials at each dose. Quantal and quantitative measurements described in Methods.
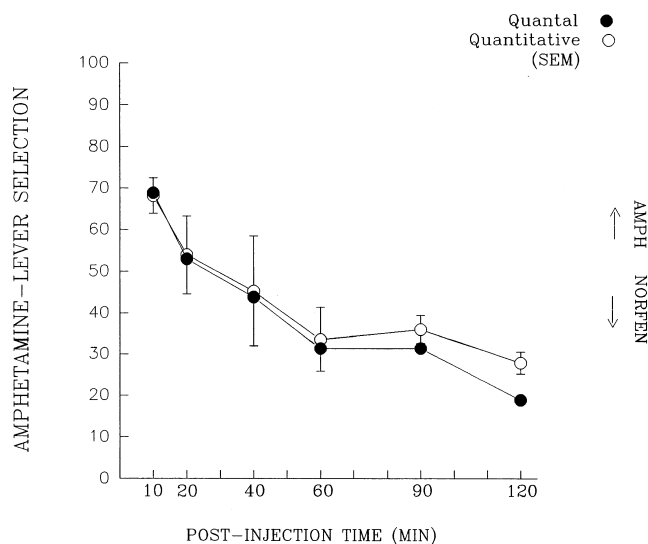

FIG. 2. Discriminative performance of rats ($n = 8$) administered the training doses of 0.7 mg/kg norfenfluramine + 0.4 mg/kg *d*-amphetamine at different post-administration times.

decrease with decreasing doses administered/tested. Calculation (29) of the ED_{50} value for amphetamine (with 95% confidence limits) indicates 0.112 (0.079-0.159) mg/kg, whereas the ED_{50} value for norfenfluramine was calculated to be 0.160 (0.121-0.213) mg/kg.

When saline was administered on two occasions, once following a maintenance session with 0.4 mg/kg *d*-amphetamine and once following a maintenance session with 0.7 mg/kg norfenfluramine, the 10 rats were seen to select the amphetamine-appropriate lever on 11 of the possible 20 occasions producing a quantal measurement of 55% and a mean (SD) quantitative measurement of 50.9 (0.64) as indicated in Fig. 1. After these experiments, one rat died and later a second rat died of unrelated causes; this is represented in $n = 9$; 8. Co-administration of the norfenfluramine and amphetamine doses on contralateral sides tested 20 min after the second injection produced 18 amphetamine-lever selections in the 34 trials, with 9 rats tested twice before and 8 rats tested twice after generalization testing; this yielded a quantal measurement of 52.9% and a mean (SD) quantitative measurement of 53.9 (3,21). Thus, the saline (nondrug) test and NF-AMPH co-administration test yielded roughly random responding, when tested at the post-injection time of 20 min. When the two doses of NF and AMPH used in training were co-administered and tested at 10, 40, 60, 90 and 120 min post-administration, the quantal and quantitative measurement results are graphically represented in Fig. 2. It would seem that the 0.7 mg/kg norfenfluramine dose, administered with the 0.4 mg/kg amphetamine

dose, produced slightly more amphetamine-lever selections at an earlier post-administration time, but that this discriminative effect shifted to more norfenfluramine-like selections as the injection-to-test interval was extended.

Generalization of Amphetamine to Various Doses of Methcathinone and 7-OH-DPAT

When the dose-response data are graphed by using a (double-cycle) log-dose abscissa with a probability ordinate, the dose-response relationship generally takes a linear form (Fig. 3). After administration and testing of 0.5 and 1.0 mg/kg methcathinone, both were observed to produce 88.9% of selected lever choices on the amphetamine-appropriate lever. Thus, when the 9 surviving amphetamine-norfenfluramine trained rats were administered these two doses on each of two occasions, they selected the amphetamine-appropriate lever on 16 sessions; thus, 16/18 gives a quantal measurement of 88.9%. The lower dose of 0.25 mg/kg methcathinone produced 66.7% and 0.125 mg/kg produced 44.4% amphetamine-lever selections. Therefore, both 0.5 and 1.0 mg/kg methcathinone may be said to generalize to the amphetamine-induced stimulus cue and calculation of the dose-response relationship indicates an ED_{50} value (with 95% confidence limits) for methcathinone equal to 0.152 (0.093-0.248) mg/kg.

Likewise, the testing of four doses of the putative D_3 agonist 7-OH-DPAT allowed for increased amphetamine-like responding with increasing doses. The testing of 0.5 mg/kg on two occasions produced 16 of 18 possible selected-lever responses on that lever yielding a quantal measurement of 88.9% and, thereby, generalization. Decreasing doses of 0.25, 0.125 and 0.063 mg/kg 7-OH-DPAT produced quantal measurements of 66.7%, 61.1% and 38.9%, respectively. Analysis (29) allowed calculation of an ED_{50} value for 7-OH-DPAT of 0.096 (0.0551-0.167) mg/kg.

Further analysis for parallelism (29) between 7-OH-DPAT, methcathinone compared to the amphetamine dose-response line (Fig. 3) indicates that the lines are parallel within statistical
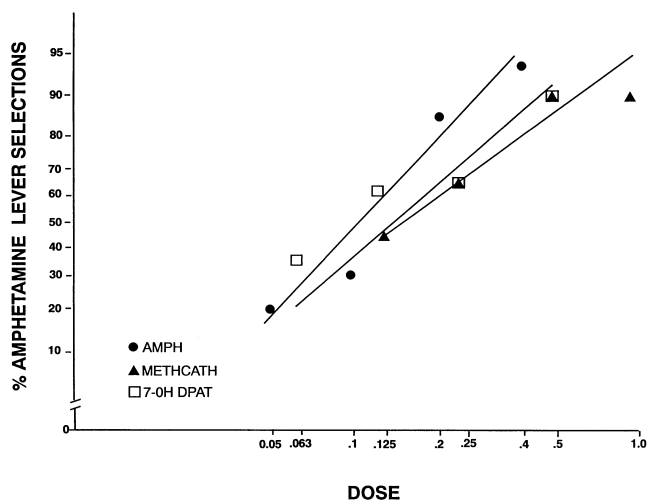
FIG. 3. Generalization of amphetamine doses in animals trained to discriminate between 0.4 mg/kg amphetamine and 0.7 mg/kg norfenfluramine, as well as generalization to methcathinone and 7-OH-DPAT. Abscissa: dose on a double-cycle log scale; Ordinate: percent amphetamine lever selections on probability scale. Each point represents $n = 9$ and two trials at each dose.

FIG. 4. Generalization of norfenfluramine, as well as MDMA and LSD, in rats trained to discriminate 0.7 mg/kg norfenfluramine from 0.4 mg/kg d-amphetamine. Scales as in Fig. 3.

limits ($p < 0.05$). In addition, ED_{50} values are not significantly different from each other.

Generalization of Norfenfluramine to Various Doses of MDMA and LSD

Test days were also employed in the amphetamine-fenfluramine trained animals to test drugs whose putative mechanisms are serotonergic in nature. Thus, doses of MDMA at 2.0, 1.5, 1.0, 0.5, 0.25 and 0.125 mg/kg were administered and produced quantal performance on the amphetamine-lever of 16.7, 11.1, 61.1, 61.1, 50.0, and 72.2%, respectively. Thus, at the highest dose tested (2.0 mg/kg), MDMA allowed for 83.3% of responses upon the norfenfluramine-appropriate lever, indicating a generalization to this (trained) drug state. This generalization persisted, albeit increased (to 88.9%), at 1.5 mg/kg and decreased with lowered doses (Fig. 4). Calculation of the ED_{50} value for MDMA: 0.327 (0.100-1.063) mg/kg.

Likewise, doses of LSD, tested on two occasions each, starting at the highest dose of 0.12 mg/kg, produced 16.7% quantal responding on the amphetamine-appropriate lever and, thus, 83.3% on the norfenfluramine-appropriate lever, indicating generalization. Lower doses of 0.06, 0.03, and 0.015 mg/kg produced 33.3, 50.0 and 61.1% responding upon the amphetamine-appropriate lever (Fig. 4). This dose-response relationship allowed an ED_{50} value for LSD equal to 0.0267 (0.045-0.156) mg/kg. Analysis of the dose-response lines generated (Fig. 4) indicates parallelism within statistical limits ($p < 0.05$) but a significantly more potent effect of LSD (potency ratio equal to 6.003; $p < 0.05$;38) when compared to the dose-response effect of norfenfluramine tested.

DISCUSSION

The behavioral paradigm of drug discrimination, a.k.a. stimulus properties of drugs, generally involves training rats to discriminate the presence or absence of a drug by using the drug to train one response, usually pressing one lever, and the vehicle to train a second equivalent response, usually to
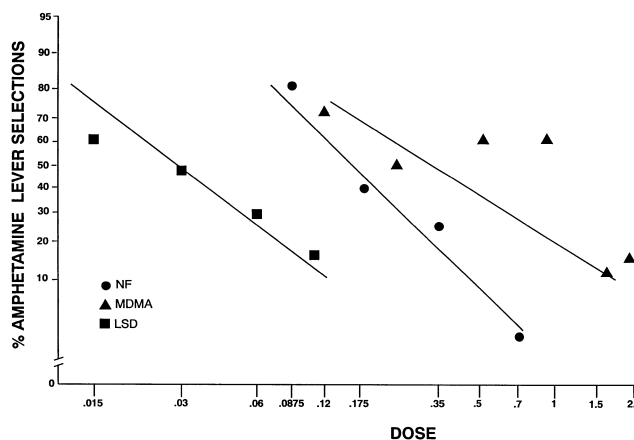
press a second lever. Rats, nevertheless, have also been trained to discriminate between one drug and another drug and have been shown to acquire the training criterion faster than those animals trained between drug and its vehicle (33, 47). Previous work from this laboratory (34) indicated that when two drugs are used to train rats, i.e., the psychostimulant amphetamine vs. the central depressant pentobarbital, novel drugs such as nicotine and fenfluramine can be administered in an effort to test the effects of these drugs upon, (what might be viewed as) a stimulant-depressant continuum. In addition, animals can be trained to differences in physiological states, such as food satiation vs. food-deprivation and, subsequently, tested with drugs along this "hunger" continuum (37). In the present study, rats were trained, as previously shown possible (6), to discriminate between two known appetite-suppressant compounds, amphetamine and norfenfluramine, the first active metabolite of fenfluramine, in a two-lever, food-motivated behavioral paradigm. The previously-cited work employed a dose of amphetamine of 0.8 mg/kg and that of norfenfluramine of 1.4 mg/kg, both administered intraperitoneally. The present study indicated that half of each dose not only can produce discriminative performance at criterion levels but, in fact, produced this in fewer sessions, i.e., the STC1 in the present study occurred at the end of a mean (S.D.) of 16.2 ± 3.34 sessions, whereas it took 9 animals trained to the high doses of 1.4 mg/kg norfenfluramine vs. 0.8 mg/kg amphetamine 23.9 ± 2.6 sessions to reach criterion performance. Similarly, the ED_{50} values for both norfenfluramine and amphetamine in the present study were lower than previously shown in rats trained to the higher doses (6). Nonetheless, a different line of rats, the N/Nih, was used in the current experiment, whereas Sprague-Dawley rats were used previously.

Analysis of the dose-response lines indicated similar ED_{50} values after administration of various doses of both amphetamine and norfenfluramine suggesting a relatively equivalent saliency between the two drug cues. This was, furthermore, exemplified by the administration of saline, which produced a quantal measurement of 55%, and the co-administration of the 0.7 mg/kg norfenfluramine plus 0.4 mg/kg amphetamine, producing a quantal measurement of 52.9%. Thus, the qualitative characteristics of norfenfluramine discrimination are different than, yet equivalent to, those of amphetamine discrimination as both are known anorectic drugs. The most

parsimonious explanation for this is that even though these drugs act on different neurotransmitters in the brain, they are being trained at relatively equivalent discriminable doses at the 20 min post-administration interval used in this study. The co-administration of norfenfluramine and amphetamine produced more amphetamine-like responding at the earlier test time of 10 min post-injection, with more norfenfluramine-lever selection at later times; this effect may simply be attributed to pharmacokinetics. In effect, temporal studies using amphetamine trained in a discriminative task indicated a rapid onset, with a maximal effect of 15-30 min, as well as minimal or absence of discriminative performance at 120 min post-injection (25,35). In contrast, the one study that employed norfenfluramine as a drug to control discriminative performance (6), observed that at 240 min post-administration, the animals chose the norfenfluramine-appropriate lever on 80% of the tests. Thus, it appears that norfenfluramine is a drug of longer duration than is amphetamine and, therefore, as seen in Fig. 2, the animals are capable of discriminating it to a greater extent as the post-injection interval increases.

The tests with saline, as used to assess stimulus saliency, also exhibited relative equality, inferring that the animals can divide their responding relatively evenly between the two interoceptive cues allowing responses to be equivalent. The administration of saline to animals who have been trained to discriminate the effects of one drug vs. the effects of a second drug produces, what might be thought of as, a third internal state, i.e., one that is neither dopaminergic nor serotonergic. This "non-ergic" state would differ in quality from the drug-states trained. The equipotence of the discriminative stimulus control of the trained dose of norfenfluramine, used in conjunction with the training dose of amphetamine, provides discriminative control of relatively even distribution precluding either of the two drugs' stimulus overshadowing the other. The combined administration of the training doses of both drugs is a different case in point than administering saline in that a mixture of the quantitative and qualitative effects of both cues are available to the animal. By having rough equipotence, it allowed for a relative random responding both in terms of percentage of animals selecting a particular lever (quantal %) and quantitative responding.

The ingenious use of a three-choice discrimination procedure in pigeons has been used to show the differences between *d*-amphetamine, fenfluramine and saline (10). In this paradigm, not only can serotonergic agonists be shown to be fenfluramine-like, but compounds with complex discriminative stimulus properties can be investigate, as well as drugs with neither drug-appropriate stimulus effects. Results using this apparatus indicated exclusive discrimination between fenfluramine and amphetamine in that low doses of either resulted in saline responding. This three-choice discrimination procedure has also been recently used in rats (13) which reiterates the inherent advantage to the three-lever design over that of the drug-drug design used herein. This resides in the fact that when the drugs used to train the subjects upon a "continuum", as appears to exist in the present study, then the trained drugs, in combination, where Drug 1 vs. Drug 2 vs. saline has been trained, will produce largely saline-lever responding (15). However, when the training drugs produce independent states with no apparent site/mechanism in common, then the combination of the two drugs in the three-lever task produces responding on both drug levers but no responding on the saline-trained lever (14).

The dose-response relationships of the training drug *d*-amphetamine, as well as the discriminative performance to

numerous doses of both methcathinone and 7-OH-DPAT, are depicted in Fig. 3. Methcathinone is a phenylisopropylamine that has recently been placed on the DEA Schedule I category as it is produced by clandestine laboratories and has been identified as an illegal drug of abuse. It has been shown to produce positive reinforcing effects in self-administration experiments in baboons (26). In rats, cocaine has been shown to generalize to methcathinone which was, in fact, shown to be approximately six times as potent (50). In amphetamine-trained animals, methcathinone was found to be both more potent than its parent-compound, cathinone, as well as amphetamine. Thus, this suggested to the authors (17) that methcathinone is to cathinone, much what methamphetamine is to amphetamine, in potency and abuse potential.

The testing of a non-drug of abuse with dopamine D_3 agonism effects, viz., 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetraline (7-OH-DPAT;30), allowed evidence as to it producing amphetamine-like effects at the highest dose (0.5 mg/kg) tested. Of interest, is the recent finding that 7-OH-DPAT produces rewarding effects in the place conditioning paradigm when tested at 0.5-5.0 mg/kg (31). In addition, this D_3 receptor-selective ligand has been shown to produce hypermotility (2), whereas 0.1 mg/kg 7-OH-DPAT has been successfully employed to produce drug discriminative control in the rat (32). The contribution of dopamine D_3 receptors to the euphorigenic effects of both methcathinone and amphetamine will be of future scientific interest as the generalization from amphetamine to methcathinone and 7-OH-DPAT occurred in a dose-responsive manner with slopes that were parallel (Fig. 3; $p < 0.05$). This observation may suggest that all three agents are working at the same site/mechanism of action (28). More recently, other D_3 receptor ligands have been observed to produce cocaine-like discriminative effects in the rat when administered in doses that produce response deficits (1).

Generalization experiments using two drugs thought to be serotonergically mediated, LSD and MDMA, indicated a dose-response effect in the norfenfluramine-amphetamine trained rats; this allowed for the highest dose of each drug to generalize to the norfenfluramine stimulus cue. Previous work indicated that both fenfluramine and LSD would generalize in animals trained to discriminate the serotonin receptor agonist m-chlorophenylpiperazine from saline (8). In fenfluramine-trained rats, LSD produces partial substitution (45) and fenfluramine produces intermediate results in animals trained with LSD (46). The possibility exists that norfenfluramine, the first active metabolite of fenfluramine, may, in fact, be more potent and act not only by releasing pre-synaptic serotonin, but also as a post-synaptic serotonin agonist (21,24). In fact, using the same operant discrimination task, norfenfluramine was shown to be approximately twice as potent as fenfluramine and to produce generalization to LSD (27).

Likewise, the alleged (12,40) serotonin neurotoxin, 3,4 methylenedioxymethamphetamine (MDMA), has been shown to promote the release of serotonin and block its reuptake; the same mechanism as proposed for fenfluramine (18). The possibility also exists that MDMA releases both serotonin and dopamine (18,36). In the present experiment, the highest two doses of MDMA administered, 1.5 and 2.0 mg/kg, produced greater than 80% of responses upon the norfenfluramine-appropriate lever. Careful examination of the dose-response effects of MDMA (Fig. 4) indicates an interesting phenomenon in that, whereas the other drugs, norfenfluramine and LSD, have clear and linear dose-effect discriminative responses along the amphetamine-norfenfluramine continuum, there are, at least, three doses of MDMA, 0.25, 0.5 and

1.0 mg/kg, each producing a percent amphetamine discrimination that would indicate approximately 50% discrimination; as much as if the animal had been given the combination of norfenfluramine and amphetamine. MDMA administration produced a situation that allowed for approximately equivalent responding on one lever corresponding to responses linked to amphetamine with an equal number of responses upon the lever linked to norfenfluramine; this has been shown to occur when chlorphentermine is administered to rats trained to discriminate between amphetamine and fenfluramine (48). Thus, both drug stimuli complexes apparently were equally attended to by the animals after these three doses of MDMA. This would support the suggestion that low doses of MDMA are more dopaminergic, whereas higher doses are more serotonergic, with intermediate doses producing both effects (36).

Although the use of a drug vs. drug vs. saline, three-lever, paradigm offers distinct advantages (above), the drug vs. drug model, using drugs of different purported mechanisms of action, may also allow for definitive information regarding generalization to novel drugs. This is especially true when a serotonergic vs. a dopaminergic drug is trained such as in the present experimentation, to allow for the animal's ("detection") behavior to ascertain the putative neurotransmitter mediation of the quantitative and qualitative effects of the drugs tested for their interoceptive cueing properties. This is especially important in that some drugs of abuse, such as MDMA (36) and cocaine, as well as its congeners (4), may affect both serotonergic and dopaminergic neurons in the central nervous system.

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