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Drug Discrimination and Cross Generalization Between Two Methylxanthines[1]

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MODROW, H. E. AND F. A. HOLLOWAY. *Drug discrimination and cross generalization between two methylxanthines.* PHARMACOL BIOCHEM BEHAV **23**(3) 425–429, 1985.—Twenty-one rats were trained to discriminate 32 mg/kg caffeine from saline in a two-lever drug discrimination task (variable ratio) while another ten rats were trained to discriminate 56 mg/kg theophylline from saline. For each group, dose-effect curves (% drug-lever responses and overall response rate) were obtained for both caffeine and theophylline. Significant dose-related generalization of each training drug was found for both the caffeine- and theophylline-trained rats. Concomitant dose-related decreases in overall response rate also were apparent. Similar dose-related effects were seen with cross-generalization tests for various doses of the other xanthine. The nature of the training session preceding the test session was found to have an effect on discrimination performance at intermediate test doses. Drug appropriate responding was higher and overall response rate was lower after saline- than after drug-training days. Such data may suggest the possibility of short-term tolerance to caffeine's cue. That the discriminative cue was specific to the xanthines was shown by the lack of generalization seen after either amphetamine or metrazol.

Drug discrimination Caffeine Theophylline Amphetamine Metrazol Methylxanthines Training procedures

ALTHOUGH typically classified as a psychomotor stimulant [6,17], caffeine can produce both rate-increasing and rate-decreasing effects on operant behavior [1, 5, 9–12] and locomotor activity [7, 8, 18]. Further, like other psychomotor stimulants, caffeine can function as a discriminative cue in the rat [14, 15, 19]. In the drug discrimination paradigm, rats are trained to respond differentially to drug and saline injections. This procedure is used to assess the similarity of stimulus properties of other drug/dose combinations to the stimulus produced by the training drug dose (see [2,16]). With a caffeine training dose of 60 mg/kg, Winter [19] found generalization to another xanthine, aminophylline, and partial generalization to amphetamine. Modrow and colleagues [14] found a training cue produced by 32 mg/kg caffeine generalized to theophylline but not to d-amphetamine, nicotine, methylphenidate, or thyrotropine-releasing hormone. Caffeine and theophylline are methylxanthines. Theophylline is a metabolite of caffeine and has one fewer methyl group [17]. The relative efficacy and/or potency of caffeine and theophylline varies with the specific process being examined or measurement procedure used [7, 17, 18].

Modrow and colleagues [14] found minor differences in caffeine discrimination performance as a function of test conditions. When generalization tests were given 24 hours after a saline training day, doses of caffeine as low as 10 mg/kg promoted caffeine-lever responding at or above criterion level. Tests given 24 hours after a caffeine-training session required nearly 20 mg/kg caffeine to produce criterion performance. Since a subsequent study indicated that after injections of 32 mg/kg caffeine, caffeine-lever responding declined more rapidly than the caffeine plasma levels [15], the differential caffeine dose-effect curves after saline or drug training days may reflect some sort of acute tolerance process.

The specific objectives of this study were to examine: (a) the acquisition of caffeine-saline and theophylline-saline discrimination using a two-lever operant task for food reward; (b) the generalization of the training drug cue to other doses of the training drug; (c) cross-generalization to the other xanthine; and (d) differences in the dose-effect curves after saline or drug training days. During all tests, overall response rate and % training drug lever responses were meas-

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ured. The measurement of both response rate and drug discrimination provided an assessment of possibly related drug effects. That is, if generalization to the other xanthine were based on simple psychomotor stimulation, we would then expect doses which produce equal stimulation to be equally discriminable. If, however, the discriminability of the two drugs was not related to specific levels of stimulation, doses of theophylline and caffeine producing equivalent rates of lever pressing would not be equally discriminable.

METHOD

Subjects

Thirty-one adult, male Sprague-Dawley rats (Sasco, Inc.) weighing between 350 and 500 grams were used in this study. Prior to training, all animals were gradually reduced to approximately 85% of their free-feeding weight. After beginning the study, the animals were allowed to gain 10% of their initial free-feeding weight every two months, up to 500 grams. Once the latter weight was reached, the rats were maintained at that weight for the remainder of the study. All rats were individually housed in hanging wire-mesh cages with ad lib access to water and were fed lab chow 2 hours after their daily operant session. The animal quarters were kept on a 12:12 light-dark cycle with light onset at 0800.

Apparatus

Four identical (Lafayette, model 800L), two-lever operant chambers were used. Food pellets (45 mg, BioServ, Inc.) were delivered by a Lafayette pellet dispenser. Each test apparatus was enclosed in a Lafayette sound-attentuating chamber. All programming and recording was accomplished with a Rockwell Aim-65 microprocessor system.

Procedure

A total of 21 rats were trained to discriminate intraperitoneal (IP) injections of 32 mg/kg of caffeine (free-base) from saline; 10 rats were trained to discriminate 56 mg/kg theophylline (free-base) from saline. These caffeine and theophylline doses were judged to be approximately equivalent in terms of concentrations detected in brain (Carney and Christensen, unpublished observations). The methylxanthines (Eastman Kodak Chemicals) were dissolved in normal saline. Both drug and saline solutions were administered in a volume of 1 ml/kg body weight.

As soon as the animals achieved 85% of their free-feeding weights, they were trained to lever press for food reinforcement on a fixed ratio-1 schedule (FR-1). Each animal was trained and tested at the same time during the light cycle each day (from 9:00 a.m. to 3:00 p.m.). One-half of the animals were initially trained to press the right lever; the other half, the left lever. Upon completion of the initial shaping procedure (i.e., at least 50 reinforcements within a 30 min session), Phase 1 of drug discrimination training began and lasted 12 days with daily sessions of 11 minutes.

Phase 1 training was designed to develop schedule-controlled lever responding on the initially trained lever after IP injections of normal saline (No drug, lever N), and then on the other lever after IP injections of the training drug (Drug, lever D). All rats received an IP injection of normal saline or the training drug 20 min before placement in the operant chamber, and lever presses on the appropriate lever resulted in schedule-controlled delivery of food pellets. During Phase 1 training, six drug and six saline sessions were given with the reinforcement schedule for all sessions progressively shifting from fixed interval (FI) 1, to FI3, FR3, FR5, variable ratio (VR) 5–10, and then to VR 5–15. This terminal schedule produces a sufficient level of responding to permit simultaneous assessment of the drug's stimulus properties and its effect on rate during test sessions (see below). Half of the caffeine-discriminating and theophylline-discriminating animals received six days of training (all schedules) on the saline lever prior to training on the drug lever; the other half of each group was trained at each schedule alternatively on both levers. No differences in acquisition of discrimination performance were found between the latter procedures.

Phase 2 training began immediately after the completion of Phase 1. All training sessions consisted of an 11-minute session. During the first minute of each session no reinforcement was available (extinction); during the last ten minutes reinforcement was available on the VR 5–15 schedule for injection-appropriate lever responding (i.e., N or D). A double-alternation sequence of training under N and D conditions was used for the remainder of the experiment. With sessions run only 5 days a week, the sequence repeated itself every 4 weeks. All rats continued on the latter training regimen until they reached a criterion of 8 out of 10 sessions with 70% or greater drug-appropriate responding during the initial 1-minute extinction period. Sessions to criterion was designated as the number of sessions from the beginning of Phase 2 training to the first session of the 10-day criterion sequence. After reaching criterion, rats received additional training sessions until an asymptotic discrimination performance criterion was met (i.e., 10 sessions of 70% or better discrimination and all sessions within 10% of the mean).

Drug discrimination test sessions lasted 2 minutes and no reinforcement was available. If criterion level performance was maintained, test sessions were given on Tuesdays and Fridays. The latter test schedule was superimposed on the double-alternation training sequence and repeated every four weeks. Every test drug/dose combination was assessed after both N and D training days. All caffeine-trained rats received a random sequence of saline and several doses of caffeine (1, 5, 10, 15, 20, and 32 mg/kg) followed by theophylline (5, 10, 20, 32, 44, and 56 mg/kg). All theophylline-trained rats received a random sequence of saline and several doses of theophylline (1, 5, 10, 20, 32, 44, and 56 mg/kg) followed by caffeine (5, 10, 15, 32, 44, and 56 mg/kg).

Following completion of the xanthine discriminability testing, both the caffeine and the theophylline-trained animals were tested for generalization to saline and four doses (calculated as the salt) of d-amphetamine sulfate (0.05, 0.1, 0.5, and 1.0 mg/kg for caffeine-trained animals and 0.1, 0.5, 1.0, and 2.0 mg/kg) for theophylline-trained animals). In addition, the theophylline-trained animals were tested for generalization to metrazol (5.0, 10.0, and 20.0 mg/kg).

Separate two-way repeated measures analyses of variance (ANOVA) were performed on overall response rate and % drug-lever responses. The two within-subjects factors were dose and the nature of the injection (drug or saline) prior to the training session the day before each test session. Post-hoc comparisons among doses were obtained with the Duncan's Multiple Range Test ($p<0.05$).

RESULTS

Generalization Tests for the Training Drug

Rats trained on the caffeine cue began criterion performance after a mean of 14.9 days. After the stability criterion

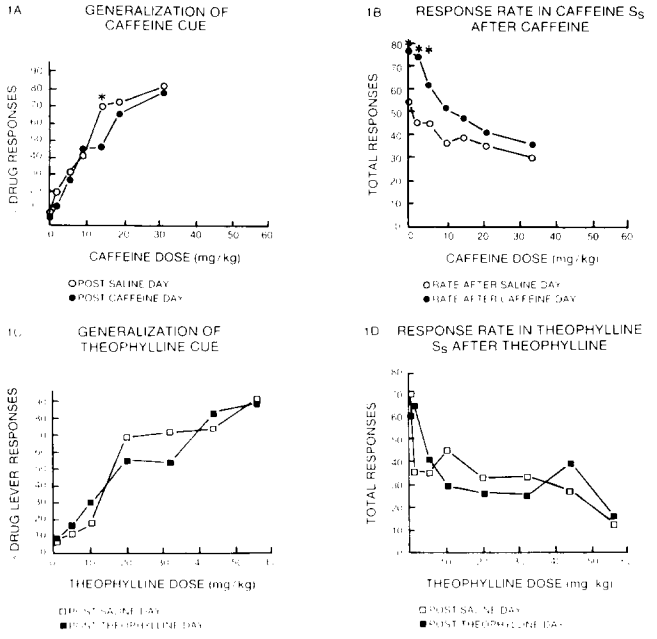

FIG. 1. Generalization tests for training drug. Effects of various test doses of caffeine on caffeine-trained animals: 1A—% caffeine-lever responses and 1B—total response rate. Effects of various test doses of theophylline in theophylline-trained animals: 1C—% theophylline-lever responses and 1D—total response rate. Probabilities associated with tests after saline or drug training days (ANOVA Simple Effects test): *=$p<0.05$, **=$p<0.01$.

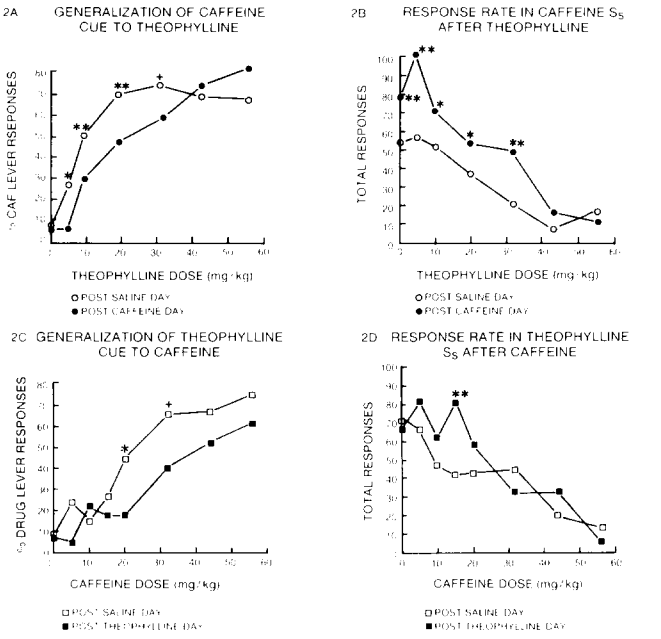

FIG. 2. Cross-generalization tests. Effects of theophylline on caffeine-trained animals: 2A—% caffeine-lever responses and 2B—total response rate. Effects of caffeine on theophylline-trained animals: 2C—% theophylline-lever responses and 2D—total response rate. Probabilities associated with tests after saline or drug training days (ANOVA Simple Effects test): +=$p<0.10$, *=$p<0.05$, **=$p<0.01$.

was attained, testing on various doses of caffeine began. Figure 1A illustrates the % caffeine lever responses (N=21). After both caffeine and saline training days, significant dose-related generalization to the caffeine cue was found (overall: $F(6,120)=47.5$, $p<0.01$). The % caffeine-lever responses was overall significantly higher after saline than after drug training days, $F(1,120)=5.81$, $p<.005$, but at individual test doses this effect was only significant at 15 mg/kg ($p<0.05$). The response rate dose-effect curves from the same 2-minute test sessions are illustrated in Fig. 1B. Increasing doses of caffeine decreased the response rate after both post-caffeine and post-saline tests (overall: $F(6,120)=6.34$, $p<0.01$). Significantly lower response rates were found, $F(1,120)=7.41$, $p<0.05$, after saline training session than after caffeine training sessions.

The animals trained to discriminate theophylline from saline began baseline criterion performance after a mean of 13.7 days. The theophylline dose-effect curves are shown in Fig. 1C (N=10). There was a significant dose-related increase in theophylline-lever responding as the theophylline dose was increased (overall: $F(7,163)=30.2$, $p<0.001$) after saline or theophylline training days, but no significant differences between the latter curves were obtained. Figure 1D illustrates the response rate of the theophylline-trained animals during the theophylline tests. Under both test conditions, significant dose-related decreases in responding were found (overall: $F(7,63)=4.16$, $p<0.01$). Again, no overall difference in the two dose-effect curves was found.

Cross-Generalization Tests

Following completion of the dose-effect curve for caffeine, caffeine-trained rats were tested for generalization of the caffeine cue to theophylline (Fig. 2A, N=20). Significant dose-related increases in caffeine-appropriate responding with increasing amounts of theophylline were found for both dose-effect curves (overall: $F(6,114)=35.2$, $p<0.01$). Overall caffeine-lever responding was significantly higher after saline than after drug training days, $F(1,19)=9.8$, $p<0.01$, and also after test doses of 5, 10, and 20 mg/kg theophylline ($p<0.05$). After both saline and caffeine training days, the total number of responses emitted by caffeine-trained rats (Fig. 2B) declined significantly as the theophylline test dose increased (overall: $F(6,114)=23.8$, $p<0.001$). Overall, response rates were significantly lower after saline- than after caffeine-training days, $F(1,19)=28.6$, $p<0.01$.

When theophylline-trained animals were tested with caffeine (Fig. 2C. N=9), significant dose-related changes in theophylline-lever pressing were produced under both test conditions (overall: $F(7,56)=10.1$, $p<0.001$). Higher levels of theophylline-lever responding were found after saline than after drug training days, $F(1,8)=8.34$, $p<0.05$. No significant difference in total responses between the two test conditions was found (Fig. 2D), with both showing a significant decrease as the caffeine dose was increased (overall: $F(7,56)=7.75$, $p<0.01$).

Amphetamine and Metrazol Tests

Amphetamine produced similar and significant dose-related changes on overall response rate during test sessions for both the caffeine-, $F(4,12)=8.20$, $p<0.01$, and the theophylline-trained animals, $F(4,24)=9.02$, $p<0.01$. Relative to saline tests, lower amphetamine doses (0.05 and 0.1

mg/kg) significantly increased, and higher doses (1.0 and 2.0 mg/kg) decreased, response rate. Amphetamine failed to generalize to the training cue in both the caffeine and the theophylline discriminating animals. Finally, no dose of metrazol produced theophylline-lever responding significantly different from that produced by saline, although the highest dose (20.0 mg/kg) yielded significantly fewer lever presses than either saline or the other doses ($p<0.05$).

DISCUSSION

Not surprisingly, rats learned to discriminate both methylxanthines from saline. In addition, the cue generalized from one methylxanthine to another. This is an extension of our previous findings [14] showing that caffeine-trained animals would generalize to theophylline. This study indicates that while theophylline-trained rats will generalize to caffeine, they do not generalize to other stimulants (e.g., amphetamine and metrazol). However, when one compares the caffeine dose-effect curve for the two groups of animals, some differences are observed. The theophylline-trained animals required much higher doses of caffeine to demonstrate responding on the drug lever. While the caffeine-trained animals responded on the drug lever approximately 60–70% of the time after an injection of 20 mg/kg caffeine, after the same dose of caffeine, the theophylline-trained animals responded less than 50% of the time on the drug lever. The differential responding was not due to less sensitivity to the drug because both groups had similar response rates at the same doses. It is probably due to differences in relative training doses in the caffeine-trained and theophylline-trained animals.

This study further replicates our previous finding of differential drug discrimination performance on tests occurring after either a drug or a saline training day [14]. Generally, the percent of drug-appropriate lever responding was higher and the overall response rate was lower on test sessions following a saline training day than on tests following a drug training day. This effect occurred primarily when the training drug was caffeine, regardless of whether the test drug was caffeine or theophylline. In theophylline-trained rats, this effect was apparent only with caffeine tests. The principal locus of differences in discrimination lies in the intermediate range of doses tested.

Colpaert and colleagues noted a larger degree of intra-subject [3] and inter-subject [4] variability when testing at the lower discriminable doses of a training drug. The present results suggest that one factor contributing to variability in drug discrimination performance is the nature of the prior training session on subsequent test sessions at intermediate doses. The present study was not designed to address mechanisms mediating the differences in discrimination performance after saline or drug training sessions. However, one possible explanation for these results may be the presence of short-term tolerance or tachyphylaxis to the effects of methylxanthines [7, 13, 15]. A reduced sensitivity to caffeine's effects on locomotor activity appears within 20 minutes [7]. Further, drug-appropriate responding is significantly reduced on tests for the caffeine cue with injections more than two hours prior to the test sessions [15]. The present report and an earlier study [13] suggest that the duration of reduced sensitivity to the drug cue can be moderately long, lasting at least 24 hours but less than 48 hours. An alternative explanation for the latter data may be that the animal contrasts the present drug state with that present during the previous training session. This hypothesis would explain the higher level of drug-lever responding on tests following a saline training day. However, it would not be able to explain any differences in response rates between the two tests. In order to differentiate between the two hypotheses a third type of test and additional research would have to be done. Following a saline training session, the rats would be injected with the training drug. The next day they would be tested with a moderate dose of that drug. If the contrast explanation is true, the responses would be predominantly on the drug lever. If, however, the tolerance explanation were true, responses would be primarily on the saline lever.

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