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Female Rats That Rapidly Acquire a *d*-Amphetamine Discrimination Generalize More to *d*-Amphetamine

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TOMIE, A. AND E. M. MOSAKOWSKI. *Female rats that rapidly acquire a* d-*amphetamine discrimination generalize more to* d-*amphetamine.* PHARMACOL BIOCHEM BEHAV **54**(4) 699–703, 1996.—Female Long–Evans rats were trained to discriminate *d*-amphetamine (0.8 mg/kg) vs. saline in a food-reinforced two-lever operant task. Fifteen rats (fast group) acquired the discrimination rapidly, achieving criterion (eight correct choices within ten sessions) during the first 10 sessions (mean sessions to criterion = 10.0). The remaining eight rats (slow group) made at least three errors during the first 10 sessions and required additional drug discrimination training to achieve criterion (mean sessions to criterion = 15.9). When a rat had completed a minimum of 30 two-lever discrimination training sessions and, in addition, provided 10 correct choices within 10 sessions, generalization testing with lower doses of *d*-amphetamine was initiated. The fast group made more *d*-amphetamine–appropriate choices during the generalization test and generalized more to the 0.2 mg/kg *d*-amphetamine test dose than did the slow group, though the number of training sessions prior to generalization testing was comparable across groups. Results suggest that when the training drug is easily discriminated, fast learners generalize more, even when groups receive comparable amounts of training prior to generalization testing, and this effect is observed in female rats.

Drug Discrimination Rats Amphetamine Sensitivity Generalization Individual Differences

DRUG discrimination procedures have been widely used to characterize drugs on the basis of their stimulus properties. In this procedure, subjects are injected presession with either drug or vehicle, and then reinforcement during the session is made contingent on injection-appropriate choice behavior (e.g., left-lever responding is reinforced following drug administration while right-lever responding is reinforced following vehicle administration). Investigators have reported that individual differences between subjects in the number of sessions required to establish reliable discriminative control by the drug cue is substantial (1,3,5,6,8,9). Some have suggested that these differences may predict the tendency to provide drug-appropriate choices when subjects are administered lower doses of the training drug during postacquisition generalization testing (1,3,5,8,9); however, the relationship between acquisition speed and generalization remains unclear. Some studies have reported that subjects that acquired the drug discrimination rapidly generalized more to lower doses of the training drug. These studies have employed male rats trained to discriminate apomorphine (8), or delta-9-tetrahydrocannabinol (5), or rhesus monkeys trained to discriminate pentobarbital (9).

These studies reporting more generalization by fast learners share in common several features. Among them, fast learners achieved the discrimination acquisition criterion with relatively few sessions of drug discrimination training, suggesting that the training drug was readily discriminated. In addition, generalization testing was initiated on the session after the discrimination acquisition criterion was achieved. The fast learners, therefore, received fewer sessions of discrimination training prior to the start of generalization testing relative to the slow learners.

There is one study where rats that more rapidly learned to discriminate (cocaine vs. saline) tended to generalize less to lower test doses of the training drug (3), although variability within the groups of fast and slow learners was extreme and the effect failed to achieve statistical significance. In that study, the subjects were female rats and the training drug was not readily discriminated. Most of the subjects that achieved the discrimination criterion more rapidly required several dozen sessions of drug discrimination training to do so. In addition, subjects were given postcriterion discrimination training prior to the start of generalization testing, such that all subjects,

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regardless of how quickly they achieved criterion, received a total of 60 sessions of drug discrimination training prior to the start of generalization testing.

The present study addresses the discrepancy in results obtained across previous studies by providing groups of fast and slow drug discrimination learners a comparable number of sessions of drug discrimination training with a readily discriminated dose of the training drug prior to the initiation of generalization testing. Whereas studies employing male rats have reported more generalization by fast learners (5,8), and the only study employing female rats has reported the opposite result (3), female rats were employed in the present study. In addition, whereas the study employing female rats had evaluated only quantal trial choice measures (3), and studies employing male rats had employed either quantal trial choice measures (8) or measures of the distribution of responding within the choice trial (5), the present study will evaluate both percent choice and percent response measures of discrimination and generalization.

METHOD

Subjects

Subjects were 23 female Long–Evans rats weighing 210–290 g at the beginning of the study. Rats were housed individually in suspended stainless steel cages in a colony room with a 12 L:12 D (on 0800 h) cycle. Rats had continuous access to water in their home cages and were maintained at 80% of their free-feeding body weights by limiting their access to Purina Rat Chow that was provided, as needed, 30 min after completion of the session.

Apparatus

Four Plexiglas operant conditioning chambers (23 cm × 23 cm × 21 cm) for rats, with stainless steel grid floors were enclosed in sound-attenuating, ventilated outer casings. One house light (GE 1821) was mounted directly above the operant chamber, on the ceiling of the outer hull. The front panel of each chamber was equipped with two retractable levers (BRS/LVE #RRL/005), each mounted 8.5 cm above the floor, and 7 cm off to either side of the centerline. A food receptacle was mounted on the centerline of the front panel, 3 cm above the floor. Operation of a PDC/PPD pellet dispenser delivered 45 mg Noyes pellets into the food receptacle. Session events were programmed and data were collected by interfacing each chamber with standard 24 volt relay equipment and Commodore 64 microprocessors.

Procedure

Throughout the experiment, training sessions were conducted five or six days per week, and 15 min before the start of each session, each rat received an intraperitoneal (IP) injection of either 0.8 mg/kg *d*-amphetamine (drug session) or 0.9 percent saline solution (saline session). The training dose of *d*-amphetamine (0.8 mg/kg) has been reported to be readily discriminated by rats trained on drug discrimination procedures employing food reinforcement (4,7). The daily injection sequence followed a random schedule within blocks of 10 sessions with the following two constraints: (a) a particular treatment was not administered for more than two consecutive sessions, and (b) a total of five drug and five saline injections were included within each block of 10 sessions. After each session, response levers were swabbed with alcohol to eliminate olfactory cues (2). Subjects were randomly assigned to operant chambers. For two of the four boxes, the right lever was drug appropriate and the left lever was saline appropriate, while the reverse designations were in effect in the remaining two chambers.

Single-Lever Response Training

During each session of single-lever response training the subject was provided with only the injection-appropriate lever, and was reinforced with food for pressing the lever according to a fixed-ratio (FR) schedule. The FR requirement was gradually increased, across sessions, from FR1 to FR10. Two-lever drug discrimination training was initiated when a subject had made at least 150 responses on the FR10 schedule during a drug session and at least 150 responses on the FR10 schedule during a saline session.

Two-Lever Drug Discrimination Training

Each two-lever drug discrimination training session began with the insertion of the two response levers into the chamber. If the subject made a total of 10 responses on the injection-appropriate lever before doing so on the alternative lever, the choice was recorded as correct, regardless of the number of responses (0 to 9) completed on the injection-inappropriate lever. Following a correct choice, a 45 mg Noyes pellet was delivered and both response levers were retracted from the chamber. One second later only the injection-appropriate lever was reinserted into the chamber and the subject was provided with the opportunity to earn an additional nine reinforcers on a FR10 schedule. If, on the other hand, the subject completed the FR10 requirement on the injection-inappropriate lever, then both levers were retracted, and 1 s later, the injection-inappropriate lever was reinserted into the chamber during a 5-min period of extinction. The extinction period was followed by a 30-s intertrial interval. Regardless of the correctness of choice on the first trial of each session, a total of three choice trials were scheduled during each session. Thus, it was possible for each subject to procure a maximum of 30 food presentations per session. The procedures used during the second and third choice trials were identical to the first. During the 30-s interval between trials the houselight located in the ceiling was turned off and both levers were retracted. The session was terminated when the subject completed three trials or when 30 min had elapsed from the beginning of the session. Only the data from the first choice trial of each session was used to evaluate discriminative control by the drug. The data recorded was first trial choice (drug-appropriate or saline-appropriate), which was defined as the lever on which the subject first completed ten responses. The first generalization test was conducted on the session after a subject received a minimum of 30 two-lever drug discrimination training sessions, and, in addition, completed ten correct first trial choices in 10 consecutive sessions.

Generalization Testing

Each generalization test consisted of only one choice trial, and reinforcement was delivered regardless of the lever on which the subject first completed 10 responses. The first generalization test was preceded by a presession injection of saline (0.0 mg/kg *d*-amphetamine). All subsequent generalization tests were conducted in lieu of a discrimination training session on the session after the subject achieved a criterion of three correct first trial choices in three consecutive training sessions.

Each time the subject achieved criterion, one of four test doses of *d*-amphetamine was administered. Each test dose of *d*-amphetamine was administered once, and in order of descending doses (i.e., 0.8, 0.4, 0.2, then 0.1 mg/kg).

Drugs

d-Amphetamine sulfate (supplied by NIDA) was dissolved in 0.9% saline to a volume equivalent to 1.0 ml/mg. All drug doses refer to the total salt.

Data Analysis

Sessions to criterion scores as well as other indices related to training experience were computed for each subject. Effects of Groups were assessed by one-way analysis of variance (ANOVA) using SAS-GLM procedure. Analyses of discrimination functions were performed on percent correct choices on the first trial of each session and on percent correct responses on the first trial of each session. Analyses of generalization functions were performed on percent drug-appropriate choices and percent drug-appropriate responses on the single choice trial provided during each generalization test session. The effects of groups and blocks or groups and doses were assessed by mixed-design, two-way, repeated-measures analysis of variance (MANOVA) using SYSTAT (Version 5 for Windows). Planned pairwise posthoc comparisons, using an alpha level of 0.05, provided comparisons between individual points.

RESULTS

Fifteen subjects (fast group) acquired the *d*-amphetamine vs. saline discrimination rapidly, achieving the acquisition criterion of eight correct first trial choices within 10 consecutive sessions during the first 10 sessions (mean sessions to criterion = 10.0). The remaining eight subjects (slow group) made at least three errors during the first trial of the first 10 sessions and required additional drug discrimination training sessions to achieve criterion (mean sessions to criterion = 15.9).

Mean percent correct first trial choices for each group during the first 20 sessions of two-lever discrimination training are plotted as a function of blocks of two trials (see Fig. 1). Mean percent correct first trial choices for the fast group on block one (sessions 1–2) was 56.67 and on each of the remaining nine blocks (sessions 3–20) was greater than 86.67. Mean percent correct first trial choices for the slow group on block one was 62.50 and did not exceed this value until block four (sessions 7–8). Mixed-design, two-way, repeated-measures MANOVA revealed a reliable main effect of groups, $F(1, 21) = 32.98$, $p < 0.01$, a reliable main effect of blocks, $F(9, 189) = 7.48$, $p < 0.01$, and a reliable groups by blocks interaction, $F(9, 189) = 4.49$, $p < 0.01$. Planned pair-wise post hoc comparisons (alpha = 0.05) revealed that the effect of groups was significant on blocks 2, 3, 5, and 7.

Mean percent correct first trial responses for each group during the first 20 sessions of two-lever discrimination training are plotted as a function of blocks of two trials (see Fig. 2). Mean percent correct first trial responses for the fast group on block one (sessions 1–2) was 59.63 and on each of the remaining nine blocks (sessions 3–20) was greater than 77.09. Mean percent correct first trial responses for the slow group on block one was 58.19 and did not exceed this value until block four (sessions 7–8). Mixed-design, two-way, repeated-measures MANOVA revealed a reliable main effect of groups, $F(1, 21) = 29.92$, $p < 0.01$, a reliable main effect of blocks, $F(9, 189) = 8.49$, $p < 0.01$, and a reliable groups by blocks

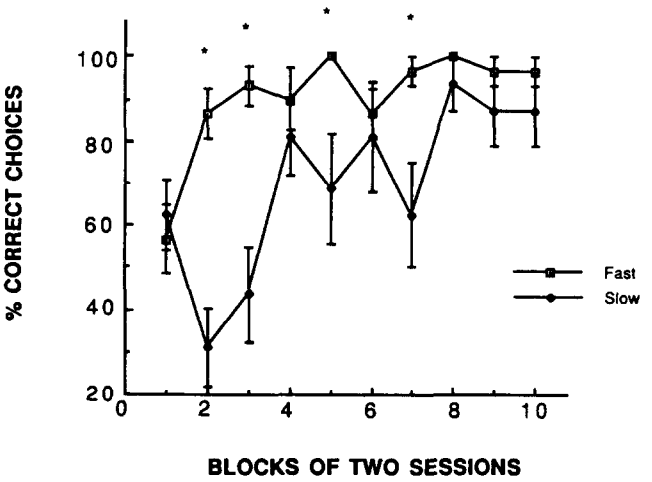

FIG. 1. Mean percent correct first trial choices for the fast group and the slow group as a function of blocks of two sessions. Vertical bars represent the standard error of the mean (SEM). The asterisk (*) indicates that the effect of groups was statistically significant at the 0.05 level of confidence.

interaction, $F(9, 189) = 2.98$, $p < 0.01$. Planned pair-wise post hoc comparisons (alpha = 0.05) revealed that the effect of groups was significant on blocks 2, 3, 4, 5, and 7.

During blocks 11–20 (sessions 21–40) mean percent correct choices and mean percent correct responses for the fast group and the slow group was at least 80%. One-way ANOVA on percent correct choices on block 20 revealed no reliable effect of groups $F(1, 21) < 1$, and one-way ANOVA on percent correct responses on block 20 revealed no reliable effect of groups $F(1, 21) < 1$.

Mean percent drug-appropriate choices for each group are plotted as a function of the dose of *d*-amphetamine given

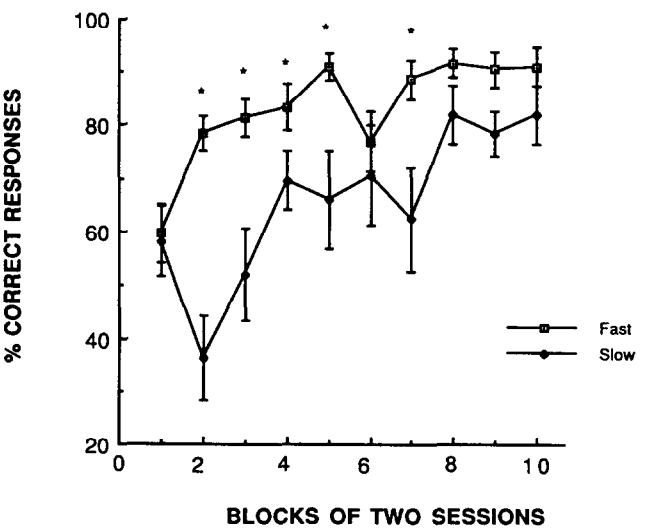

FIG. 2. Mean percent correct first trial responses for the fast group and the slow group as a function of blocks of two sessions. Vertical bars represent the standard error of the mean (SEM). The asterisk (*) indicates that the effect of groups was statistically significant at the 0.05 level of confidence.

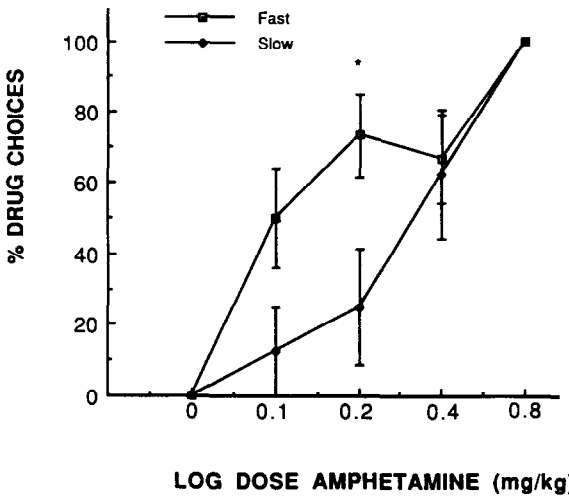

FIG. 3. Mean percent drug-appropriate choices during generalization testing for the fast group and the slow group as a function of the log (base 2) dose of *d*-amphetamine tested (0.0, 0.1, 0.2, 0.4, and 0.8 mg/kg). Vertical bars represent the standard error of the mean (SEM). The asterisk (*) indicates that the effect of groups was statistically significant at the 0.05 level of confidence.

during generalization testing (see Fig. 3). Mean percent drug-appropriate choices for the fast group were at least 50% for four of the five *d*-amphetamine doses tested (i.e., 0.10, 0.20, 0.40, and 0.80 mg/kg), while mean percent drug-appropriate choices for the slow group were less than 50% for three of the five *d*-amphetamine doses tested (i.e., 0.00, 0.10, and 0.20 mg/kg). Mixed-design, two-way, repeated-measures MANOVA revealed a reliable main effect of groups, $F(1, 21) = 4.60, p < 0.05$, a reliable main effect of doses, $F(4, 84) = 19.68$, $p < 0.01$. The groups by doses interaction was not significant, $F(4, 84) = 1.76, p > 0.05$. Planned pairwise post hoc comparisons (alpha = 0.05) revealed that the groups differed significantly on the 0.20 mg/kg *d*-amphetamine test dose, where 11 of 15 subjects in the fast group and 2 of 8 subjects in the slow group made drug-appropriate choices.

Mean percent drug-appropriate responses for each group are plotted as a function of the dose of *d*-amphetamine given during generalization testing (see Fig. 4). Mean percent drug-appropriate responses for the fast group were at least 50% for four of the five *d*-amphetamine doses tested (i.e., 0.10, 0.20, 0.40, and 0.80 mg/kg), while mean percent drug-appropriate choices for the slow group were less than 50% for three of the five *d*-amphetamine doses tested (i.e., 0.00, 0.10, and 0.20 mg/kg). Mixed-design, two-way, repeated-measures MANOVA revealed a reliable main effect of groups, $F(1, 21) = 5.79, p < 0.05$, a reliable main effect of doses, $F(4, 84) = 22.71$, $p < 0.01$. The groups by doses interaction was not significant, $F(4, 84) = 1.35, p > 0.05$. Planned pair-wise post hoc comparisons (alpha = 0.05) revealed that the groups differed significantly on the 0.20 mg/kg *d*-amphetamine test dose.

The mean number of single-lever response training sessions for the fast and slow groups were 29.33 and 23.63, respectively. One-way ANOVA revealed that this difference was not significant, $F(1, 21) < 1$. The mean number of two-lever drug discrimination training sessions before the start of generalization testing for the fast and slow groups were 46.87 and 52.25, respectively. One-way ANOVA revealed that this difference was not significant, $F(1, 21) = 2.14, p > 0.05$. The mean number of *d*-amphetamine injections administered before the start of generalization testing for the fast and slow groups were 40.20 and 38.50, respectively. One-way ANOVA revealed that this difference was not significant, $F(1, 21) < 1$. The mean number of two-lever drug discrimination training sessions given after the start and before the completion of generalization testing for the Fast and Slow Groups were 24.75 and 24.38, respectively. One-way ANOVA revealed that this difference was not significant, $F(1, 21) < 1$.

DISCUSSION

The results reveal that the 0.8 mg/kg *d*-amphetamine training dose was readily discriminated, as 15 of the 23 subjects (fast group) achieved the discrimination criterion of eight correct first trial choices in 10 sessions by the 10th session, and the remaining eight subjects (slow group) also achieved the criterion relatively quickly, within 12–23 sessions. Other drug discrimination researchers have previously reported that this training dose of *d*-amphetamine (0.8 mg/kg) was reliably discriminated by all rats within 32 sessions (4,7).

The results also reveal that the fast group provided a higher percentage of correct first trial choices and correct first trial responses during the first 20 discrimination training sessions, as compared to the slow group, and these group differences in discriminative control by the drug cue were evident early during discrimination training (i.e., during blocks 2 and 3).

On the other hand, although the slow group took longer to achieve criterion, they later attained the same high level percent correct choices and percent correct responses as did the fast group. The comparability of choice accuracy across groups later during discrimination training is also revealed by the similarity in the number of sessions required to attain the criterion to begin generalization testing (ten correct first trial choices in 10 sessions).

The results also reveal that the subjects that acquired the *d*-amphetamine vs. saline drug discrimination more rapidly

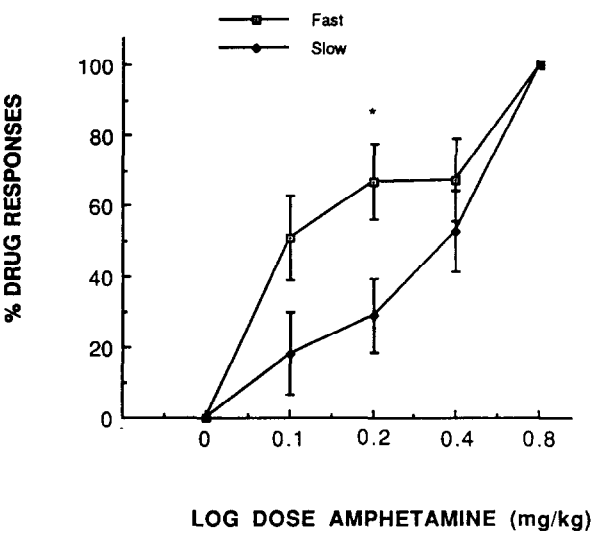

FIG. 4. Mean percent drug-appropriate responses during generalization testing for the fast group and the slow group as a function of the log (base 2) dose of *d*-amphetamine tested (0.0, 0.1, 0.2, 0.4, and 0.8 mg/kg). Vertical bars represent the standard error of the mean (SEM). The asterisk (*) indicates that the effect of groups was statistically significant at the 0.05 level of confidence.

(fast group) were more likely to generalize to lower test doses of *d*-amphetamine than were subjects that made at least three errors during the first ten sessions (slow group). The fast group provided higher mean percent *d*-amphetamine-appropriate choices at the 0.1, 0.2, and 0.4 mg/kg *d*-amphetamine test doses, relative to the slow group, and the difference in tendency to generalize was most apparent and statistically reliable at the 0.2 mg/kg *d*-amphetamine test dose.

The tendency of fast learners to generalize more to lower test doses of the training drug is consistent with reports provided by previous drug discrimination researchers (5,8,9). The present study provides the first report of this effect in female rats, and, in addition, the first report of this effect under conditions where the fast and slow learners received comparable amounts of drug discrimination training prior to generalization testing. In all of the earlier studies reporting this effect, generalization testing was initiated on the session after each subject achieved criterion. Consequently, slower subjects differed from faster subjects in several ways, receiving more sessions of drug discrimination training, more injections of the training drug, more injections of saline, generalization testing at an older age, more sessions of training between initial exposure to the training drug and generalization testing, more sessions of discrimination training between response training and generalization testing, etc.

While the results of the present study are similar to those of previous investigators reporting more generalization by faster learners, the data from the present study cannot be attributed to differences in amount of training.

The procedures employed in the present study are similar, in many respects, to those employed by Goudie et al. (3), who reported somewhat more generalization in female rats who were slow learners. In the present study, as in the study by Goudie et al. (3), female rats were given extended postcriterion discrimination training and tested for generalization following a comparable number of sessions of discrimination training. The studies differ most notably in the number of drug discrimination training sessions required to establish reliable discriminative control by the drug cue. In the study by Goudie et al. (3), even the majority of the fast learners required several dozen discrimination training sessions to establish control by the drug cue, suggesting that the training drug was not readily discriminated, whereas in the present study, the training drug was apparently quite readily discriminated, as fast learners required only 10 sessions to acquire the discrimination. The results of the present study, therefore, confirm the hypothesis originally formulated by Goudie et al. (3), that fast learners are particularly likely to generalize more when the training drug is readily discriminated.

The results reveal that the tendency to respond correctly during drug discrimination training and the tendency to provide drug-appropriate choices during generalization testing covary between groups. Schechter (8) has suggested that the two measures both relate to an individual subject's physiological sensitivity to the training drug cue. Thus, for fast learners, the training drug cue is more intense or robust, and hence, more readily discriminated from vehicle. During generalization testing, presumably lower test doses of the training drug, are also perceived by more sensitive subjects as more intense or robust, and, hence, are more likely to engender drug-appropriate choices.

When the training drug is difficult to discriminate, even by the faster learners, the training drug cue is presumably more likely to be perceived as similar to vehicle, owing to the more expanded range of cue intensities produced by the training drug that overlap those produced by vehicle. It follows, therefore, that during discrimination training, all subjects, regardless of their sensitivity to the drug cue, would be less proficient in discriminating the drug cue, particularly early during discrimination training. When all subjects tend to make more errors, differences in the probability of errors between subjects tends to decrease, making the discrimination performance of sensitive and insensitive subjects more homogenous. In the extreme case where the training drug is not discriminable, even to the most sensitive subject, then all subjects would make many errors, and the relative differences in errors between fast and slow learners would be very small and unrelated to differences in sensitivity to the training drug cue. Thus, highly discriminable training drugs are more likely to differentiate between fast and slow learners, as well as sensitive and insensitive subjects, in drug discrimination procedures.

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