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Rats That Acquire a THC Discrimination More Rapidly are More Sensitive to THC and Faster in Reaching Operant Criteria

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O'NEAL, M. F., L. W. MEANS, J. H. PORTER, J. A. ROSECRANS AND D. J. MOKLER. *Rats that acquire a THC discrimination more rapidly are more sensitive to THC and faster in reaching operant criteria.* PHARMACOL BIOCHEM BEHAV **29**(1) 67–71, 1988.—Male Sprague-Dawley rats were trained to discriminate delta-9-tetrahydrocannabinol (THC) from saline in a two-lever operant task using successive training criteria. Untreated animals were first shaped to barpress for a milk reward with one lever available. As each animal reached criterion the second lever was installed, the first lever was removed, and the animal was treated with 3.0 mg/kg THC 30 min prior to barpress training. When criterion on the second lever was reached the rats were trained to discriminate THC from vehicle injections with both levers available. Following acquisition of the discrimination, test doses of THC at 0.00, 0.375, 0.75, 1.5 and 3.0 mg/kg revealed that the half of the 24 rats who reached criterion (STC) more rapidly exhibited significantly greater sensitivity to THC at the 0.75 mg/kg test dose than did the 12 slow-learner rats; the former group generated an ED50 of 0.77 mg/kg, whereas the ED50 for the later group was 1.63 mg/kg. The fast learners acquired both the initial barpress response and the discrimination more rapidly than did slow-learners. Results suggest that some animals are inherently more sensitive to THC and faster in meeting learning criteria.

Discrimination learning Drug discrimination Individual differences Learning Operant learning Sensitivity THC

DELTA-9-Tetrahydrocannabinol (THC) is one of many psychoactive drugs capable of producing differential responding in discrimination tasks in laboratory animals [4, 9, 16]. Behavioral paradigms in which the discriminative stimulus properties of THC have been demonstrated include the two-lever approach-avoidance discriminated lever press [8], discriminated arm selection in a T-maze [3, 5, 7] and, more routinely, the two-lever appetitive discriminated operant [1, 2, 6, 10]. In the appetitive two-lever operant paradigm, a food deprived subject is trained to press one lever following drug administration and the other lever following administration of vehicle. THC has been found to be "highly discriminable" [12] in this paradigm in comparison with other drugs as indicated by speed of acquisition of the discrimination.

As Schechter [15] has noted, researchers who train laboratory rats to discriminate between drug and vehicle are aware of the different rates at which the animals acquire the discrimination. Using sessions to criterion (STC) as a measure (which includes both shaping and discrimination training), Schechter evaluated the drug sensitivity of "early" and "late learner" rats trained to discriminate 0.16 mg/kg apomorphine from saline. The dose-response curve generated suggested that rats that acquired the discrimination more rapidly were more sensitive to the drug; i.e., "early learners" were found to discriminate lower doses of apomorphine in stimulus generalization tests as indicated by a lower ED50 to apomorphine.

The goal of the present experiment was to partially replicate Schechter's [15] study by evaluating sessions to criterion and sensitivity of animals trained with a discriminative stimulus of 3.0 mg/kg THC. In addition, it was reasoned that by separating out the data for barpress acquisition, performance on an operant task could be assessed separately from performance on drug discrimination. Thus the general tendency of animals to perform on specific acquisition phases and across all phases could be evaluated.

The separation of barpress training from total number of sessions to the criterion of the drug discrimination (STC) was initiated also in response to Overton and Hayes' [13] research. These investigators maintained that the number of times the correct bar is reversed or switched from vehicle to drug and vice-versa before criterion performance is achieved

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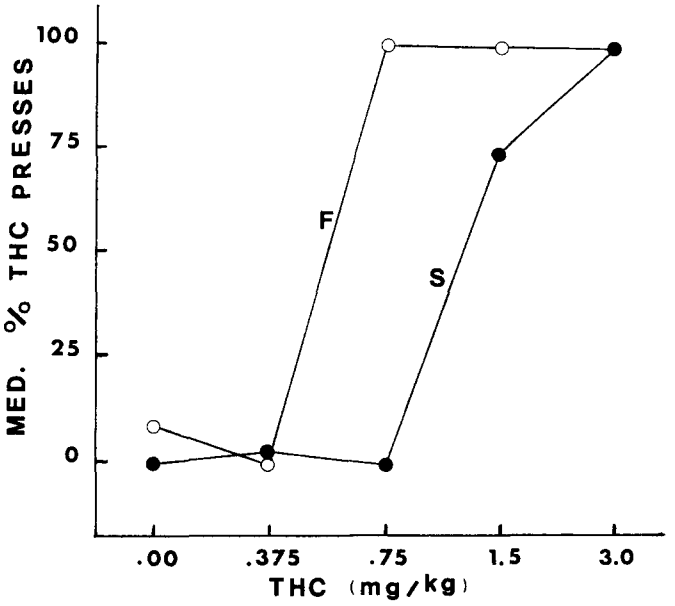

FIG. 1. Proportion of responding on the THC lever by fast and slow learners

(reversals to criterion—RTC in a successive alternation procedure) is the measure that most accurately represents drug discriminability. These authors criticized the use of STC indices as a measure of acquisition of discrimination in that they include "the variable number of days devoted to shaping."

THC is known to initially decrease barpressing in rats before they become behaviorally tolerant [16]. By using a criterion procedure in training the animals to barpress on the drug lever as well as on the vehicle lever prior to discrimination training, we sought to insure that each subject had acquired approximately the same level of tolerance to the rate-decreasing effects of THC before discimination training was initiated. Therefore, the animals were treated as individuals, and advancement to subsequent phases of training was not dependent upon group membership or on the performance of other animals.

Thus, this study sought to address two questions: (1) Are rats that acquire a THC discrimination more rapidly more sensitive than slow-learning animals to test doses of THC? and (2) Are rats that acquire a THC discrimination more rapidly more likely to reach all phases of behavioral training more rapidly than slow-learning animals?

METHOD

Subjects

Subjects were 24 experimentally naive male Sprague-Dawley rats (Dominion Laboratories, Herndon, VA) weighing from 250 to 300 g at the beginning of the experiment. Animals were housed individually with water supplied ad lib. Body weights were maintained at approximately 80% of free-feeding weight with commercial chow (Ralston Purina) provided after each day's training. A 12 hr light-dark cycle, with the lights turned on at 6:00 a.m. was in effect throughout the experiment.

Drugs

The subjects were injected IP with 3.0 mg/kg delta-9-tetrahydrocannabinol (National Institute on Drug Abuse) or its vehicle 30 min prior to training. The vehicle was a solution of 1.5% emulphor: 1.5% ethanol: 97% saline (v:v:v). Injections were made in a volume of 1 ml/kg.

Apparatus

The experimental equipment consisted of four standard operant chambers, each equipped with two levers 14 cm apart and 3 cm above the compartment's grid floor (Coulbourn Instruments, LeHigh Valley, PA). A dipper provided a reinforcement of sweetened milk. The test cages were enclosed in sound-attenuating chambers and each was equipped with 9 W house lights. Computerized programming equipment (MCS-Aim 65 microcomputers) controlled the operant chambers and recorded the data.

Training

Magazine training. Prior to training, each animal was

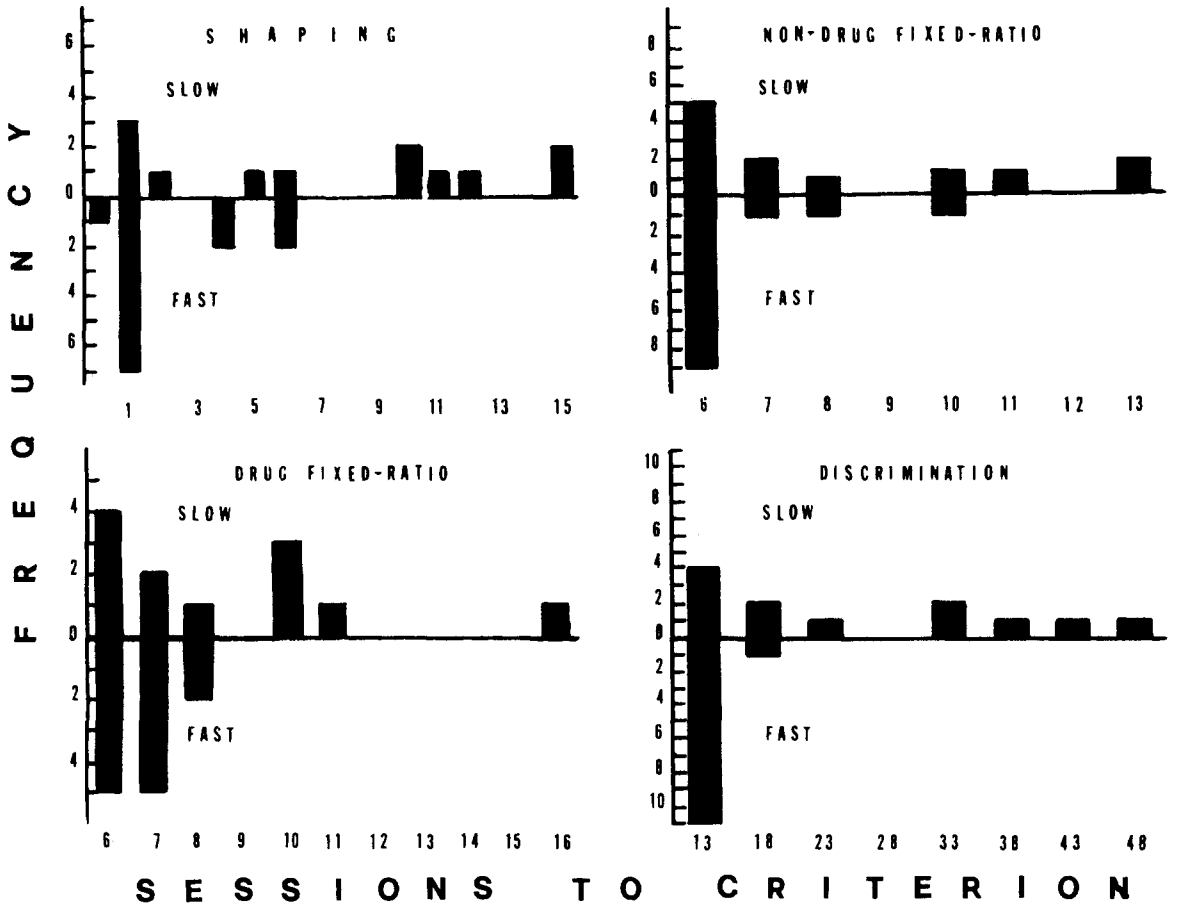

FIG. 2. Sessions to criterion for fast and slow learners for shaping, no-drug FR, drug FR and discrimination training.

weighed and handled daily for three minutes for three consecutive days. Magazine training was conducted on the following three days, with each animal spending twenty minutes per day adapting to the dipper and light and consuming milk in the chamber in which he would subsequently receive barpress training. No levers were present during this phase of training. The dipper was manually engaged whenever the rat approached it. The number of approaches to the dipper was not recorded, but animals were required to consume milk on at least one occasion for three consecutive days before shaping began.

Shaping. Shaping began on the day after magazine training criterion was met, according to the method of successive approximations. Training was based upon the Overton [11] finding that discrimination training is expedited when the mechanisms of state dependent learning are incorporated in barpress training. Thus, training was conducted first in the non-drug state on the lever that would subsequently serve as the lever reinforced following vehicle injection; the other lever was not present during this phase. For half of the subjects the left lever was designated as the vehicle response lever; for the other half of the animals the right lever was the vehicle lever. Daily shaping sessions lasted 20 min and were conducted only on weekdays. Performance for each animal was individually monitored. Once a subject completed a minimum of ten reinforced barpresses during a session, shaping criterion was met, and FR training began for that animal on the following day.

Animals that did not acquire the barpress response within seven training sessions received an additional training procedure beginning on Day 8. A closed plastic box was placed in the back of the chamber, restricting the animal to the third of the chamber where the lever was located. The facilitated training continued until the animal completed a minimum of ten reinforced responses during a shaping session. On the following day, the plastic box was removed, and FR training was begun for that animal.

FR Training.

Phase 1: No-drug FR training. Animals were trained on the no-drug (i.e., subsequently vehicle) lever on weekdays (20 min sessions) until the barpress criterion of 50 reinforced responses on fixed ratio (FR) 1 within a session was reached. Once an experimental subject had attained the criterion of 50 reinforced responses on FR 1, the fixed ratio schedule was increased to FR 3 for the next session. When the rat met the criterion of 50 reinforced responses on that FR schedule, the fixed ratio was increased to FR 5, FR 8, FR 10 and finally to FR 15, completing his single-lever training on the vehicle lever. The response criterion had to be met for each schedule before increasing the ratio. When an animal reached criterion on FR 15 on the no-drug lever, drug lever training was begun for that animal.

Phase 2: Drug FR training. Following training on the no-drug lever (vehicle) lever, it was removed from the chamber, and the drug lever was installed. Animals that had been trained on the left lever for vehicle were trained on the right lever for drug and vice versa. The training dose of 3.0 mg/kg

THC was injected 30 min before each session, and each animal was again trained until the barpress criterion of 50 reinforced responses on each FR schedule (1, 3, 5, 8, 10, and 15) was met. An "over-learning" criterion of FR 15 was employed in barpress training to insure the stability of the response.

Discrimination training. Discrimination training began on FR 10 for each animal on the first day after achieving barpress criterion during the drug training phase. Both levers were installed in the chamber, and the rat was injected IP with 3.0 mg/kg THC or vehicle 30 min prior to training. Sessions were 15 minutes long for the first six sessions; they were reduced to 10 minutes for all subsequent training.

Drug treatment during discrimination training was selected randomly on every other day; i.e., on the first day of drug discrimination, one condition (drug or vehicle) was randomly selected and that treatment used for all animals in drug discrimination training on that day. On the second day, the other condition was used. With this procedure, treatments sometimes alternated and were sometimes identical on two successive days.

Discrimination training continued until the subject achieved the discrimination criterion used by Schechter [15]: 8 of 10 consecutive sessions in which the first reinforcer was obtained within 12 or fewer responses.

Testing

After attaining discrimination criterion, each subject was given stimulus generalization tests of 0.375, 0.75, 1.5 and 3.0 mg/kg THC and 3.0 mg/kg vehicle. Dose order was randomized for each animal.

On test days each animal was injected IP 30 min prior to the test session. During testing the rat was allowed to press in extinction until ten responses were completed on one lever, subsequently called the "selected lever;" the animal was then removed from the chamber. Extinction testing was employed in order to preclude training at a dose different from the original training dose.

To maintain and confirm presence of the discrimination, animals received a minimum of three training sessions between each test session. In order to qualify for a subsequent test, the animal was required to have selected the correct lever on two of three consecutive training days including a THC and a vehicle treatment day.

Analysis

Animals were divided post hoc into "slow" and "fast" learners on the basis of sessions to combined criteria (STC); i.e., sessions to shaping + sessions to no-drug FR + sessions to drug FR + sessions to discrimination. Due to the fact that neither the dose response test data nor the sessions to attain the various acquisition criteria were normally distributed, non-parametric measures were generally employed. ED50s were determined using extrapolation from a linear regression analysis.

RESULTS

Sessions to Criterion (STC)

Using a median split, the animals were divided into two groups of 12 animals each, fast and slow learners, based on the total number of sessions to criterion (STC). For the fast learners, the mean STC (±SEM) was 27.3±0.86; for the slow learners, the mean STC (±SEM) was 50.0±2.87. Means for the two groups differed significantly, $t(22)=7.60$, $p=0.001$.

A comparison of mean weights at one, two and three months into the study revealed no significant differences between fast (mean 1=262 g; mean 2=296 g; mean 3=297 g) and slow (mean 1=265 g; mean 2=291 g; mean 3= 298 g) learners at any point.

Dose-Response Relationships

Both fast and slow learners increased their proportion of responses on the THC lever as drug dose increased, as measured by relative response frequencies on the drug lever during testing (Fig. 1). Friedman Anova by Ranks [17] revealed that the increase was significant for both groups: for early learners, $\chi^2(4)=12.217$, $p=0.026$ and for late learners, $\chi^2(4)=9.317$, $p=0.038$.

Examination of Fig. 1 suggested greater responding on the THC lever by fast learners than by slow learners at intermediate drug doses. The two groups differed significantly on proportion of THC-lever presses only at the 0.75 mg/ dose level (Kruskal-Wallis H Test [10] ($H=4.381$, $p=0.0344$). Fast learners also exhibited more sensitivity to drug as indicated by regression analysis used to determine the ED50s by extrapolation. (Fast learners, ED50=0.77; slow learners, ED50=1.63).

Analysis of the dose-response data according to test order via Friedman Anova by Ranks demonstrated no significant effects for either the fast, $\chi^2(4)=0.700$, $p=0.453$, or slow, $\chi^2(4)=3.400$, $p=0.138$, learners. Thus, neither group was found to show a significant change in proportion of THC presses across test sessions.

Learning Criteria

All 24 animals consumed milk from the dipper on each of the three days of magazine training. Fast and slow learners were compared on each subsequent phase of pre-test operant training, and on each the two groups differed on sessions to reach training criteria (see Fig. 2). These differences were significant for shaping ($H=6.059$, $p=0.0135$) and for discrimination training ($H=10.854$, $p=0.0014$), but not significant for no-drug FR training ($H=2.564$, $p=0.1057$) or for drug FR training ($H=1.933$, $p=0.1612$). Examination of Fig. 2 revealed that the two groups differed primarily because the slow group consisted of several members that required many more sessions to reach criterion than the worst performing subject in the fast group required. In brief, because of the few extremely poor performers, the slow-learner group had both greater variability and higher central tendency. The two groups did not differ significantly on the number of training sessions that they received between tests.

DISCUSSION

The present findings revealed that, as in the Schechter study [15], the fast-learner animals achieved discrimination criterion in almost half the number of sessions required for the slow-learner rats. The fast learners were significantly faster in reaching both shaping and discrimination criteria, and they demonstrated less variability on the individual operant criteria. Though both groups of animals exhibited increased recognition of the drug as dose increased, fast learners were more likely than slow learners to select the drug lever at 0.75 mg/kg, a dose only one quarter of the training dose. This greater sensitivity of the fast learners was also reflected in a lower ED50.

The possibility of an effect of motivational differences in

acquiring the criteria was considered, but there was no significant difference between the weights of fast and slow learners at any point in the study.

Since training sessions were conducted between tests, and since the total number of these sessions varied among animals, the possibility of differential effects upon fast versus slow learners on training and/or sensitivity was considered. However, there was no significant tendency to increase responding on the THC lever for either fast or slow animals over the course of testing. The absence of a significant difference between number of between test training sessions for fast and slow-learners also suggested that the discrimination had been acquired prior to testing and that the two groups did not differ in practice needed for maintenance of the discrimination.

As Schechter [15] found regarding apomorphine, fast learners on the present discrimination required a significantly lower dose to discriminate THC. Schechter suggested, and these data seem to support his prediction that, using an easily discriminable training dose, "as (STC) increases, so does the ED50." Rats that acquire behavioral criteria more rapidly appear to have an inherently greater "physiological sensitivity," in Schechter's terms, to THC. Schechter noted that his finding of greater drug sensitivity among the fast-learner rats might dissuade the frustrated researcher from concluding that some rats are "simply stupid." We suggest, however, based upon current findings, that both sensitivity AND "learning aptitude" are factors, since fast learners displayed more rapid shaping prior to drug injection. Apparently, some underlying mechanisms that influence acquisition of operant criteria also influence sensitivity to THC. Since this is a behavioral study, any attempt to identify the mechanism would be purely speculative.

As Schechter [15] proposed, the individual differences in drug sensitivity among rats may explain the discrepancies in ED50s reported in the literature for the same drug and same training dose, since the differences appear to be strongly correlated with behavioral acquisition speed, and studies vary in the acquisition criteria they require. We concur with Schechter's suggestion that acquisition data as well as ED50s be presented in future drug discrimination studies.

In regard to the use of STC as the standard measure of acquisition in drug discrimination studies, Overton and Hayes' [13] advocacy of the use of RTC instead represents a valid point; in cases in which animals are shaped to press a lever other than the one which will subsequently serve as the vehicle lever during discrimination training, conceptually RTC is the only measure of "pure" discrimination acquisition. However, if animals are shaped and FR trained as Overton [11] suggested, so that their drug state is consistently paired with the appropriate bar for subsequent discrimination training, it may be assumed that discrimination training is being conducted from the onset of the study. The present experiment, carried out in accordance with these principles, has demonstrated that animals which reach STC criterion more rapidly are significantly more likely to reach the individual discrimination criterion more rapidly as well. It seems then, that STC is an appropriate measure of discrimination acquisition when initial shaping methods accord with the principles of state dependent learning.

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