Pharmacology Biochemistry & Behavior, Vol. 19, pp. 1–4, 1983.

Drug Sensitivity of Individual Rats Determines Degree of Drug Discrimination

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Received 1 October 1982

SCHECHTER, M. D. *Drug sensitivity of individual rats determines degree of drug discrimination.* PHARMACOL BIOCHEM BEHAV **19**(1) 1–4, 1983.—Rats were observed to learn to discriminate between the stimulus properties of intraperitoneal 0.16 mg/kg apomorphine and saline, in a two-lever operant task, at different rates. Half of the 12 rats reached criterion performance in a mean of 22.5 session, whereas the other half reached criterion in a mean of 44.2 sessions. These two groups, i.e., the early and later learners, were tested with a range of apomorphine doses and the former group had an ED50 of 0.01 mg/kg, whereas the later group generated an ED50 of 0.07 mg/kg apomorphine. These results suggest that the early learners were significantly more sensitive to apomorphine than the later learners and this may explain the discrepancies in the drug-discrimination literature regarding different ED50's generated at the same drug training dose.

Apomorphine Drug-induced stimulus Sensitivity Learning

THE ability to assume discriminative control of behavior has been observed to be the property of virtually every psychoactive drug tested and numerous reports have indicated that apomorphine is capable of producing a discriminative stimulus complex in rats [2, 5, 13]. Within a discriminative stimulus (DS) paradigm, a subject comes under the stimulus control of a drug whereby correct operant responses in a choice situation are contingent upon which drug was previously administered. Thus, a hungry subject is trained to emit one response, i.e., to press one lever of a two-lever operant box for a food reward, following the administration of a drug. The same subject must make the opposite response, i.e., press the other lever, following the injection of a vehicle solution (saline).

This area of behavioral psychopharmacology has expanded rapidly in the last ten years as evidenced by three major textbooks in this field [6–8] and anyone working in this research area has observed that rats learn to discriminate between a drug and saline at different rates. Indeed, one of the first and most productive workers in this area has used the term "sessions-to-criterion" (STC) as an indication of how long it takes rats to attain the criterion of discriminative training [11]. Once rats are trained, subsequent stimulus generalization tests with lower doses of the training drug yield a dose-response curve which provides an indication of the sensitivity of the subjects to the training drug's cueing properties [3].

In training rats to discriminate between 0.16 mg/kg apomorphine and saline, this laboratory was provided with the opportunity to answer the question: "Do rats learn to discriminate between a drug and saline at different rates because of differences in "intelligence" (learning ability) or by virtue of differences/variabilities in sensitivity to the training dose employed?" This opportunity resulted from the observation that half of twelve rats that were being trained to discriminate apomorphine from saline learned much more rapidly than the other half of the subjects in the study. Instead of continuing to train these "early learners" along with the "late learner" rats, they were removed after they had attained criterion performance and were subjected to various tests to determine their drug sensitivity. Subsequently, the late learners were subjected to the same tests after they had reached the criterion. Thus, it was the purpose of this study to determine the possible differences in sensitivity to apomorphine in those animals learning quickly and those that took a greater length of time to reach discriminative training criterion.

METHOD

Subjects

The subjects were 12 male ARS/Sprague-Dawley rats weighing 240±10 g at the beginning of experimentation. They were housed in individual living cages and their weights were adjusted, by daily rationing of commercial rat chow, to approximately 85% of their free-feeding weights as determined by daily weighing of 3 control free-feeding rats purchased from the supplier (Zivic-Miller, Allison Park, PA) at the same time. Water was continuously available.

Apparatus

The experimental space was a standard rodent Skinner test cage (Lafayette Instruments Corp.) equipped with two operant levers placed 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and 9 W houselight. Solid-state programming equipment (LVB Corp.) was used to control and record the sessions and was located in an adjacent room.

Discrimination Training

Training was based upon procedures described by Overton [12] and there were two training phases. In the first phase, food-deprived subjects learned to lever press on both levers for food reinforcement (45 mg Noyes pellets) on an FR 10 schedule. The drug lever was activated first for all subjects. Animals were initially shaped to press this lever on an FR 1 schedule. The schedule was then made progressively more difficult, in daily 30 min sessions, over 12 days until a FR 10 schedule was achieved. Throughout drug lever-press training, animals received daily intraperitoneal (IP) injections of freshly prepared 0.16 mg/kg apomorphine hydrobromide (as base) 15 min prior to being placed into the two-lever operant box. Immediately following attainment of the FR 10 schedule after drug administration, the opposite lever was activated and rats were trained on an FR 1 schedule after the administration of an equal volume (1 ml/kg) of saline. Daily sessions of 30 min were continued over 7 days with saline administration until an FR 10 schedule was attained.

Phase II, discrimination training, then began. Subjects were trained 5 days per week with alternation of reinforcement proceeding in a pseudo-random sequence. Thus, in each 2-week period there were 5 days with drug lever (D) correct and 5 days with saline lever (S) correct. The pattern was DSSDD; SDDSS. Criterion was set at 8 of 10 consecutive sessions during which the first food pellet was received within 12 or less total responses. As stated (above), it became apparent that half of the 12 rats attained this criterion faster than the other 6 animals. These 6 animals reached criterion after 8 weeks, whereas the other 6 required an additional 8 weeks to attain this criterion. However, both groups of animals continued with the following procedures.

Dose-Response Relationships

After each group of animals attained the training criterion, testing and training sessions of 15 min duration, with alternating administrations of 0.16 mg/kg apomorphine and saline were continued on Mondays, Wednesdays and Fridays. This procedure endeavored to insure and maintain behavioral discrimination to the trained drug conditions. It was intended that if a rat was observed to make more than 2 incorrect first choice selections in any of ten daily consecutive maintenance sessions, the data on that rat's performance would be deleted from the results. On Tuesdays and Thursdays, the rats were injected IP with different doses of apomorphine ranging from 0.04 to 0.24 mg/kg and, 15 min later, they were placed into the experimental chamber and were allowed to lever press, in extinction, until 10 responses were made on either lever. To preclude training at an apomorphine dose different than employed to train the animals, the rats were immediately removed from the experimental chamber upon making 10 responses on either lever. Each of 3 doses of apomorphine was tested in each animal on 4 occasions with each test preceded by both a 0.16 mg/kg apomorphine and a saline maintenance session. The lever first pressed 10 times was designated as the "selected" lever.

Once the ED50's of each group were graphically or actually determined, that dose was administered on 4 trials to each of the rats in the "early" or "later" learner groups and they were tested, in extinction, to determine selected lever.

Extended Schedule Testing

Once the animals had been tested with various doses of apomorphine, they were tested with a procedure termed "extended schedule testing" as described elsewhere [14]. Training sessions of 15 min duration with alternating administration of 0.16 mg/kg apomorphine or saline were continued on Mondays, Wednesdays and Fridays for the remainder of the experimentation. On Tuesdays and Thursdays, the rats were administered either the training dose of apomorphine or saline and, 15 min later, they were placed in the experimental chamber and were allowed to lever press, in extinction, until 10 responses were made on the lever that was not the first lever selected. Thus, for example, when a rat pressed the apomorphine lever 10 times after apomorphine administration, that lever was designated as the "selected" lever and the rat was allowed to continue pressing, without reinforcement, until it accumulated 10 presses upon the saline lever. The number of presses made on the apomorphine-appropriate lever prior to 10 presses on the saline lever was recorded. Likewise, if the saline lever was the selected lever after saline injection, the rat was allowed to continue pressing until 10 responses were made on the apomorphine lever. These extended schedule performance (perserverance) measurements were conducted in 4 trials each with the training dose of apomorphine and with saline.

Statistic

The sessions-to-criterion [11] were analyzed in each of the "early learner" and "later learner" groups of animals, The dose-response results were expressed as the percent of first 10 responses (selected lever) on the apomorphine-appropriate lever. Subsequently, these dose-response data were analyzed by the method of Litchfield and Wilcoxon [10] which plots data on probit vs. log-dose paper and generates ED50's for each group and tests for parallelism and potency differences. The extended schedule testing was subjected to paired t-tests between apomorphine and saline in each group and to unpaired t-test of means between groups.

RESULTS

Sessions-to-Criterion

The mean ($\pm$SEM) sessions-to-criterion (STC) for the early learner rats was 22.5 ± 5.24 sessions. In the later learner rats, the mean STC ($\pm$SEM) was 44.2 ± 7.36. These means were significantly different, $t(5)=5.87$, at the $p<0.001$ level.

Learning Rates

The learning curves for the early learner and later learner groups of animals is presented in Fig. 1. The former group attained criterion performance after 4 session blocks, or 8 weeks, of training, whereas it took 8 session blocks (40 training sessions with each of apomorphine and saline) for the later learners to attain approximately the same discriminative criterion.

Dose-Response Relationships

The dose-response results appear in Fig. 2 which indicates that with administration of decreasing apomorphine doses the percent of apomorphine-lever selections decreases. Analysis of the best-fitted curves by the method of Litchfield and Wilcoxon [10] indicates an ED50 for the early

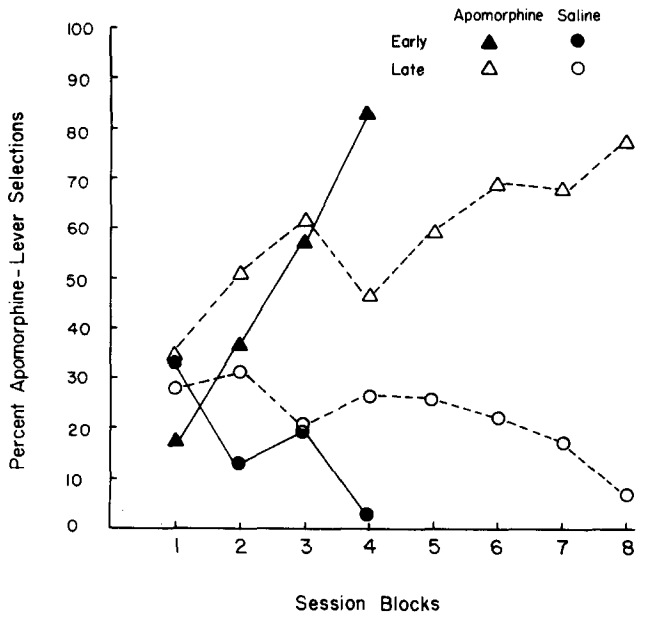

FIG. 1. Learning curves for early (n=6) and late (n=6) learners. Ordinate: Percent first choices (selected lever) on apomorphine-correct lever; Abscissa: Session blocks consisting of 5 apomorphine (A) and 5 saline (S) trials each according to biweekly schedule of A-S-S-A-A; S-A-A-S-S.

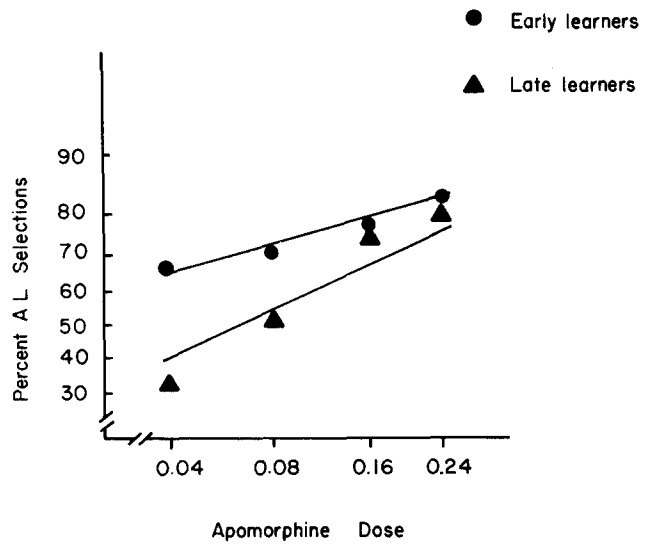

FIG. 2. Dose-response curves for early and late learners. Ordinate: Percent first choices (selected lever) on apomorphine-correct lever graphed on probit paper; Abscissa: Log dose (mg/kg) apomorphine tested in each of 12 rats in 4 trials except for 0.16 mg/kg training dose (16 trials).

learners of 0.01 mg/kg apomorphine and an ED50 of 0.07 mg/kg for the later learners. In addition, when subjected to the Litchfield-Wilcoxon method, the lines are parallel within statistical significance (95% confidence limits) and there is a significant difference in potency with the early learners being 3.39 times more sensitive than the later learners.

When the early learner rats were tested with the 0.01 mg/kg dose of apomorphine they first selected the apomorphine-correct lever on 43.8% of trials, whereas the late learner rats choose this lever on 50% of trials after administration of 0.07 mg/kg apomorphine.

Extended Schedule Performance

The mean (±SEM) responses for the group of early learner rats on the saline lever, after saline administration, was 43.7± 11.7 responses before 10 responses were made on the (incorrect) apomorphine lever. This group perseverated a mean (±SEM) of 84.1±25.1 responses on the apomorphine lever, after apomorphine administration, before crossing over and making 10 responses on the saline lever. When analyzed with a paired t-test, the differece between perseverance rates on the saline lever was not significantly different ($p>0.1$) than that mean rate on the apomorphine lever. In the late learner animals, the mean (±SEM) responses during extended schedule testing for the apomorphine lever was 94.5±18.7 and for the saline lever was 61.5±10.1 and neither value was significantly different from each other or from that of the same lever as seen in the early learner animals.

DISCUSSION

The observation that rats being trained to discriminate between 0.16 mg/kg apomorphine and saline were learning at different rates afforded the opportunity to test drug sensitivity in early and late learning rats. The former group was observed to reach criterion in significantly fewer sessions than the latter group; indeed, they learned in approximately half the time. When each group was given decreasing doses of apomorphine they both exhibited decreased discrimination. However, the observation that the early learners required a lower drug dose in order to discriminate is indicated by their lower ED50. Furthermore, the dose-response curves for each of these two groups were parallel and this suggests that apomorphine was working at the same site since parallel dose-response lines is indicative of a common site/mechanism of action [9]. Although it appears that the early learning group were more sensitive to the various doses of apomorphine in their discriminative performance, the results of the extended schedule performance task indicates that within each group there was no significant difference as to the strength of each of the drug and non-drug state cues. In addition, the differences between the sensitivities between the two groups may be explained on the basis of drug distribution, i.e., the drug levels of apomorphine may be higher in the rapid learners. No attempt was made to investigate this possibility.

It has often been reported [1,12] that as the training dose of a drug increases so will the ED50 in dose-response experiments. The present study indicates that at the same training dose different animals will have different drug sensitivities that will allow them to reach criterion at different rates. Thus, it is not what many frustrated researchers may believe, viz., that the rats are simply "stupid," but rather that they are possessed of inherent differences in their physiological sensitivity to the training drug dose. Indeed, the cueing properties of drugs are based upon these physiological effects [3]. In addition, this may explain why there are different ED50's at the same training dose in the same behavioral paradigm, as seen in the literature. Rats might be learning the task at different rates and this can be evidenced if more

publications would report the sessions-to-criterion (STC) data. The results of the present study would indicate that as the sessions-to-criterion increases so does the ED50. This conclusion is, however, at some variance with the previous report of Colpaert *et al.* [4] which indicated that, for fentanyl, relative ED50 is lower as training dose is lower and, hence, as STC is higher.

ACKNOWLEDGEMENTS

The author would like to thank Ms. Denise M. Lovano for her excellent technical assistant and Ms. Jance Lentz-Hatch for the graphic illustrations.

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