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Research Article

Analysis of the Acquisition of Drug Discrimination Reveals Differences Between a High Versus Low Training Dose of ±3,4-methylenedioxymethamphetamine (MDMA)

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Abstract *Background.* Studies of the discriminative stimulus effects of the recreational drug, ±3,4-methylenedioxymethamphetamine (MDMA), typically use a dose of 1.5 mg/kg during training. This dose is relatively low compared to those used in other behavioral paradigms. *Purpose.* The present study assessed the ability of this low dose of MDMA and a higher dose of 3.0 mg/kg to support drug-discrimination learning in rats. *Procedures.* Daily training sessions were preceded by an injection of either MDMA (1.5 mg/kg or 3.0 mg/kg) or saline. Injections alternated in a pseudorandom fashion for a total of 63 sessions. Criteria for the acquisition of the MDMA/saline discrimination were increased from 4 to 10 successive, and successful, discriminations. As the acquisition criteria became more stringent, the impact on the low dose discrimination was greater than the impact on the high dose discrimination. *Conclusions.* These results suggest that the drug discrimination produced by 1.5 mg/kg MDMA may be less reliable than when a higher dose is employed, especially when the number of training sessions is limited. The data further suggest that 3.0 mg/kg MDMA produced a robust discriminative stimulus effect which may be better suited to experiments of this nature.

Keywords ±3,4-methylenedioxymethamphetamine; MDMA; drug discrimination; discriminative stimulus; acquisition; training dose

1. Introduction

Drug discrimination procedures are a versatile way to model the subjective effects produced by centrally active drugs and to investigate neurobiological mechanisms [1]. In a typical experiment, subjects are trained to make one response following administration of a drug, and to make a different response following administration of the vehicle solution. Training can take several weeks, or even months, before reliable discrimination is produced. Once training is complete, different doses of the training drug, and of other compounds, can be administered to test whether the stimulus effects resemble those produced by the initial training drug. Interpretation of these data relies on the assumption that subjects are able to consistently and accurately discriminate between the two stimuli, and therefore the choice of acquisition criteria is critical.

Rats are typically trained in daily sessions that consist of a number of trials. Each trial within a session requires the completion of a fixed ratio of responses on the drug-appropriate lever—often FR10—in order for a reinforcer to be delivered. Under this regimen, only responding during the first trial is controlled solely by the interoceptive effects of the drug/vehicle injection since responses in subsequent trials may be guided by the delivery or nondelivery of a reinforcer. Therefore, the distribution of responses during the first FR10 is often used to indicate whether the discrimination has been acquired. The acquisition criteria usually have two components: (1) accuracy within the first trial of a session, and (2) the number of daily sessions for which this within-session accuracy must be maintained [2]. A within-session accuracy of 80% is commonly reported. Thus, at least 80% of responses emitted before the delivery of a reinforcer must be on the drug appropriate lever during drug sessions, or the vehicle appropriate lever during vehicle sessions. Requirements for the second component vary between studies and are sometimes not even reported, but often require that within-session accuracy must remain above 80% for 10 consecutive sessions (e.g., [3,4]).

Training dose can markedly impact the acquisition of a drug discrimination; discrimination is acquired more rapidly when higher training doses are used [5]. Different doses of the same drug may also produce different subjective effects [6]. For example, a range of stimulants—including cocaine and amphetamine—substituted for the subjective effects of a low dose of caffeine but failed to substitute for the subjective effects of a high dose [7]. Similarly, the local anesthetics lidocaine, dimethocaine, procaine, and chloroprocaine substituted fully for a low dose of cocaine but failed to substitute for the subjective effects of a high dose [8]. In most drug discrimination studies, doses used for training are usually those that produce other behavioral

effects so as to ensure that the stimulus effect will be readily produced. Thus, a dose of 10 mg/kg of cocaine and 1 mg/kg of amphetamine are typically selected.

The relatively small number of studies examining ±3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") have usually employed a training dose of 1.5 mg/kg. This is notably lower than doses required to produce other behavioral responses such as conditioned place preference [9] or hyperlocomotion [10]. A low dose of 1.25 mg/kg was insufficient to produce reliable discrimination behavior in rats even after 65 training sessions [11], raising the possibility that the typical dose of 1.5 mg/kg is just at the threshold for producing discriminative stimulus effects.

Most MDMA discrimination studies to date have applied a less stringent acquisition criterion than is typically used for discrimination of other drugs; within-session accuracy must only be maintained for "at least 8 out of 10 sessions" [11, 12]. As with other studies, the order in which MDMA or vehicle is administered typically alternates in a pseudorandom fashion so that vehicle (S) and MDMA (M) sessions are evenly distributed. For example, a ten-day sequence may run as follows: SMMSSMSMMS. Thus, in a sequence of 10 sessions, there are a maximum of 6 occasions where the drug administered at the start of a given training session differs from that of the previous session (i.e., there is a change in the discriminative stimulus). It is possible for accuracy to fall below the 80% threshold in two (out of six) of these "change" sessions and to still meet the "8 out of 10 sessions" acquisition criterion. This raises the possibility of spurious results during discrimination training that might be particularly apparent when doses of drug that are just at threshold are tested.

The present study was designed to determine the impact of criteria on the acquisition of the MDMA discrimination. Additionally, the role of training dose in the acquisition of the MDMA discrimination was determined by comparing the acquisition profile of the discrimination of the typical low training-dose of MDMA (1.5 mg/kg) with that of a higher training-dose (3.0 mg/kg). It was expected that (1) a higher dose of MDMA would produce a more potent/salient discriminative stimulus and therefore be more readily/rapidly discriminated from vehicle, and (2) employing increasingly stringent acquisition criteria would have greater impact when the lower training-dose of MDMA was used.

2. Methods

2.1. Subjects

Male Sprague-Dawley rats ($n = 24$) weighing 270–300 g at the beginning of experiment were bred in the vivarium at Victoria University of Wellington, New Zealand. The colony was maintained on a 12 h light/dark cycle (1900–0700 light) in a temperature- (21 °C) and humidity- (55%) controlled environment. Rats were housed in pairs and food was restricted to maintain a bodyweight of approximately 85% of free-feeding weight. Food was made available in an amount calculated to maintain a constant body weight (85% free feeding). The food was available until fully consumed. Thus, the exact time for consumption varied depending on amount of food given, the rat, and drug/dose given that day but was typically in the range of 10–60 min. Deprivation target weights were periodically adjusted to allow for natural growth. Food was given immediately following training sessions while water was available ad libitum throughout the study.

2.2. Apparatus

Fourteen commercially available operant chambers (ENV-008: Med Associates Inc.) were used, each containing two retractable levers and a sugar pellet dispenser. The dispenser was located in the center of the front panel of the chamber with one lever positioned on either side. Standard white lights were positioned directly above each of the levers. Experimental events and data collection were controlled by a computer (MED-PC IV) and dustless precision sucrose pellets were obtained from Bio Serve (Frenchtown, NJ, USA).

2.3. Drugs

±3,4-methylenedioxymethamphetamine (MDMA) was obtained from the Institute of Environmental Science and Research (Porirua, New Zealand). MDMA was dissolved in 0.9% saline and administered in a volume of 1 mL/kg via intraperitoneal injection. Drug weights refer to the salt.

2.4. Procedure

Rats were randomly assigned to one of two groups. Each group was trained to discriminate saline from MDMA (1.5 mg/kg or 3.0 mg/kg) in a standard two-lever task. Training procedures were identical in both conditions.

Training began with a series of autoshaping sessions to establish lever-pressing behavior. During these sessions, a randomly selected lever was inserted into the chamber and the corresponding light above the lever was illuminated for 12 s. A single response on the lever (or failure to respond within 12 s) resulted in the immediate delivery of a sugar pellet, the deactivation of the light, and the withdrawal of the lever. After a 30 s delay, a new trial was initiated and a new randomly selected lever was inserted. Autoshaping sessions ran for 30 min or until 120 reinforcers had been delivered. The number of responses required to complete a trial was gradually increased each session until all rats were reliably responding on both levers at a fixed ratio of 10 responses per reinforcer (FR10).

For all subsequent sessions, an IP injection of either saline or MDMA (1.5 mg/kg or 3.0 mg/kg) was administered

15 min prior to the first trial. The order of these injections consisted of a six-session repeating cycle as follows: SMMSSM where S represents saline sessions and M represents MDMA sessions. For half of the rats in each condition, responding on the left lever was reinforced following saline injections and responding on the right lever was reinforced following injections of MDMA. The lever allocation was reversed for the other half of each group.

Fifteen errorless training sessions (7 saline, 8 MDMA) were carried out in which only the injection-appropriate (correct) lever was inserted into the chamber. During each trial, ten responses on the lever were reinforced with the delivery of a sugar pellet. Each trial was followed by a 30 s delay during which the levers were retracted and the lights were deactivated. The session ended after 30 min or once 60 trials had been completed.

Full drug discrimination training began once all autoshaping and errorless training sessions were complete. In drug discrimination training sessions, rats were injected with either MDMA or saline and, 15 min later, they were placed in the operant chambers. Each trial began with the presentation of both levers. Ten consecutive responses on the correct lever were required to deliver a sugar pellet and to complete the trial. Responses on the incorrect lever reset the count. A delay of 30 s separated each trial and the session ended after 30 min or once 60 trials had been completed. All measurements of discrimination performance were based on the allocation of responses during the first trial (FR) of a session. Responses on the MDMA or saline lever were expressed as a percentage of the total number of responses emitted before a reinforcer was delivered. A total of 63 daily sessions were conducted.

All procedures were approved by the Victoria University of Wellington Animal Ethics Committee.

2.5. Acquisition criteria

A major purpose of this study was to investigate how changes in criteria would impact the acquisition of the discrimination between MDMA and saline. In order to assess this impact, a range of different criteria were employed to evaluate responding during drug discrimination sessions.

Table 1 summarizes each of the five different sets of criteria used in the study. The first set of criteria represented the standard model used in the majority of MDMA-discrimination studies to date. These standard criteria were met when at least 80% of responses were on the correct lever for at least 8 out of 10 consecutive sessions. Four additional sets of criteria were also applied. For each of these additional criteria, a within-session accuracy of at least 80% responses on the correct lever was required for an increasing number (4–10) of consecutive sessions (4C–10C).

Table 1: Requirements for the various criteria employed.

Criteria	Requirements
Standard	$>80\%$ correct for at least 8 out of 10 sessions
4C	$>80\%$ correct for 4 consecutive sessions
6C	$>80\%$ correct for 6 consecutive sessions
8C	$>80\%$ correct for 8 consecutive sessions
10C	$>80\%$ correct for 10 consecutive sessions

2.6. Analysis

Only data from full drug discrimination training sessions (i.e., not autoshaping or errorless sessions) are included in the analyses. The number of rats meeting each criterion as a function of test session was determined and the results were used to generate Kaplan Meier survival functions. Data points represent the first session of a sequence that led to the fulfilment of a criterion. The number of sessions required for 50% of rats to meet each criterion was used as a measure of acquisition latency. Response rates were calculated by dividing the total number of responses made during the first trial by the time taken to complete the FR10 requirement. An alpha-level was set at $P < .05$ for all statistical tests.

3. Results

Figure 1 (top panels) shows the effect of manipulating criterion on the acquisition of drug discrimination for the 1.5 mg/kg (left) and 3.0 mg/kg (right) training-dose conditions. Response rates for each condition during each of the daily saline or MDMA sessions are also shown (bottom panels). Table 2 displays the percentage of rats that met each criterion at the conclusion of the experiment (63 sessions) as well as the number of sessions required for at least 50% of rats to meet each criterion.

Discrimination of a low dose of MDMA (1.5 mg/kg) as determined by the standard criterion was learned rapidly with at least 50% of rats reaching criterion after 11 sessions (see Table 2). Manipulating the criteria had a marked impact on the acquisition of the low dose discrimination. As the criterion became more stringent, a smaller percentage of rats acquired the discrimination and more test sessions were required to meet each criterion.

Acquisition curves in the low training-dose condition differed significantly as a function of criterion ($\chi^2(4) = 24.45$, $P < .001$). Follow-up tests (Bonferroni corrected) confirmed that the survival function generated by the standard criterion was significantly different from that of the most stringent (10C) criteria ($\chi^2(1) = 11.61$, $P = .007$). A separate analysis was conducted to determine whether increasing the required number of consecutive correct sessions led to a decrease in the percentage of rats that acquired the discrimination. This analysis confirmed that acquisition rates in the low MDMA-dose condition decreased as the required number of consecutive sessions increased ($\chi^2(1) = 17.81$, $P < .001$).

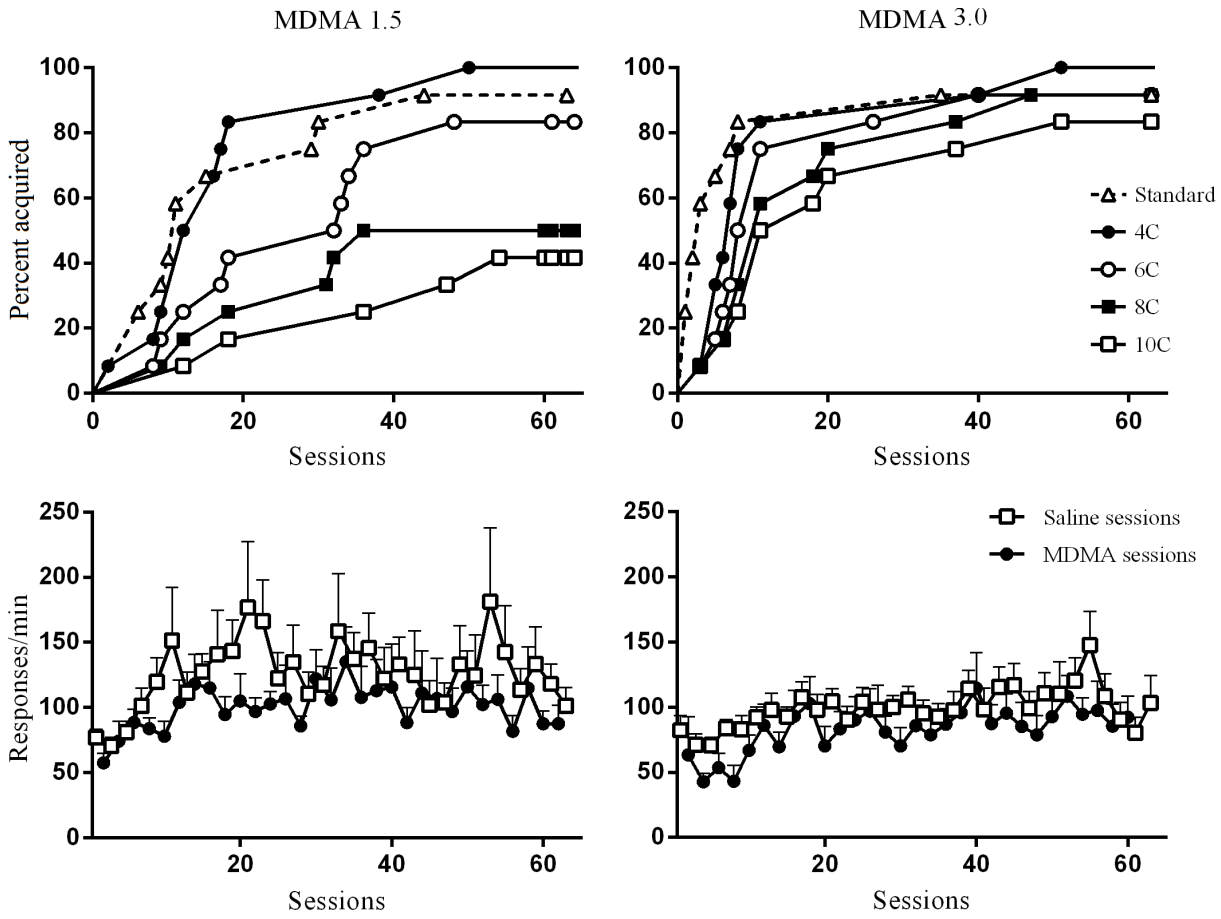

Figure 1: Top panels: effect of manipulating criteria on the acquisition of the discrimination between MDMA 1.5 mg/kg (left) or MDMA 3.0 mg/kg (right) and saline. Symbols represent the cumulative percent of rats that met criterion as a function of test session. Bottom panels: response rates during saline and MDMA sessions remained stable throughout the 63 test sessions. Symbols and error bars represent the mean number of responses/min and standard error of the mean.

Table 2: Summary of latency and proportion of rats meeting each criterion.

	MDMA 1.5 mg/kg		MDMA 3.0 mg/kg	
Criteria	*Percentage of rats that reached criterion*	*First session in which 50% of rats met criterion*	*Percentage of rats that reached criterion*	*First session in which 50% of rats met criterion*
Standard	92%	11	100%	3
4C	100%	12	92%	7
6C	83%	32	92%	8
8C	50%	36	92%	11
10C	42%	—	83%	11

The impact of criterion on the acquisition of the 3.0 mg/kg discrimination was less pronounced. The discrimination using the standard criterion was learned very rapidly with at least 50% of rats reaching criterion after 3 sessions (see Table 2). A log rank test showed that the percentage of subjects that met the acquisition criterion in the 3.0 mg/kg training-dose group did not significantly change as a function of criterion ($\chi^2(4) = 9.07$, $P = .059$).

The experiment was conducted during a total of 63 sessions which alternated between saline (32 sessions) and MDMA (31 sessions) as described in Section 2. Response rates in both groups remained stable throughout testing with ANOVA revealing no interactions ($F(31,682) = .53$, $P = .98$; $F(31,682) = .36$, $P = .99$).

4. Discussion

The present study assessed the ability of typical low dose of MDMA (1.5 mg/kg) versus a higher dose (3.0 mg/kg) to support drug discrimination learning in rats. Both doses were successfully discriminated from saline but

the high dose discrimination was more rapidly acquired than the low dose discrimination. Furthermore, only the low dose discrimination was affected by increasing the acquisition criterion. Taken together these results suggest that a higher training-dose of MDMA produced a more robust discriminative stimulus effect than the 1.5 mg/kg dose which is typically used. It must be acknowledged that the present training period was only 63 days in duration. While this was apparently sufficient for the acquisition of the high dose MDMA discrimination, it is possible that the lower dose discrimination may have been acquired more reliably with more extensive training.

The drug discrimination paradigm is based on the assumption that the interoceptive effects of a drug can guide operant behavior in the absence of some other response strategy. Thus, once the task is learned, administration of drug should consistently lead to the allocation of responses to the drug-appropriate lever. The typical requirement of at least 80% correct responses within a session allows for the occasional accidental lever press during the first trial while still preventing the use of "trial and error" as an effective strategy for earning a reinforcer. This requirement alone, however, does not necessarily stop a strategy of "predominantly pressing the left lever" from being effective. Therefore, the ability to correctly switch responding from the drug lever to the vehicle lever in response to a change in discriminative stimulus becomes a critical determinant of a reliable discrimination. This is verified by observing successful switches between responding for drug versus vehicle stimuli over consecutive sessions. A question remains as to how many successful switches should be required in order to confidently establish that the drug is serving as a discriminative stimulus. The present findings suggest that requiring 8 accurate sessions out of 10 may be insufficient in this respect since an inability to switch may be masked by the allowance of two inaccurate sessions. The requirement of accurate responding for at least 10 consecutive sessions may provide a better indication of whether reliable discrimination has been acquired especially when a low training-dose is used.

The acquisition of the discrimination of a low dose of MDMA was disrupted by changes in the acquisition criteria whereas discrimination of the higher dose was not. This could be interpreted to suggest that the higher dose produced a more salient/potent discriminative stimulus. Studies with other drugs have also shown that higher doses facilitate the acquisition of drug discrimination. For example, acquisition of a cocaine (10 mg/kg) discrimination required an average of 35 sessions whereas an additional 75 sessions were required when the cocaine dose was reduced to 2 mg/kg [13]. Similarly, discrimination of a high dose (56 mg/kg) of caffeine from saline was learned by a greater proportion of rats and required fewer sessions than the discrimination of a lower dose (10 mg/kg) [7].

In some cases, alterations of the training dose also resulted in qualitative changes in discriminative stimulus effects. The psychostimulants, cocaine, and amphetamine, generalized to the discriminative stimulus effects of a low dose of caffeine but failed to generalize to those of a high dose [7]. Similarly, the local anesthetics lidocaine, dimethocaine, procaine, and chloroprocaine substituted fully for a low dose of cocaine but failed to generalize to the subjective effects of a high dose [8]. Few studies have examined the role of training dose in the MDMA discrimination. However, in a three-lever task in which rats were trained to discriminate 1.5 mg/kg MDMA, 0.5 mg/kg amphetamine, and saline, high doses of MDMA (3.0–4.5 mg/kg) produced a notable increase in responding on the amphetamine lever suggesting that these higher doses produced discriminative stimulus effects that became more amphetamine-like [14]. In the present study, it is possible that recruitment of additional neurotransmitter systems by a high dose of MDMA may have produced discriminative stimulus effects that were more easily distinguished from saline compared to those of the lower dose. Future studies investigating the discriminative stimulus properties of high dose MDMA are required to support this idea.

Conflict of interest The authors declare that they have no conflict of interest.

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