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Tolerance to the locomotor-activating effects of 3,4-methylenedioxymethamphetamine (MDMA) predicts escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking in rats

Kevin T. Ball* and Mylissa Slane

Department of Psychology, Bloomsburg University of Pennsylvania, 400 E. 2nd St., Bloomsburg, PA, 17815, USA

Abstract

Pre-clinical studies of individual differences in addiction vulnerability have been increasing over recent years, but the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) has received relatively little attention in this regard. Previously, we reported large individual differences both in rats' initial behavioral response to experimenter-administered MDMA and their degree of behavioral sensitization to repeated administration. To determine whether these differences could predict subsequent patterns of MDMA-taking or -seeking behaviors we used the self-administration-extinction-reinstatement model to examine addiction-like behavior (i.e., escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking) in rats a priori characterized for either locomotor sensitization or tolerance to MDMA. Rats that developed tolerance to the locomotor-activating effects of MDMA had a significantly larger locomotor response to the first MDMA injection relative to rats that developed sensitization. Importantly, rats that developed tolerance subsequently displayed an escalation of MDMA self-administration over days, as well as clear cue-induced reinstatement of MDMA seeking following extinction. Conversely, rats that developed locomotor sensitization to MDMA subsequently maintained relatively stable levels of MDMA self-administration over days and showed no cue-induced reinstatement of MDMA seeking. These results show that differences in the level of psychomotor activation following acute and repeated MDMA administration can reliably predict two important addiction-like behaviors in rats, which may have implications in the prediction of compulsive MDMA use in humans.

Keywords

Cue; MDMA; Reinstatement; Self-Administration; Sensitization; Tolerance

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*Please address correspondence to: Kevin T. Ball, Department of Psychology, Bloomsburg University of Pennsylvania, 400 E. 2nd St. Bloomsburg, PA 17815, TEL: 570-389-4513, FAX: 570-389-2019, kball@bloomu.edu.

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Although pre-clinical studies of individual differences in addiction vulnerability have been increasing over recent years, the amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) has received relatively little attention in this regard. This is somewhat surprising given both the increasing use of MDMA and increasing reports that some users meet criteria for dependence or consume MDMA in very large amounts [1, 2]. Pre-clinical studies are in line with these findings and have shown that while acquisition and maintenance of MDMA self-administration is, in general, less robust compared to other psychomotor stimulants [3, 4], there is a vulnerable subgroup of animals that displays an escalation of intake over days [5, 6], a pattern of behavior suggested to be indicative of an addiction-prone phenotype [7].

In addition to differences in the pattern of MDMA self-administration, we have found large individual differences both in rats' initial behavioral response to experimenter-administered MDMA and their degree of behavioral sensitization to repeated administration [8]. Moreover, although we have reported that discrete MDMA-associated cues can reinstate extinguished MDMA seeking in an animal model of relapse [5, 9], a large degree of variability among animals exists with regard to this behavior as well. Thus, in the present study, we tested the possibility that differences in the behavioral response to acute or repeated MDMA administration are predictive of subsequent MDMA-taking or -seeking behaviors. Specifically, we used the self-administration-extinction-reinstatement model [10, 11] to examine addiction-like behavior (i.e., escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking) in rats a priori characterized for either locomotor sensitization or tolerance to MDMA.

Data were collected from adult male, Sprague-Dawley rats weighing 350–400 g at the commencement of experiments were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Rats were housed individually under standard laboratory conditions (12-hr light cycle from 7:00 AM to 7:00 PM) with *ad libitum* access to water. Rats had *ad libitum* access to food until 5 days before operant training, at which time they were placed on a restricted diet and maintained at 85 to 90% of free-feeding body weight for the remainder of the study with the exception of a period before and after surgery during which animals were given free access to food. All procedures were conducted between 9:00 AM and 5:00 PM, were in compliance with NIH guidelines, and were approved by the Bloomsburg University Institutional Animal Care and Use Committee. A total of 44 rats underwent MDMA-induced locomotor testing and then continued to the MDMA self-administration portion of the study; an additional 8 rats underwent locomotor testing only while receiving saline injections. Of the 44 rats that initially underwent MDMA-induced locomotor testing, 7 rats that began self-administration were subsequently removed from the experiment due to catheter- or health-related problems before completion of the 10 sessions.

Apparatus

Open-field ambulatory distance was measured in standard rat open-field arenas (Coulbourn Instruments, Whitehall, PA) that use a photo beam grid to track animals' location. Arenas were housed in sound-attenuating, ventilated cubicles and connected to a PC with Tru Scan

activity-monitoring software (Coulbourn Instruments). The ambulatory distance measure is the floor plane total movement distance less stereotypic movement (i.e., repetitive movements that do not contribute to large location changes progressively further from the starting point), and is a measure of locomotion.

Self-administration occurred in operant conditioning chambers (Coulbourn Instruments) that were housed in sound-attenuating, ventilated cubicles and connected to a PC with the Graphic State software interface system (Coulbourn Instruments). Each chamber was outfitted with a fluid swivel and spring leash assembly connected to a counterbalanced arm assembly. Chambers also included an active and an inactive response lever, a house light, a row of multicolored LED cue lamps, and a tone generator. A motor-driven syringe pump for drug delivery was located outside of each cubicle.

Locomotor testing

Animals were transported from their home cages and received once daily injections of ($\pm$)-MDMA (5.0 mg/kg, i.p.; dissolved in 0.9% sterile saline; weight of salt) subsequent to placement in open-field arenas for 1 hr for 5 consecutive days. Two weeks following the fifth injection, animals received a challenge injection (5.0 mg/kg) and were again placed in the open field recording chamber. A separate group of rats ($n = 8$) received saline injections according to the same schedule. Ambulatory distance was recorded on Days 1, 5, and challenge. We found previously that this treatment regimen resulted in the expression of locomotor sensitization on challenge day in a proportion of animals tested without the occurrence of focused stereotypy in any animals [8]. This was confirmed by casual observation of behavior in the present study also.

Operant training

Five days following the conclusion of locomotor testing, the rats that received MDMA injections began operant training. We used food self-administration pretraining to acclimate rats to operant responding for MDMA as in our previous studies of MDMA self-administration and reinstatement [5, 9]. This is a well-established procedure shown to facilitate acquisition of drug self-administration, but have negligible effects on maintenance, extinction, and reinstatement [12, 13]. Training began with 2 days of shaping sessions during which responses on the active lever were reinforced with 45-mg grain-based food pellets (Bio Serv, Frenchtown, NJ) contingent upon a fixed ratio (FR)-1 schedule of reinforcement. Over the course of the next 7 days the reinforcement schedule was gradually increased to FR-5 in daily 30-min sessions. Analysis of food-reinforced responding showed that there were no significant differences in response rate on any day based on subsequent behavioral phenotype classification (see below).

Surgery

Five days following the conclusion of operant training, rats underwent catheterization surgery. After an injection of atropine sulfate (0.05 mg/kg; s.c.), animals were anesthetized with ketamine HCl (90 mg/kg; i.m.) and xylazine HCl (10 mg/kg; i.m.), with supplemental injections as needed. A catheter constructed from polyethylene tubing (PE10 and PE50;

Fisher Scientific, Pittsburgh, PA) and a 22 gauge cannula-guide connector assembly (Plastics One, Roanoke, VA) was inserted into the right jugular vein. The catheter was routed subcutaneously and mounted to the skull with dental cement. During 1 week of recovery catheters were flushed with heparinized physiological saline (50 U/ml heparin) twice daily, and 0.1 ml (10 mg/ml; i.v.) of gentamycin (Lonza, Walkersville, MD) was administered once daily. During the period of MDMA self-administration, catheter patency was evaluated by injecting 0.1 ml Brevital (1%) as necessary. Loss of muscle tone within 5 s after injection indicates a patent catheter.

MDMA self-administration training

Following recovery from surgery, rats began a series of 10 daily, 2-hr MDMA self-administration sessions. During these sessions rats responded on a modified FR5 schedule under which the first response on the active lever resulted in an intravenous infusion of 0.50 mg/kg of ($\pm$)-MDMA (dissolved in 0.9% sterile saline; weight of salt) accompanied by conditioned stimuli (CS), which consisted of a tone + flashing cue light compound stimulus presented for 5 s. Drug concentration (5.0 mg/ml) and pump delivery rate (10 μ l/s) were kept constant, and dose of drug (0.50 mg/kg/infusion) was controlled by varying the duration of pump action (i.e., volume of injected solution) based on body weight. The dose was chosen based on our previous work [5, 9]. Delivery of the drug and CS was followed by a 15 s time-out period signaled by illumination of the house light. During MDMA infusions and time-outs responses were recorded but had no programmed consequences. After the first infusion, MDMA infusions and presentations of the CS were contingent upon an FR5 schedule.

Extinction sessions

On the day following the last self-administration session, a subset of rats ($n = 12$) began daily 60-min extinction sessions. This subset of rats represents the first squad of rats to undergo self-administration, minus the rats that were removed because they did not reach the criterion for sensitization or tolerance following locomotor testing (see below) or because of catheter failure before the completion of self-administration. Because our reinstatement results with this subset of rats were clear cut and statistically significant, the second squad of rats did not undergo extinction or reinstatement testing, but instead continued on to different experiments in order to collect pilot data for a separate study. During extinction sessions responses were recorded but had no programmed consequences. Extinction sessions continued for at least 5 days or until the total presses for a session were 20% of the mean number of total presses during the last three self-administration sessions.

Cue-induced reinstatement testing

Following extinction, animals underwent 1-hr CS-induced reinstatement sessions. Sessions began with a non-contingent presentation of the CS over a 5-s period followed by a 15-s time-out. Thereafter, responding was reinforced by the CS alone, contingent upon an FR-5 schedule except that the first response was reinforced by the CS.

Statistical Analyses

Following locomotor testing, rats were categorized as displaying either sensitization (SENS group) or tolerance (TOL group) based on whether their locomotor response (total ambulatory distance) to the MDMA challenge increased or decreased, respectively, by at least 20% compared with Day 1. This is a standard criterion for drug sensitization studies [14, 15]. Dependent variables were analyzed by means of one-way and two-way, ANOVAs with behavioral classification (SENS or TOL) as a between factor and day as a within factor. For time-course analyses of locomotor activity, a two-way ANOVA was used, with group (SENS, TOL, or saline) as a between factor and time (in 20-min blocks) as a within factor. One-way and two-way ANOVAs were followed by Dunnett's multiple comparison tests and Bonferroni post-tests, respectively. Finally, the relationship between locomotor activity on Day 1 of locomotor testing and subsequent behaviors (self-administration and reinstatement) was evaluated with correlation and regression analyses.

Results showed that 12 rats (27%) displayed sensitization on challenge day compared with Day 1 [mean ($\pm$ SEM) increase was 75.98 ($\pm$ 21.49) %], and 21 rats (48%) displayed tolerance [mean ($\pm$ SEM) decrease was 45.22 ($\pm$ 3.54) %]. As seen in Fig. 1a, overall locomotor activity was higher in MDMA-treated rats compared with saline treated rats [main effect of group, $F(2, 38) = 17.37, p < .001$]. However, SENS rats displayed a significantly smaller locomotor response to MDMA on Day 1 compared with TOL rats, resulting in a significant group $\times$ day interaction, $F(4, 76) = 18.98, p < .001$. The time-course analyses showed that TOL rats displayed a greater locomotor response to MDMA compared with SENS rats across the entire session on Day 1 [main effect of group, $F(2, 38) = 23.28, p < .001$; see Fig. 1b]. Locomotor activity in SENS rats was similar to saline-treated rats across the entire session on Day 1. In all groups, there was a decrease in activity across the session [main effect of time, $F(2, 76) = 12.50, p < .001$]. On Day 5 locomotor activity was similarly high in SENS and TOL rats across the entire session and both groups showed significantly higher activity compared to saline-treated rats [main effect of group, $F(2, 38) = 16.64, p < .001$; see Fig. 1c]. In all groups, there was a decrease in activity across the session on Day 5 [main effect of time, $F(2, 76) = 23.75, p < .001$], but a sharp drop in activity after the first 20 min in saline-treated rats resulted in a significant group $\times$ time interaction, $F(2, 76) = 5.18, p < .001$. Activity also was similar in SENS and TOL rats on challenge day (see Fig. 1d), and was significantly higher in both groups compared with saline-treated rats during the middle and final 20-min periods [$F(2, 38) = 8.75, p < .001$ and $F(2, 76) = 17.26, p < .001$ for main effect of group and time, respectively].

As seen in Fig. 2a, drug intake in the SENS ($n = 9$) and TOL ($n = 17$) groups was similar on Day 1 [mean ($\pm$ SEM) intake was 4.28 ($\pm$ 0.28) mg/kg and 5.62 ($\pm$ 0.61) mg/kg for SENS and TOL groups, respectively]. Relative to the TOL group, intake in the SENS group remained relatively stable across days, although there was a significant increase across days, $F(9, 8) = 2.07, p < .05$. The TOL group, however, displayed a significantly greater increase in MDMA intake across self-administration sessions ($F(9, 16) = 3.60, p < .001$), which was most prominent on Days 9 and 10 as indicated by post-hoc analyses (see Fig. 2). On Day 10 of self-administration, intake in the TOL group [mean ($\pm$ SEM) = 14.21 ($\pm$ 2.27) mg/kg] was nearly double that of the SENS group [mean ($\pm$ SEM) = 7.67 ($\pm$ 1.74) mg/kg]. When

comparing locomotor activity on Day 1 of locomotor testing and MDMA infusions/2 hr across the last 3 days of self-administration in individual rats, we found no significant correlation ($r = .14$, $p > .05$; see Fig. 2b).

As shown in Fig. 3a, overall extinction responding over the first 5 days was slightly, but not significantly, higher in TOL ($n = 7$) animals compared with SENS ($n = 5$) animals. All rats reached extinction criterion, and the days of extinction training necessary to reach extinction criterion was not significantly different between groups [mean ($\pm$ SEM) days to criterion was $8.20 (\pm 1.39)$ and $6.57 (\pm 0.48)$ for SENS and TOL groups, respectively; see Fig. 3b]. Finally, there was a significant decrease in responding in both groups when comparing mean responses across the last three self-administration sessions to the last day of extinction [$t(4) = 2.74$, $p < .05$, one-tailed and $t(6) = 4.93$, $p < .01$, one-tailed for SENS and TOL groups, respectively].

As shown in Fig. 4a, mean response rates on the active lever on the last day of extinction training for SENS and TOL groups were similar. During CS-induced reinstatement sessions, however, there were distinct group differences; while the TOL group showed clear reinstatement, the SENS group showed no reinstatement at all, with responding that was comparable to extinction. Statistical results showed a significant main effect of group [$F(1, 10) = 12.33$, $p < .01$] and session [$F(1, 10) = 11.37$, $p < .01$], as well as a significant interaction [$(1, 10) = 16.52$, $p < .01$]. Inactive lever responses were very low and not significantly different between groups or sessions (see Fig. 4b). When comparing locomotor activity on Day 1 of locomotor testing and active lever responses/hr during CS-induced reinstatement testing in individual rats, we found a strong positive correlation ($r = .84$, $p < .001$; see Fig. 4c).

Consistent with our previous results [8], we found that outbred, male Sprague-Dawley rats displayed large individual differences in their initial locomotor response to experimenter-administered MDMA and also that this initial response predicts whether rats will display psychomotor sensitization or tolerance to repeated administration. The tolerance displayed by TOL animals was not due to increases in competing focused stereotyped behaviors, which would indicate greater sensitization [8, 16]. Given that locomotor activity in SENS and TOL groups was similar on challenge day, our results could be interpreted as a highly variable first test and subsequent regression to the mean. It is noteworthy, however, that these results are consistent with those obtained with cocaine, which show that rats displaying a smaller locomotor response to acute cocaine more readily exhibit locomotor sensitization to repeated administration relative to high acute responders [17, 18].

A novel finding in the present study was that TOL rats displayed an escalation of MDMA intake across self-administration days and SENS rats did not. Indeed, by the 10th day of self-administration, the amount of MDMA taken by TOL rats was nearly double that of rats in the SENS group. Escalation of self-administration is a pattern suggested to model a key feature of human addiction [7]. Previous studies have shown that when animals are given limited access to drugs of abuse (~ 2 hr/day), they tend to display relatively stable levels of intake over days, but when given extended access (~ 6 hr/day), intake gradually escalates. In the present study, all rats had only limited access to MDMA, but a subset of rats (TOL

group) displayed an escalation of intake over days. This suggests that, at least with regard to MDMA, under some circumstances even limited access conditions can promote addiction-like patterns of self-administration in a vulnerable subpopulation of rats. Our finding that rats displaying tolerance to the locomotor activating effects of MDMA showed an escalation of intake compared to SENS rats is in line with findings that escalation of cocaine or heroin self-administration is not related or inversely related to psychomotor sensitization [19-22] (but see [23]). Importantly, however, the present study showed that *a priori* characterization of a subgroup of rats that developed tolerance to MDMA's locomotor-activating effects predicted *subsequent* escalation of MDMA self-administration, whereas the previously mentioned studies assessed sensitization *subsequent to* long-access conditions that promoted accelerated drug intake. Thus, our results extend other investigators' findings, further supporting the idea that increased psychomotor activation and increased motivation for drugs have at least partially dissociable neural substrates [24].

Another main finding in our study was that TOL rats displayed clear cue-induced reinstatement of MDMA seeking following extinction, whereas SENS rats showed no reinstatement at all. Additionally, there was a strong positive correlation between the initial locomotor response to MDMA and subsequent reinstatement responding. This finding is in line with a recent study showing that, relative to low responders, rats displaying a high locomotor response to an initial injection of cocaine displayed enhanced reinstatement of cocaine seeking following low-dose cocaine priming injections [25]. Although previous studies have shown that escalation of drug intake as a result of extended access or simply extended access in the absence of escalation are both related to enhanced reinstatement of cocaine or heroin seeking relative to short-access rats [19, 20, 22, 23, 26, 27], the present results suggest that preexisting differences among rats (as expressed in the present study by differential sensitivity to acute and chronic MDMA administration) may drive both increased intake and reinstatement of drug seeking. Although we cannot rule out the possibility that more drug seeking during reinstatement tests in TOL rats was simply the result of a greater number of drug-cue pairings during prior self-administration, this explanation would suggest that the SENS group should have shown *some* reinstatement, even if weaker than the TOL group since the SENS group had many pairings, albeit less than the TOL group. Given that the SENS group showed no reinstatement whatsoever, it is unlikely that the number of drug-cue pairings is the sole reason for the observed differences in reinstatement.

In sum, the present results show for the first time that differences in the behavioral response to acute and repeated MDMA administration can reliably predict two important addiction-like behaviors in rats: escalation of MDMA self-administration and cue-induced reinstatement of MDMA seeking after extinction. These results add to the growing pre-clinical literature showing that individual differences in addiction-like behavior are greater with MDMA compared to other psychomotor stimulants [28, 29] and may have implications in the prediction of compulsive MDMA use in humans, thus informing novel addiction treatments.

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Abbreviations

CS	conditioned stimulus
FR	fixed ratio
MDMA	3,4-methylenedioxymethamphetamine
SENS	group of animals that expressed sensitization to the locomotor-activating effects of MDMA
TOL	group of animals that expressed tolerance to the locomotor-activating effects of MDMA

Research Highlights

- Rats were characterized for locomotor sensitization or tolerance to MDMA
- Tolerance predicted escalation of MDMA self-administration over days
- Tolerance predicted cue-induced reinstatement of MDMA seeking following extinction
- Thus, locomotor tolerance to MDMA predicts addiction-like behaviors in rats

Fig. 1.

(A) MDMA-induced locomotion across treatment days. In SENS rats, a sensitized locomotor response was observed both on Day 5 and challenge compared with Day 1. In TOL rats, MDMA-induced locomotion was significantly lower both on Day 5 and challenge compared with Day 1. In saline-treated rats, locomotor activity remained relatively low and stable across days. The acute locomotor response to MDMA (Day 1) was significantly higher in TOL rats compared to SENS rats. $^{++}p < .01$ and $^{+++}p < .001$, Bonferroni post-test; $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ indicates difference from Day 1, Bonferroni post-test. (B, C, D) Time course (20-min blocks) of locomotor activity on Days 1, 5 and challenge in saline, SENS, and TOL groups. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ indicates difference from saline group, Bonferroni post-test; $^{++}p < .01$ and $^{+++}p < .001$ indicates difference from SENS group, Bonferroni post-test. All points represent mean $\pm$ SEM.

Fig. 2.

(A) MDMA self-administration (number of 0.05 mg/kg infusions per 2-hr session) across days in SENS and TOL rats. Compared to SENS rats, which showed relatively stable intake, TOL rats displayed an escalation of intake over days. $*p < .05$ and $***p < .001$ indicates difference from Day 1, Dunnett's multiple comparison test. Data are represented as mean $\pm$ SEM. (B) Regression slope for the correlation between locomotor activity on Day 1 of locomotor testing and MDMA infusions/2 hr across the last 3 days of self-administration in individual rats. There was no significant correlation between these two variables ($r = .14$, $p > .05$).

Fig. 3.

(A) Extinction responding (when responses on active lever had no programmed consequences) during daily 1-hr sessions across first 5 days in SENS and TOL rats. Responding was similar in both groups. (B) Number of days necessary to reach extinction criterion (at least 5 days or until the total presses for a session were 20% of the mean number of total presses during the last three self-administration sessions) in SENS and TOL rats. Days of extinction training was not significantly different between groups. Data are represented as mean $\pm$ SEM.

Fig. 4.

(A) Active lever presses during 1-hr CS-induced reinstatement sessions in SENS and TOL rats. Whereas the TOL group displayed clear CS-induced reinstatement of MDMA seeking, the SENS group showed no reinstatement at all. $+++p < .001$ indicates difference from SENS group, Bonferroni post-test; $***p < .001$ indicates difference from extinction, Bonferroni post-test. (B) Inactive lever presses during reinstatement sessions. Inactive responses were very low and not significantly different between groups or sessions. Data are represented as mean + SEM. (C) Regression slope for the correlation between locomotor activity on Day 1 of locomotor testing and active lever responses/hr during CS-induced reinstatement testing in individual rats. There was a strong positive correlation between these variables ($r = .84$, $p < .001$)