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Weekend Ecstasy use disrupts memory in rats

Leah M. McAleer^a, Timothy Schallert^{b,c}, and Christine L. Duvauchelle^{a,c,*}

^aThe University of Texas at Austin, College of Pharmacy, Division of Pharmacology and Toxicology, 2409 University Avenue, Stop A1915, Austin, TX, 78712, USA

^bThe University of Texas at Austin, Department of Psychology, Behavioral Neuroscience, 108 E. Dean Keeton Avenue, Stop A8000, Austin, TX 78712-1043, USA

^cWaggoner Center for Alcohol & Addiction Research, The University of Texas at Austin, 2500 Speedway, Stop A4800, Austin, TX 78712, USA

Abstract

3,4-Methylenedioxymethamphetamine (MDMA, or “Ecstasy”) is a popular recreational drug. However, its exposure is often limited to the weekends in a highly stimulating environment. The goal of this study was to investigate the behavioral domains of working and recognition memory within a model of “weekend” Ecstasy use. Rats self-administered MDMA during 2-hr sessions on two consecutive days followed by five drug-free days. Coupling this intermittent dosing schedule with a novel object recognition task, we found non-spatial memory impaired after only two “weekends” of self-administered MDMA. Our findings suggest that MDMA at recreational use levels can disrupt memory processes.

Keywords

Habituation; MDMA; Memory; Rats; Recognition; Self-Administration

1. Introduction

3, 4-Methylenedioxymethamphetamine (MDMA, or “Ecstasy”) is a popular recreational drug, most often taken by adolescents and young adults at nightclubs or raves that feature sensory-rich environments: loud music, flashing lights, crowds, and elevated temperatures [6, 7, 22]. Ecstasy users report higher euphoric effects of the drug within such highly stimulating environments [2, 14]. Indeed, 80-95% of Ecstasy use is associated with dance or rave settings while 5-15% of Ecstasy use occurs under other conditions [21].

MDMA consumption has remained steady over the past few years, but the perceived risk associated with its use has been on the decline. This is a major concern because as the perceived risk of use decreases, renewed interest in the drug and vulnerability to youthful drug use can increase [11]. Several studies report MDMA’s potential neurotoxic effects and subsequent deficits. In rats, high doses of MDMA reduce regional forebrain serotonin (5-HT) and cause degeneration of 5-HT nerve terminals [1, 15]. Among Ecstasy users, the most

*Correspondence: Christine L. Duvauchelle, PhD, Associate Professor, The University of Texas at Austin, College of Pharmacy, Division of Pharmacology and Toxicology, 2409 University Avenue, Stop A1915, Austin, TX 78712, Tel: 512-471-1090, FAX: 512-475-6088, duvauchelle@mail.utexas.edu.

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prominent behavioral deficits appear to involve memory, although the relative contribution of MDMA to these deficits is difficult to determine in poly-drug users [4, 10, 13, 16, 27].

The objective of this preliminary investigation was to assess the effects of MDMA on recognition memory by implementing a model of human recreational MDMA use, including intermittent MDMA self-administration (e.g., weekends only) within a sensory stimulating environment (e.g., flashing lights and heated test chamber). Though a number of pre-clinical studies have shown debilitating cognitive effects related to MDMA experience [17, 19], none have shown these effects as the result of “weekend-only” MDMA self-administration and environmental conditions common to MDMA use.

2. Materials and methods

2.1 Animals

Male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Ad libitum access to food and water was provided. The temperature in the colony was maintained at 20° C and the animals were kept on a 12-hour reverse light/dark cycle (lights off at 8am). Rats were handled for 10 minutes daily for 2 weeks prior to the start of lever response training and were experimentally naïve prior to the start of the study. The University of Texas at Austin Institutional Animal Care and Use Committee (IACUC) approved this experimental protocol in compliance with NIH standards. The work described in this article was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments.

2.2 Self-Administration preparation: Operant training and catheterization surgery

Rats were first trained to lever press using food reward (45 mg sucrose pellets; P.J. Noyes, Lancaster, NH) on a fixed ratio (FR1) schedule of reinforcement. Animals were food restricted (approximately 6 g of laboratory rat chow/day) until lever responding was acquired (e.g., approximately 50+ lever responses/session). Food-reinforced operant sessions were 10 minutes/day for approximately 8 days with chambers maintained at room temperature (23° C (+/- 1° C)). After completion of operant training, all animals were surgically implanted with a Silastic jugular catheter (constructed in-house using Silastic tubing and metal cannula; Plastics One, Roanoke, VA) under anesthesia. Anesthesia was maintained and delivered through a gas delivery system (VetEquip, Inc, Pleasanton, CA) and consisted of an isoflurane (2.5 – 4 %; AErrane, Baxter Healthcare, Deerfield, IL) and oxygen (0.8 l/min; Airgas Southwest, Corpus Christi, TX) mixture. Animals were allowed to recover for 7 days, with catheter patency maintained throughout, as previously described [12]. Post-surgery, animals were housed individually in polypropylene cages (45 × 23.75 × 20 cm) with wood chip bedding.

2.3 Apparatus, sensory conditions and self-administration sessions

Operant training and self-administration (SA) sessions were conducted in one-lever operant chambers (28 × 22 × 21cm) housed within sound-attenuating boxes (Med-Associates, St. Albans, VT) to limit outside noise and light. A house light within the chamber and a single retractable lever located on the right wall were activated at the start of each session. During the 2-hr SA sessions, each lever press activated a motor-driven syringe pump (Razel, St. Albans, VT) that delivered 0.1 ml of saline (e.g., Control condition) or MDMA solution over a 6-sec interval into the implanted jugular catheter. An additional stimulus light directly above the lever remained on for the injection duration. Immediately after each lever response, the lever retracted from the chamber and was inaccessible for a 20-sec time-out period. The sensory environment within the operant chamber consisted of elevated temperature and flashing lights. The temperature was kept at 32 °C (+/- 2 °C) (Repticare

Ceramic Infrared Heat Emitter, Zoo Med Laboratories, San Luis Obispo, CA) and flashing lights were activated throughout the 2-hr session (10,000 millicandelas at 4 Hz; Knog Frog Superbright Microlight, Richmond, Australia).

Self-administration sessions were conducted on 2 consecutive days/wk over a 4-wk period (4 “Weekends”, or 8 total sessions). During the five drug-free days between self-administration sessions animals remained in their home cages. For Weekends 1-2, the MDMA group received a unit dosage of 1.0 mg/kg/inj, and for Weekends 3-4 the unit dosage was 2.0 mg/kg/inj. Mean ($\pm$ SEM) session mg/kg intake and MDMA-reinforced lever responses for Weekends 1-2 were both 5.43 ($\pm$ 1.04). MDMA intake for Weekends 3-4 was 5.53 ($\pm$ 0.76) mg/kg and MDMA-reinforced lever responses were 2.76 ($\pm$ 0.37).

2.4 Test Objects

The objects used in this experiment were a variety of unfinished wooden shapes (Lara’s Crafts, Union, SC). Each week, three different, previously un-experienced shapes were used. These objects consisted of $\frac{3}{4}$ -1 $\frac{3}{4}$ ” wooden beads of various shapes (e.g., round, square, spool, peg, etc). The shapes were counterbalanced amongst the rats and across weeks.

2.5 Object Recognition Test

The Object Recognition Test (ORT) used in the current study is a 3-day procedure made up of Familiarization, Habituation and Recognition components. The ORT takes advantage of the fact that rats typically spend significantly more time exploring a novel stimulus compared to familiar stimuli [26]. The ORT procedure begins with three identical stimuli (e.g. FAM 1, 2, and 3) placed in the home cage for a 24-hr period known as the “Familiarization” phase. The next day (Day 2), Habituation trials consist of repeated exposures to a novel stimulus (e.g., N1) in the presence of FAM stimuli. On Day 3, the Recognition test consists of the introduction of a new novel object (N2) in addition to a FAM and recently novel (e.g., N1) stimulus (see Diagram 1). In control conditions, rats typically spend the most time exploring a new novel stimulus (e.g., N2), and the least amount of time attending to the most familiar (e.g., FAM) stimuli [3, 26].

2.6 ORT Testing Procedure

ORTs were conducted on self-administration Weekends 2 and 4. Day 1 of the ORT commenced one hour after completion of the first MDMA or saline self-administration weekend session, with the placement of 3 identical wooden objects (FAM 1, 2, and 3) in the home cages of all rats.

On Day 2, Habituation trials commenced one hour after completion of the second MDMA or saline self-administration weekend session. A novel object (N1) and two familiar objects (e.g., FAM 1 and 2) were placed within the home cage during a series of three 1-minute trials. Each 1-minute trial was initiated when the rat first approached any of the objects. Trials were videotaped and subsequently analyzed for the amount of exploration time spent with each object. Object exploration was defined as the direct interaction with any object and included sniffing, gnawing, climbing and pawing. All objects were removed from the cages during 1-minute inter-trial intervals. Ten minutes after the completion of Habituation trials, the Familiar objects (FAM 1, 2 and 3) were in placed back in the home cages and rats were returned to the animal colony.

24 hrs later, the Recognition test (Day 3) occurred, with all rats in a drug-free state. Rats were returned to the testing area and objects FAM 1, 2 and 3 were removed from their cages. One hour later, a New Novel object (N2) was presented alongside the recently novel (N1)

and a familiar object (FAM 1, 2 or 3). The amount of time spent exploring each object was then determined during a 1-min trial.

3. Results

Differences in object exploration time could affect subsequent performance on the ORT the following day. Therefore, independent t-tests (2-sample) were used to compare the time spent exploring objects (secs) between the Control and MDMA groups during the three 1-min Habituation trials on self-administration Weekends 2 and 4. Separate one-way ANOVAs were used to analyze the proportion of time spent exploring Familiar (2 of 3 FAM) and Novel (N1) objects in Control and MDMA groups during Habituation trials. Posthoc tests (LSD) to determine specific between-object exploration time differences were performed when ANOVAs revealed significant differences. Dependent t-tests (Correlated groups) were used to determine within group differences in the proportion of exploration time spent with New Novel (N2) and FAM objects during the Recognition Test.

3.1 Habituation Trials

Independent t-tests showed no significant differences between Control and MDMA groups in the time spent exploring objects during Habituation Trials (Weekend 2: $t(16) = -0.8491$; Weekend 4: $t(16) = -0.4046$, n.s. for both).

Control - Weekend 2—A one-way ANOVA showed significant differences in the proportion of time spent exploring the Novel (N1) and FAM objects ($F(2,8) = 35.38$; $p < 0.001$) during Habituation trials. Posthoc tests revealed that the time spent with the N1 object was significantly greater than with FAM objects (LSD, $p < 0.01$), while time spent exploring either FAM object did not differ.

Control - Weekend 4—A one-way ANOVA performed on the same measures two weeks later were similar to Week 2, with significant group differences ($F(2,8) = 18.78$; $p < 0.005$). Posthoc tests revealed more exploration time spent at N1 compared to either FAM objects (LSD, $p < 0.01$).

MDMA - Weekend 2—Exploration times between FAM and N1 objects were significantly different during Habituation trials ($F(2,8) = 6.91$; $p < 0.005$). Posthoc tests revealed time spent at N1 object was significantly greater than FAM objects ($p < 0.01$), and no differences between exploration times at FAM objects.

MDMA - Weekend 4—One-way ANOVA showed no significant differences between the amount of time spent exploring FAM and N1 objects ($F(2,8) = 1.338$, n.s.; see Fig 1A)

3.2 Recognition Tests

Control – Weekends 2 and 4—T-tests comparing the amount of time spent exploring N2 and FAM objects revealed differences in exploration times of N2 and FAM objects that were significant and approaching significance for Weekend 2 ($t(3) = 2.86$; $p = 0.06$) and Weekend 4 ($t(2) = 9.548$; $p = 0.01$).

MDMA – Weekends 2 and 4—No significant differences in exploration times between N1 and FAM objects were detected by t-tests after Weekend 2 ($t(3) = 1.24$; n.s.) or Weekend 4 ($t(3) = 2.45$; n.s.) of MDMA self-administration sessions (see Fig 1B).

4. Discussion

In the present study, ORTs revealed both short-term (Habituation) and 24-hr memory (Recognition) disruption after minimal ‘weekend’ MDMA (Ecstasy) use. Control animals preferred exploring novel (e.g., N1 or N2) rather than familiar (FAM) stimuli, as consistent with previous findings [26].

The elimination $\frac{1}{2}$ life of MDMA in rats is 1 hr, compared to 8 hrs in humans [5, 18]. Therefore, at the time of the Habituation test (e.g., 1-hr post self-administration session), when the concentration was (at most) $\frac{1}{2}$ of the self-administered dose, the pharmacological effects did not significantly interfere with Habituation during Weekend 2. However, by Weekend 4 Habituation performance significantly declined, with rats apparently unable to differentiate between objects present in the home cage for 24 hrs (FAM 1, 2 and/or 3) and an object that had not been previously encountered (N1). However, novel object recognition was more sensitive to disruption by MDMA than was habituation to novelty. After Weekends 2 and 4 of MDMA self-administration, and while in a drug-free state, animals did not appear able to distinguish between previously encountered objects (FAM and N1) and a novel object (e.g., N2).

In this study, unique patterns and environments of human Ecstasy use were modeled using MDMA self-administration on an intermittent schedule in the presence of enhanced sensory stimuli (e.g., flashing lights and elevated temperatures). Under these conditions, even small quantities of MDMA appear to disrupt memory function. As we have previously reported [8, 9], MDMA administration results in exaggerated neurochemical and behavioral responses in the presence of auditory stimuli and elevated temperatures. Evidence suggests that similar conditions may increase detrimental effects on memory processes [24].

In response to a developing tolerance of MDMA, users have been reported to “stack” multiple tablets together or “boost” successive MDMA doses over an extended period [20]. In our experiment, the MDMA dose per injection during Weekends 3-4 was increased (e.g., from 1 mg/kg/inj during Weekends 1 and 2 to 2 mg/kg/inj during Weekends 3-4) to allow animals to replicate that behavior. However, rats maintained approximately the same MDMA intake level across Weekends 1-4 (Weekends 1-2 = 5.43 (+/- 1.04) and Weekends 3-4 = 5.53 (+/- 0.76)), suggesting that the cumulative amount of MDMA intake in these animals had not been sufficient for behavioral tolerance to develop. This view corresponds with previous work from our laboratory showing that, even with alterations in MDMA unit dosage, daily intake levels remained constant for approximately 3 weeks (e.g., 15 totals sessions) before escalating to significantly higher levels [23].

Our findings are consistent with other reports of MDMA exposure leading to memory deficits. However, in the current study, cumulative MDMA intake levels accrued during Weekend sessions were quite low compared to experimenter-administered MDMA dosages [17, 19] or long-term self-administration experience [25] shown to induce memory impairment. Indeed, memory tests were conducted prior to the development of behavioral tolerance to MDMA, as indicated above by the lack of escalated MDMA self-administration. These findings suggest that an intermittent “weekend” or other model of limited MDMA experience may be a useful direction to further explore MDMA effects.

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Highlights

- Rats self-administered Ecstasy in a high sensory environment on weekends only
- Object recognition tests were used to monitor non-spatial memory
- Minimal Ecstasy exposure leads to deficits in short-term and recognition memory

Figure 1. Habituation and Recognition in Control and MDMA Groups

(A) Habituation: Prior to Weekend 4, novel object (N1) exploration times are significantly greater than familiar (FAM) objects for Control ($n=4$, Weekend 2; $n=3$ Weekend 4) and MDMA ($n=4$; both Weekends) groups (**= $p < 0.01$). However, during Weekend 4, novel object Habituation is impaired in MDMA-experienced rats. (B) Recognition: Control animals spent more exploration time at new novel objects (N2) compared to FAM objects (FAM); significantly more after Weekend 4 (**= $p < 0.01$). After Weekends 2 and 4, MDMA-experienced rats showed no significant differences in times spent exploring novel and familiar objects.

Day 1 Familiarization

Day 2 Habituation

Day 3 Recognition

Diagram 1. Object Recognition Test Schematic

Day 1 Familiarization: Three identical objects (FAM 1, 2, and 3) are placed into the home cages for 24 hrs. Day 2 Habituation: two familiar (FAM) objects and one novel object (N1) are presented during three 1-minute Habituation trials (1-min inter-trial intervals), and exploration times are recorded. Day 3 Recognition: Rats are presented with one familiar (FAM 1, 2, or 3), one recently novel (N1), and one new novel object (N2) during a 1-minute Recognition test while exploration times are recorded.