Published in final edited form as:

Brain Res Bull. 2008 October 22; 77(4): 189–196. doi:10.1016/j.brainresbull.2008.07.007.

Auditory stimuli enhance MDMA-conditioned reward and MDMA-induced nucleus accumbens dopamine, serotonin and locomotor responses

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Abstract

MDMA (3,4-methylenedioxymethamphetamine), also known as ecstasy, is a popular drug often taken in environments rich in audio and visual stimulation, such as clubs and dance parties. The present experiments were conducted to test the notion that auditory stimulation influences the rewarding effects of MDMA. In Experiment 1, a conditioned place preference (CPP) procedure was conducted in which rats received MDMA (1.5 mg/kg, s.c.) in a distinctive environment accompanied by music (65–75 dB), white noise (70 dB), or no added sound. Animals were pretreated with saline on alternating days in an alternate environment. Results revealed CPP in animals exposed to white noise during MDMA trials. For Experiment 2, rats from Experiment 1 had access to operant levers that delivered intravenous MDMA (0.5 mg/kg/inj) or saline (0.1 ml) on alternate days in the presence or absence of the same types of auditory stimuli as previously experienced. After three each of MDMA and non-reinforced (saline) sessions, animals were tested for NAcc DA and 5-HT responses to MDMA (1.5 mg/kg) or saline under the same stimulus conditions. Findings revealed that NAcc DA and 5-HT increased after an MDMA injection, and both DA and 5-HT were significantly highest in animals exposed to music during the test session. These results indicate that paired sensorial stimuli can engage the same systems activated during drug use and enhance neurochemical and behavioral responses to MDMA administration.

Keywords

3,4-Methylenedioxymethamphetamine; Conditioned place preference; MDMA reinforcement

1. Introduction

MDMA (3,4-methylenedioxymethamphetamine), also known as ecstasy, is a popular drug often taken at clubs and raves, particularly by adolescents and young adults. Raves and clubs offer environments that are rich in sensory stimulation, such as loud electronic dance music and flashing lights that may enhance the positive and subjective effects of MDMA. Indeed,

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Conflict of interest Neither of the authors have any conflict of interest to report.

it is estimated that 80–95% of people who attend raves use ecstasy, while 5–15% of young people in general report using the drug [20]. Therefore, the distinctive elements of the drug-taking environment could play a role in both the primary reinforcing effects of the drug as well as the negative consequences associated with MDMA.

Although MDMA use occurs most frequently in environments rich in sensory stimulation, little is known about the reinforcing properties of music on the ecstasy experience. Evidence suggests that music influences the dopaminergic mesolimbic pathway in the brain, the same pathway that is activated by drugs of abuse and natural rewards, such as food and sex. For instance, human fMRI studies show the mesolimbic dopaminergic system, including the NAcc and VTA, are highly activated during music processing [3,16], and music increases dopaminergic neurotransmission and neostriatal DA concentrations in spontaneously hypertensive rats (SHR) [28]. These findings indicate that music activates common neural networks involved in reinforcement and arousal, raising the possibility that MDMA and music may be mutually influential on reward pathways.

Animal models of addiction, including conditioned place preference (CPP) and drug self-administration are useful in predicting or confirming drug candidates with abuse potential in humans. However, even though MDMA is a very popular drug in party-like atmospheres, response rates and speed of acquisition for MDMA self-administration are low [24,22], especially when compared to cocaine [14]. Since human MDMA use patterns are typically intermittent [27], it is possible that the reinforcing properties of MDMA are difficult to assess using standard animal models of addiction. Another possibility is that MDMA administered in the absence of additional sensory stimuli (e.g., music, noise, lights) may not truly reflect the subjective rewarding qualities of MDMA intake within a sensory-rich environment.

One goal for the experiments presented here was to model a human condition of MDMA use by providing multiple MDMA experiences under different contingencies and environmental contexts. Through exposure to intermittent, low dosages of MDMA, we tested the hypothesis that auditory stimuli present in environments paired with MDMA influence behavioral and neurochemical responses to the drug. For the first stage of the experiment, a 6-day CPP procedure was performed in which 3 days of MDMA treatment (1.5 mg/kg) alternated with 3 days of saline injections (0.1 ml). During MDMA sessions, rats were exposed to music (65–75 dB), white noise (70 dB) or no additional sound (ambient noise approx 52 dB). CPP was assessed on Day 7 in the absence of MDMA and auditory stimuli. In Experiment 2, a subgroup of the same rats was trained to lever press for food reinforcement. The animals subsequently underwent intravenous catheterization and stereotaxic surgery to enable drug self-administration and *in vivo* microdialysis testing. During cue conditioning/operant sessions (which commenced 3 weeks after the CPP test), rats were allowed access to levers that, when activated, delivered MDMA (0.5 mg/kg/inj) or saline (0.1 ml) over 6 alternating days (3 MDMA and 3 saline sessions) in the presence of visual and auditory stimuli similar to the CPP conditions. On Day 7 of this experimental phase, rats were tested for NAcc DA and 5-HT responses to a self-administered MDMA injection or to a non-reinforced operant response in the presence or absence of auditory stimuli.

2. Materials and methods

2.1. Animals

100 adult male Sprague-Dawley rats (200–250 g at the start of the experiment), obtained from the University of Texas Animal Resource Center, were used in this study. Two weeks prior to the experiment, animals were handled daily. Rats were group housed in plastic cages; after surgery they were housed individually. They had free access to food and water and were on a 12:12 reverse light dark cycle. Animals were kept in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Public Health Service, National Institute of Health) and the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at Austin.

2.2. Drug and sensory stimuli (cues)

2.2.1. MDMA—(±)3,4-Methylenedioxymethamphetamine HCl used in this experiment was provided through the NIDA Drug Inventory Supply and Control Program (RTI International, Research Triangle Park, NC). The drug was dissolved in isotonic saline solution (0.9%) for intravenous and subcutaneous injections.

2.2.2. Sensory stimuli—Auditory stimuli consisted of music (The Very Best Euphoric House Breakdown; Telstar Records, UK) similar to the genre of music that would be played in a club or rave, and white noise (Pure White Noise, Tallahassee, FL). Two speakers produced sound at 65–75 dB, measured by a sound pressure level meter (Galaxy Audio, Wichita, KS). Visual stimuli consisted of all black or all white walls in the place conditioning apparatus and operant chambers. During operant trials, olfactory stimuli (cinnamon or rose oil-based scents) were also paired with visual and auditory cues.

2.3. Apparatus

2.3.1. Place preference—The place preference apparatus used consisted of two compartments (26.6 cm×27.6 cm×31 cm) separated by a removable piece of Plexiglas. One compartment had white walls and chicken wire over the white floor. The other compartment had black walls and a black Plexiglas floor.

2.3.2. Operant conditioning chambers—Operant chambers (28 cm×22 cm×21 cm) located within sound-attenuating compartments (Med Associates, St. Albans, VT) were used for food training, self-administration and *in vivo* microdialysis sessions. Chambers had a single lever on the right wall and a stimulus light above the lever. Three sets of photocells were located on the lower front and back walls of the chamber. Breakages in the photocell beams were recorded as locomotor activity units. Lever presses and locomotor activity were recorded during each session using a Med Pentium 100 MHz computer equipped with Med-PC software.

2.4. Conditioning and training

2.4.1. Experiment 1: Conditioned place preference

2.4.1.1. Treatment groups: In three experimental groups, animals were pretreated with MDMA (1.5 mg/kg) 5 min prior to placement into one compartment of the apparatus. After placement within the compartment, animals were exposed to music, white noise or no additional sound. On alternate days in the alternate compartment, saline pretreatment was received 5 min prior to placement and animals were not exposed to additional sound stimuli during the session. Three control groups were pretreated with saline and were placed in the compartment and exposed to music, white noise or no additional sound. On alternate days in the other compartment, these groups received saline pretreatment, but no added auditory stimulation (see CPP groups, Table 1).

2.4.1.2. CPP procedure: Animals were allowed access to both compartments of the CPP apparatus to determine the baseline preference. For this procedure, rats habituated to the apparatus for the first 5 min and were then timed for the duration spent in each compartment (e.g., black side versus white side) over the following 15 min period. Conditioning sessions commenced the next day. On Conditioning Days 1, 3, and 5, animals were injected with MDMA (1.5 mg/kg, s.c.) and confined to one side of the apparatus for 40 min. On alternate days, an equal volume of saline was injected and rats were confined to the alternate compartment for 40 min. Control groups received saline prior to placement in both sides of the apparatus for all 6 days. 24 h after the last conditioning session, animals were tested for place preference. In the test session, rats did not receive an injection, but were directly placed within the apparatus and allowed access to both compartments for 20 min. As with the baseline preference test, after a 5 min habituation period, the time spent in each compartment was measured for a total of 15 min.

2.4.2. Experiment 2: Cue conditioning/operant sessions and *in vivo* microdialysis

2.4.2.1. Food training: After completing the CPP experiment, a subgroup of animals (with equal MDMA intake experience) was food restricted (≈ 6 g of standard rat chow per day, adjusted as needed to maintain body weight) and trained to lever press for food on a FR1 schedule of reinforcement. Each lever response resulted in dispensing one sucrose pellet (45 mg; P.J. Noyes, Lancaster, NH). After the lever press response for food was acquired (approx. 3 days), 10-min food-reinforced operant sessions (FR1) were conducted for the next 6 days without food restriction.

2.4.2.2. Surgical procedure: To enable voluntary intake of MDMA and *in vivo* microdialysis testing, jugular catheterization and stereotaxic surgery to implant a guide cannula was performed. Rats were anesthetized with pentobarbital sodium (Nembutal®; 50 mg/kg, i.p.) and chloral hydrate (80 mg/kg, i.p.) as needed to prolong the anesthesia. Rats received atropine sulfate (250 μ g/rat, s.c.) for prevention of respiratory secretions, and the anti-inflammatory agent, Rimadyl (5 mg/kg, s.c.), post-surgically to alleviate pain. Catheters were constructed from 8.5 mm Silastic tubing (0.64 mm o.d.) with one end connected to a cannula endpiece (Plastics One, Roanoke, VA). The catheter was inserted into the right

jugular vein and the cannula endpiece passed subcutaneously to an incision on the head. Animals were stereotactically implanted with a unilateral guide cannula (21 g; Plastic One, Roanoke, VA) above the NAcc (AP: +0.2 mm, ML: ± 0.12 mm; DV: -2.5 mm). NAcc microdialysis probes extended 6.25 mm past the ends of the cannula. The catheter and cannula were anchored to the top of the head with four stainless steel screws and dental acrylic cement. For the first week after surgery, catheters were flushed daily with 0.1 ml of 0.9% saline that contained U/ml heparin and 67.0 mg/ml of the antibiotic drug, Timentin. Animals continued receiving the same solution daily without the Timentin component through the duration of the experiment to maintain catheter patency.

2.4.2.3. Treatment groups: Rats were assigned to one of five groups in which different auditory, visual and olfactory cues were present while rats had the opportunity to press a lever resulting in intravenous MDMA or saline injections. Group assignments for each rat were in correspondence with their previous CPP treatment conditions, except that olfactory stimuli were added as an additional cue during operant sessions. Three groups were exposed to music (MDMA+Music), white noise (MDMA+Noise) or no additional sound (MDMA Alone) during MDMA self-administration sessions, and no additional sound during non-reinforced sessions. The other groups were exposed to MDMA and no additional sound during MDMA sessions and either music (Music Alone) or white noise (Noise Alone) during non-reinforced operant sessions.

2.4.2.4. Cue conditioning/operant sessions: Conditioning sessions commenced after a 7-day surgery recovery period. During these sessions, MDMA (0.5 mg/kg per infusion) or saline (0.1 ml/inj) was available for self-administration. On Days 1, 3, and 5, rats had access to MDMA, and on Days 2, 4, and 6, lever responses resulted in saline infusions. For the first half hour of the session, the operant chamber remained dark and the lever was retracted. After 30 min, the house light came on and the cues (visual, olfactory and auditory) were presented. five s later, the lever became available for operant responding for a total of 30 min.

2.5. Microdialysis procedures

2.5.1. In vitro recovery calibration—Microdialysis probes were constructed as previously described [7] with an active membrane length of 2.5 mm at the probe tip. Prior to probe recovery, all probes were flushed with nanopure water. Recovery values for each probe were calculated by comparing the peak heights of samples to those from a standard as previously described [13]. The mean ($\pm$ S.E.M.) recovery of probes used in the experiment was $12.28 \pm 0.44\%$ for DA and $12.90 \pm 0.50\%$ for 5-HT.

2.5.2. Microdialysis probe implantation—Within 12 h after the 6th self-administration session, animals were briefly anesthetized with isoflurane and implanted with a microdialysis probe through the previously implanted guide cannula. Each microdialysis probe was connected to a 1.0 ml gastight Hamilton 1000 series syringe mounted on a syringe pump (Razel®, Model A), and freshly prepared Ringer's solution was pumped through the probe. Animals implanted with the probe remained in a holding chamber overnight with the syringe pump speed set at 0.2 μ l/min. Bedding, food, and water were

available in the holding chamber. One h prior to the test session, the pump speed was increased to 1.6 $\mu\text{l}/\text{min}$.

2.6. Test session for NAcc DA and 5-HT responses

Twenty-four h after the last self-administration session, rats were tested for their NAcc DA and 5-HT responses in one of six different test conditions. Animals that had self-administered MDMA in the presence of music or white noise were tested for DA and 5-HT responses to MDMA under the same conditions (MDMA+Music and MDMA+Noise). Animals that were exposed to music or white noise during access to the non-reinforced lever (saline) received saline under the same conditions at test (Music Alone and Noise Alone). For animals that self-administered MDMA in the absence of additional auditory stimuli, some received MDMA at test (MDMA Alone) and the others received saline (Saline Alone). For the test session, animals were placed in the operant chamber with the lever retracted for the first 30 min (baseline), as previously described for self-administration sessions. After 30 min, the house light illuminated, the lever extended into the chamber and the sensory cues associated with either MDMA or saline operant sessions were introduced into the chamber. Animals were allowed to respond once on the lever and received either a single self-administered injection of MDMA (1.5 mg/kg) or saline (0.1 ml) infused over a 6-s interval. The lever was then retracted for the remainder of the session. *In vivo* microdialysis samples were collected at 10 min intervals across the entire test session, comprising 3 10-min baseline and 3 10-min test (e.g., post-MDMA or saline injection) samples. Locomotor activity units (photobeam breakages) were assessed in correspondence with dialysis sampling.

2.7. Analysis of DA and 5-HT

High Performance Liquid Chromatography with electrochemical detection (HPLC-EC; Shizeido Capcell C-18 narrow bore column, ESA model 5200A Coulochem III Detector, Model 5041 cell and a Model 5020 Guard Cell; ESA, Inc., Chelmsford, MA) was used to determine DA and 5-HT levels. An ESA model 420 dual pistons HPLC pump pushed 0.2 ml/min Mobile Phase through the system. The mobile phase composition was as follows: sodium dihydrogen phosphate (75 mM), citric acid (4.76 mM), SDS 1 g/l, EDTA (0.5 mM), MeOH 8% and acetonitrile 11% (v/v), pH 5.6. The analytical cell potential was set at 100 mV (oxidation), guard cell potential at 400 mV, and the pump speed at 0.2 ml/min. The detection limit of DA was 0.1 nM with a signal to noise ratio of 3:1. The detection limit of 5-HT was 0.12 nM with a signal to noise ratio of 3:1. The amount of DA and 5-HT within each sample was determined by comparison with standards prepared and analyzed on the day of sample analysis. Prior to correcting for probe recovery, mean ($\pm$ S.E.M.) basal NAcc DA and 5-HT concentrations (first baseline sample) for all animals included in the dialysis portion of the experiment was calculated at 0.54 ± 0.06 nM and 0.21 ± 0.05 nM, respectively ($n = 50$). Data were collected and analyzed using an ESA Model 500 Data station.

2.8. Histology

Animals were sacrificed at the end of the experiment and brains were stored in 10% formaldehyde/30% sucrose solution. Dialysis data obtained only from animals with probe locations confirmed to be in the NAcc from 48 μ m coronal sections (see Fig. 1).

2.9. Statistical analysis

2.9.1. Conditioned place preference—Conditioned place preference was determined with the use of difference scores at baseline and test to depict the amount of time spent on each side of the place preference apparatus. For rats receiving MDMA during conditioning, difference scores were calculated by subtracting the number of seconds spent on the saline-paired side from time spent on the drug-paired side. For the control groups, difference scores were calculated by subtracting the saline-paired side from the saline+sound-paired side. Therefore, significant positive differences between the comparison of baseline and test scores reflect CPP. A one-way analysis of variance (ANOVA) was performed across all groups' baseline difference scores to confirm comparable scores across groups. CPP's were then determined by comparing MDMA-receiving groups with the Saline Control using a 2-way analysis of variance with repeated measures (Group $\times$ Time [Baseline, Test]) on the difference scores. Two additional control groups were also compared to the Saline Control in a separate 2-way ANOVA. Post hoc analyses (Fisher's Least Significant Difference) were utilized to detect significant group differences (at least $p < 0.05$) when main effects were detected in the initial analyses.

2.9.2. Operant sessions—The number of lever responses elicited during the three MDMA- and non-reinforced operant sessions was compared across groups using a three-way ANOVA (Group $\times$ Treatment Level [MDMA and Non-reinforcement] $\times$ Days).

2.9.3. Microdialysis test session—To compare the magnitude of NAcc DA and 5-HT responses between animals receiving different sensory stimuli during self-administration and test sessions, data from animals receiving MDMA and those receiving saline during the test session were analyzed using two-way ANOVA with repeated measures (Group $\times$ Time [3 Baseline and 3 Test intervals]). DA and 5-HT concentrations in nM were corrected according to probe recovery rates and converted to percent of baseline for data analyses (overall baseline averaged from within-subject means of three baseline measurements). Locomotor activity, assessed by photobeam breakages within the operant chamber during the test session, was analyzed in the same manner using two-way repeated measures ANOVAs. Post hoc analyses (Fishers LSD) for dialysis and locomotor activity data were used to detect specific group or time differences (e.g., at least $p < 0.05$) when main and/or interaction effects were indicated by overall analyses.

3. Results

3.1. Experiment 1: conditioned place preference

A one-way ANOVA performed on baseline difference scores of all experimental groups showed no significant differences [$F(5,99) = 0.506$, n.s.]. A two-way repeated measures ANOVA (Group $\times$ Time) comparing the MDMA-conditioned animals with the Saline Alone

control revealed significant Time [$F(1,70) = 28.92, p < 0.0001$] and Group effects [$F(3,70) = 3.22, p < 0.05$], but no significant Interaction [$F(3,70) = 0.814, n.s.$]. Post hoc analyses showed that at test, only the MDMA+Noise group had a significantly higher score than the Saline Alone group (see Fig. 2A). A two-way repeated measures ANOVA (Group×Time) performed on the three control conditions revealed no significant Group, Time or Group×Time Interaction [$F(2,37) = 0.290, F(1,37) = 3.660, F(2,37) = 0.06$; respectively, all n.s.; see Fig. 2B).

3.2. Experiment 2

3.2.1. Cue conditioning/operant sessions—A three-way ANOVA performed on the number of lever responses during MDMA and non-reinforced sessions showed significant effects of Group ($F(4,88) = 7.82; p < 0.0001$), Treatment Level ($F(1,88) = 119.5; p < 0.0001$), Days ($F(2,176) = 34.63; p < 0.0001$), Group×Treatment Interaction ($F(4,88) = 4.19; p < 0.01$), Group×Days Interaction ($F(8,176) = 3.45; p < 0.01$), and Treatment Level×Days Interaction ($F(2,176) = 13.91; p < 0.0001$). The Group×Treatment Level×Days Interaction effect was not significant ($F(8, 176) = 1.91; n.s.$). Main findings of post hoc tests revealed significantly higher lever responses during non-reinforced compared to MDMA sessions, and a gradual decrease in responding over the 3 days of non-reinforced sessions while MDMA responding remained stable across MDMA sessions. In addition, non-reinforced responding in the MDMA Alone, MDMA+Noise and Noise Alone groups were significantly greater on the first non-reinforced session compared to all other groups. No significant group differences were detected during MDMA sessions (see Fig. 3).

3.2.2. Microdialysis test sessions—A one-way ANOVA on baseline means ($\pm$ S.E.M.) of uncorrected DA and 5-HT nM levels showed no significant differences in absolute basal DA levels between experimental groups [$F(5,49) = 1.021$ and $F(5,49) = 1.434$, respectively, both n.s.]. Therefore, data conversion to percent of baseline did not distort between-group comparisons.

3.2.2.1. Dopamine: A two-way repeated measures ANOVA (Group×Time) showed significant effects of Group [$F(5,44) = 4.632, p = 0.002$], Time [$F(5,220) = 6.869, p < 0.001$] and Group×Time Interaction [$F(25,220) = 2.601, p < 0.001$]. Post hoc tests revealed that self-administered MDMA on test day resulted in a significant DA increase from baseline (last interval) in all groups ($p < 0.05$). In addition, the magnitude of DA response during the first 10-min interval after MDMA injection was greatest in the MDMA+Music group compared to all others ($p < 0.05$; see Fig. 4A). No significant differences in DA were observed in groups receiving saline at test (see Fig. 4B).

3.2.2.2. Serotonin: A two-way repeated measures ANOVA (Group×Time) showed significant Group [$F(5, 44) = 5.758, p < 0.001$], Time [5-HT: $F(5,220) = 11.684, p < 0.001$] and Interaction effects [$F(25,220) = 3.994, p < 0.001$]. Post hoc tests revealed that only the MDMA+Music group and MDMA Alone group showed an increase in 5-HT concentrations compared to baseline ($p < 0.05$). The MDMA+Music group had a significantly greater 5-HT response during the first 10-min interval post-MDMA injection compared to all other groups ($p < 0.05$) and higher than all groups except for the MDMA Alone group for the remainder

of the test session ($p < 0.01$; see Fig. 5A). No significant differences in 5-HT were observed in groups receiving saline at test (see Fig. 5B).

3.2.2.3. Locomotor activity: A two-way repeated measures ANOVA (Group $\times$ Time) showed significant Time [$F(5, 220) = 4.602, p = 0.001$] and Interaction effects [$F(25,220) = 1.964, p = 0.006$], but no significant Group differences [$F(5,44) = 1.887, n.s.$]. Post hoc tests revealed that the MDMA Alone and Saline Alone groups showed significantly lower locomotor activity levels during some baseline intervals ($p < 0.05$). Additional comparisons showed that the MDMA+Music group was the only group showing a significant enhancement in locomotor activity from baseline (last baseline interval) to the first 10-min post-injection interval ($p < 0.001$). As can be seen in Fig. 6A, locomotor activity during the same 10-min period was significantly higher in the MDMA+Music group than all other groups ($p < 0.05$) except for MDMA+Noise. No significant changes were observed in any group after a non-reinforced lever press, though the Saline Alone group showed lower baseline activity prior to saline infusion (see Fig. 6B).

4. Discussion

Low doses of MDMA (1.5 mg/kg/session) in the presence of white noise resulted in a significant conditioned place preference. Since white noise paired with saline did not produce CPP, these results suggest rewarding effects of white noise only in combination with MDMA. Previous studies have found that MDMA administration results in a dose-dependent CPP in rats, with optimal doses ranging from 2.0 to 10.0 mg/kg [2,15,17]. However, other non-drug factors, such as housing conditions, have been shown to influence the ability of MDMA to produce a significant CPP, with isolated animals showing a more pronounced CPP than group-housed rats [17,11]. The impact of the home cage environment on the reinforcing properties of MDMA within the CPP context suggests that non-drug factors present prior to drug use can play a critical role in the subjective rewarding value of MDMA drug experiences. Our findings indicate that non-drug factors, such as auditory stimulation, during MDMA use can also affect its conditioned rewarding value.

In Experiment 2, animals had access to MDMA under differing stimulus conditions during a total of three sessions that alternated daily with non-reinforced (saline) sessions. However, since acquisition of MDMA self-administration requires a lengthy training period of daily consecutive sessions [24,22], the self-administration opportunity of the present study was too limited to enable full acquisition of MDMA self-administration behavior. Indeed, the aim of the current study was not to establish behavioral demonstration of MDMA reward through self-administration behavior, but to allow voluntary intake of MDMA in a distinctive environment. Nevertheless, consistent with previous reports showing the eventual demonstration of MDMA reinforcement [22], rats maintained a stable number of MDMA-reinforced lever responses during these sessions. Though the number of lever responses for saline on alternate days was significantly higher than for MDMA, this observation is likely to be due to the experimental procedures of alternating reinforcement conditions. For example, one possible explanation is that early “extinction” responding on non-reinforced days accounted for the higher rate of non-reinforced operant responses. Indeed, this

phenomenon has also been shown with a similar schedule of cocaine and saline self-administration/conditioning trials [13].

In the subsequent *in vivo* microdialysis test session, animals were exposed to the same stimulus conditions as during the operant sessions and allowed to lever press for MDMA or saline. NAcc DA was significantly increased from baseline levels in all groups receiving MDMA (1.5 mg/kg, i.v.), but most dramatically when MDMA was self-administered in the presence of music. MDMA also increased 5-HT levels, but significant elevations were only observed in the MDMA Alone and MDMA+Music group. The MDMA+Noise group showed a slight, but non-significant rise in 5-HT 10 min after the self-infusion. This finding suggests that the presence of music in combination with MDMA mimics a higher dose of MDMA. If so, this pattern of DA and 5-HT responses to MDMA is consistent with a previous study in which a higher dose of MDMA (5.0 mg/kg, i.p.), significantly increased both NAcc DA and 5-HT levels, but at a lower dosage (2.5 mg/kg, i.p.), NAcc DA was significantly increased, but 5-HT levels were not [19].

As can be seen in Figs. 4B and 5B, no significant changes in DA or 5-HT were observed in response to music or white noise alone, indicating that the enhancement seen in the MDMA +Music group was synergistic. While the mechanisms underlying this effect remain unknown, evidence indicates a significant influence of auditory stimulation in the presence of MDMA and on monoamines levels. For example, significant changes in electrocortical activity were observed in rats receiving MDMA with additional acoustic stimulation compared to animals that received the same dose of MDMA without additional noise. In addition, no change in electrocortical activity was observed in animals exposed to loud white noise (95 dB) as compared to control animals that received no additional sound [12]. Other work has shown a relationship between noise alone and monoamine metabolite (DOPAC, HVA, and 5-HIAA) levels in the striatum of mice [29], though the intensity of white noise (110 dB) was much higher than that of the present study (70 dB).

It is conceivable that changes in DA and 5-HT shown in the present study could be due to increased stress in response to auditory stimulation. Indeed, noise above 80 dB can raise the pain threshold in mice, suggesting that noise at this intensity may induce a stress response [6]. However, decibel levels in the present study did not exceed 75 dB and animals exposed to music or white noise in the absence of MDMA did not show enhanced NAcc DA or 5-HT.

CPP and NAcc DA responses are commonly used measures of positive reinforcement (e.g. [13,17,11]) and were both utilized in the present study. Though both of these indices showed significant effects of MDMA in combination with auditory stimuli, it may seem curious that MDMA+Noise produced the greatest CPP while NAcc DA response was most enhanced in the MDMA+Music condition. However, it should be noted that CPP is a conditioned behavioral response, while drug-induced changes in NAcc DA are primarily pharmacological in nature. Therefore, it is not surprising that the impact of the different types of auditory stimuli on different types of reward measures is not equivalent. In addition, since CPP was determined in MDMA-naïve animals, while NAcc DA was assessed after additional MDMA and auditory stimuli experience, experience-dependent changes in

responsiveness to both the drug and auditory stimulation may have occurred. Since human MDMA experiences also commonly occur on an irregular or intermittent basis [27], the circumstances and effects of MDMA and auditory stimuli exposure in the present study may have direct clinical relevance.

Though it seems likely that the changes observed in the present study were not due to auditory stimulation-induced stress, it should be noted that noise could increase MDMA-induced toxicity. For instance, nigrostriatal DA damage was enhanced in mice following exposure to loud noise combined with MDMA [8]. In addition, mice given methamphetamine (75 mg/kg) while exposed to loud music (95 dB) experienced more seizures than mice given the same dose of methamphetamine in silence (ambient noise; 55 dB) or in the presence white noise (95 dB) [18]. These findings call for further research to determine neural mechanisms underlying synergistic effects of MDMA and auditory stimuli.

During the dialysis test session, MDMA+Music was the only group that showed a significant enhancement in locomotor activity from the last baseline interval to the first post-injection interval, correlating with the high release of DA and 5-HT seen in this group. Previous studies have shown that MDMA enhances locomotor activity in a dose-dependent manner and is capable of producing locomotor sensitization after repeated administration [5,26]. The dopaminergic and serotonergic systems have both been shown to mediate MDMA-induced locomotor hyperactivity [4,10,1]. Furthermore, like other psychostimulants, MDMA can produce conditioned locomotion after the drug has been repeatedly administered and paired with environmental cues. Ultimately, conditioned stimuli (environmental cues) alone cause an increase in locomotor activity even when the drug is not present and can act as a predictor for the animal of the drug availability [9]. Therefore, in the MDMA+Music group, it is conceivable that music acted as a conditioned stimulus that further enhanced MDMA-stimulated locomotion.

The large increases in DA and 5-HT levels observed in the MDMA+Music group may have involved inherent properties of the music. Music has variations in rhythm, pitch, loudness, and frequency that may cause an increase in arousal levels in animals. For example, avian studies have shown that rhythmic auditory stimuli, but not human speech, increased physiological arousal through enhanced noradrenaline levels [23]. The reticular activating system is known to mediate arousal levels and responds to changes in the organism's acoustic environment [25]. Therefore, it is conceivable that the reticular formation may become more sensitive to changes in auditory input in an MDMA-treated animal. On the other hand, since white noise contains an equal amount of energy per frequency band, it may suppress arousal and/or have the opposite effect of music. Our finding that the only group not showing significant MDMA-induced enhancement of 5-HT was the MDMA+Noise condition provides some support to that notion.

Evidence also suggests that music influences the same pathways in the brain that are activated by natural rewards, such as food and sex, as well as drugs of abuse. Music has been shown to improve dopaminergic neurotransmission and increase neostriatal DA concentrations in spontaneously hypertensive rats [28]. A human fMRI study showed the mesolimbic dopaminergic system, including the NAcc and VTA, are highly activated and

tightly connected during music processing [16]. The hypothalamus, an important area for the physiological and autonomic response to emotional stimuli was also activated when subjects listened to pleasant music [3]. Taken together, these findings indicate that music and MDMA may work on common neural networks involving reward and arousal and most likely influence each other. Since ecstasy is often taken in environments rich in sensory stimulation, understanding the impact of music and other auditory stimuli during the MDMA experience is crucial for evaluating MDMA effects and the potential for neurotoxicity.

Acknowledgements

This project was supported by a University of Texas Faculty Research grant to C.L.D., and a Waggoner Center for Alcohol and Addiction Research Jones Fellowship to A.A.F.

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Fig. 1. Histological diagram. Drawings are representative of active membrane regions of dialysis probes in the core and shell subterritories of nucleus accumbens (NAcc). Illustrations drawn with assistance [21]. Coronal sections of probe traces ranged from +2.70 to +1.20 mm anterior to bregma.

Fig. 2.

(A and B) Conditioned place preference. Data are represented mean ($\pm$ S.E.M.) difference scores. For MDMA-receiving groups, difference scores were time spent in the drug-paired compartment minus the saline-paired compartment before and after conditioning trials. For Control groups receiving saline on both sides, difference scores were time spent in the sound-paired compartment minus the time spent in the compartment with no additional sound. (A) Significant CPP was observed only in the MDMA+Noise group $**p < 0.01$ compared to Saline Alone). (B) No significant CPP was produced by Saline Alone, Music Alone or Noise Alone treatments.

Fig. 3.

Lever responses during cue conditioning/operant sessions. Data show combined means ($\pm$ S.E.M.) for all groups. On three occasions, rats had access to MDMA in the presence of music (MDMA+Music, $n = 11$), white noise (MDMA+Noise; $n = 10$), or with no added auditory stimuli (MDMA Alone; $n = 29$). On alternating days, lever responses were not reinforced, but animals were in the presence of music (Music Alone; $n = 6$), white noise (Noise Alone; $n = 7$), or no added sound (Saline Alone; $n = 37$).

Fig. 4.

(A and B) NAcc DA before and after voluntary intake of MDMA (A) and saline (B). Timeline represents DA levels (% of baseline mean $\pm$ S.E.M.) at 10 min intervals, from 30 min before to 30 min after a single operant response (e.g., MDMA 1.5 mg/kg, or saline 0.1 ml). (A) Following MDMA self-administration (1.5 mg/kg), all three groups showed significantly greater enhancement in NAcc DA compared to baseline following MDMA self-administration. In addition, immediately after MDMA injection, NAcc DA was significantly higher in the presence of music (MDMA+Music; $n = 11$) compared to white noise (MDMA+Noise; $n = 10$) or no additional sound (MDMA Alone; $n = 9$) ($*p < 0.05$). (B) There were no significant changes from baseline following a non-reinforced operant response in the presence of music (Music Alone; $n = 6$), white noise (Noise Alone; $n = 7$) or no additional sound (Saline Alone; $n = 7$). No other group differences were observed during the test session.

Fig. 5.

(A and B) NAcc 5-HT before and after self-administered MDMA (A) and saline (B). Timeline represents 5-HT levels (% of baseline mean $\pm$ S.E.M.) at 10 min intervals, from 30 min before to 30 min after a single operant response (e.g., MDMA 1.5 mg/kg or saline 0.1 ml). (A) Following MDMA self-administration (1.5 mg/kg), the groups receiving MDMA in the presence of music (MDMA+Music; $n = 11$) and in the absence of additional sound (MDMA Alone; $n = 9$), showed a significant increase in NAcc 5-HT compared to baseline following MDMA self-administration, while the MDMA+Noise group ($n = 10$) did not. In addition, immediately after MDMA injection, NAcc 5-HT was significantly higher in the MDMA+Music group compared to MDMA+Noise or MDMA Alone ($*p < 0.05$). (B) There were no significant changes from baseline following a non-reinforced operant response in the Music Alone ($n = 6$), Noise Alone ($n = 7$) and Saline Alone ($n = 7$) groups. No other group differences were observed during the test session.

Fig. 6.

(A and B) Locomotor activity before and after self-administration of MDMA (A) and saline (B) on microdialysis test day. The graph represents photobeam breakage means ($\pm$ S.E.M.). (A) The MDMA Alone group had significantly less baseline locomotor activity compared to all other MDMA groups ($*p < 0.05$). At the first post-MDMA interval, the MDMA+Music group was the only group showing significantly increased locomotor activity compared to the baseline interval just prior to MDMA injection ($**p < 0.001$). (B) The Saline Alone group showed significantly lower baseline activity than all other control groups ($*p < 0.05$). No change in locomotor activity was detected for any group after the non-reinforced lever response.

Table 1

Conditioned place preference groups (Experiment 1)

Groups	N-size	Compartment 1 (Days 1, 3, and 5)	Compartment 2 (Days 2, 4, and 6)
1	17	MDMA+Music	Saline Alone
2	13	MDMA+Noise	Saline Alone
3	30	MDMA Alone	Saline Alone
Control			
4	14	Saline Alone	Saline Alone
5	10	Music Alone	Saline Alone
6	16	Noise Alone	Saline Alone

Rats received MDMA (1.5 mg/kg, s.c.) 5 min before being placed in the chamber with music, white noise, or no additional sound. On alternate days an equal volume of saline (0.1 ml) was given. Control groups were pretreated with saline every day prior to conditioning and exposed to either music, white noise, or no additional sound every other day.