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Behavioral, Thermal and Neurochemical Effects Of Acute And Chronic 3,4-Methylenedioxymethamphetamine ("Ecstasy") Self-Administration

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Abstract

3,4-methylenedioxymethamphetamine (MDMA) is a popular methamphetamine derivative associated with young adults and all-night dance parties. However, the enduring effects of MDMA at voluntary intake levels have not been extensively investigated. In this study, MDMA-influenced behaviors and core temperatures were assessed over the course of 20 daily MDMA self-administration sessions in rats. *In vivo* microdialysis techniques were used in a subsequent MDMA challenge test session to determine extracellular nucleus accumbens dopamine (NAcc DA) and serotonin (5-HT) levels in MDMA-experienced and naïve animals before and after a self-administered MDMA injection (3.0 mg/kg, i.v.). During self-administration sessions, gradual and significant increases in MDMA intake and MDMA-stimulated locomotor activity were observed across sessions. Core temperature significantly decreased during initial MDMA sessions, but was unaltered by the last 10 sessions. In the MDMA challenge test, MDMA-naïve rats showed significantly higher NAcc 5-HT responses compared to MDMA-experienced rats, though MDMA experience did not affect the magnitude of NAcc DA response. The overall findings suggest that changes in MDMA-induced responses over the course of increasing levels of drug exposure may reflect the development of tolerance to a number of MDMA effects.

Introduction

Psychostimulants, such as 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy), cocaine and amphetamine cause behavioral effects, such as hyperlocomotor activity, via increased dopamine (DA) neurotransmission in specific brain areas of the mesolimbocortical pathway, including the nucleus accumbens (NAcc) and prefrontal cortex (PFC) [7,37,42]. These brain areas are part of the "reward" system associated with drugs of abuse and incentive motivational processes [24]. *In vivo* microdialysis studies in laboratory animals [12,38,73] and imaging studies in humans [68,78,79] have demonstrated the important role of mesolimbocortical DA in the rewarding effects of many drugs of abuse. However, in addition to the DA system, other systems play a crucial role in drug reward. For example, MDMA also stimulates serotonin (5-HT) release in various brain regions, and a large body of data suggests both systems might be responsible for MDMA-induced behavioral effects [7,11,13,14].

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Researchers have investigated the reinforcement efficacy of MDMA using intracranial self-stimulation [36,48], conditioned place preference [9,26] and self-administration procedures [22,62,67]. However, acquisition and response rates for MDMA self-administration are low [63,67], suggesting minimal reinforcement value, especially when compared to cocaine self-administration [47]. Nonetheless, studies demonstrate MDMA is readily self-administered by drug-naïve animals, particularly when utilizing procedures shown to produce optimal MDMA self-administration behavior [22,63,67].

Chronic exposure to drugs of abuse results in neuroadaptive processes that can produce drug tolerance and/or sensitization. One of the primary effects of sensitization is a progressive enhancement of stimulant-induced behaviors, such as increased locomotion, exploratory rearing and sniffing, and repetitive stereotyped movements, collectively termed “psychomotor” activity [27,28,58]. Tolerance occurs when drug-induced responses are decreased, making increased intake necessary to produce the former response. Tolerance to the subjective effects of Ecstasy has been described by experienced users, who also report taking 5–10 times more MDMA pills in a single session than novice users [56]. Data from experimental animals reveal that tolerance develops to a number of MDMA-mediated responses after repeated MDMA administration, including hormone secretions, heart rate and 5-HT responses [6,39]. These findings exemplify the wide range of neuroadaptations produced by MDMA exposure.

Hyperthermia is a well-known physiological effect associated with recreational MDMA consumption [64]. Animal models have shown that MDMA can produce hyperthermia or hypothermia depending on ambient temperature [49,53], and involvement of both central and peripheral mechanisms in MDMA-induced thermal dysregulation [34,71]. In addition, repeated MDMA administration has been reported to produce both sensitization- and tolerance-like effects on MDMA-induced core temperature disruption [32,39,60].

MDMA-mediated neural adaptations, including sensitization and tolerance effects, may underlie or influence positive reinforcing properties of MDMA. The purpose of this study was to investigate acute and chronic behavioral, thermal and neurochemical changes occurring as the result of voluntary MDMA intake. In this study, MDMA intake, locomotor activity and core temperature data were assessed across 20 daily MDMA self-administration sessions. In a subsequent MDMA challenge test, *in vivo* microdialysis and behavioral assessment procedures were used to determine extracellular nucleus NAcc DA, 5-HT and locomotor activity responses to a self-administered MDMA injection (3.0 mg/kg, i.v.) in MDMA-experienced and naïve rats.

Materials and Methods

Subjects

Male Sprague-Dawley rats (4 weeks, Charles River Laboratories, Inc. Wilmington, MA) were used. Rats were initially group-housed in polypropylene cages in a temperature and humidity controlled vivarium under a reversed light/dark cycle (lights on 7:00 p.m. to 7:00 a.m.). To minimize stress, animals were handled daily for three weeks prior the onset of the experiment. Food and water was available *ad libitum*, except during the initial food-training phase when animals were food restricted (≈ 6 g of rat chow). After surgery, animals were individually housed. All procedures were conducted in accordance with the Guide For The Care And Use Of Laboratory Animals (U.S. Public Health Service, National Institutes of Health).

Apparatus

Food training, self-administration sessions and *in vivo* microdialysis test sessions were conducted in one-lever operant chambers (28 × 22 × 21 cm) located within sound-attenuating compartments (Med Associates, St. Albans, VT). The self-administration chambers are housed within high-ceiling sound attenuating cubicles outfitted with air flow courses, including ventilation fans, large oval baffled air intake ports and side ports that allow cable connections from the computer. The operant chambers have stainless steel rod floors above removable waste pan. A single retractable operant lever was located on the right wall, and each lever-press was accompanied by illumination of a stimulus light located above the lever. A house light located at the top of the left metal wall was illuminated at the start of each session. Three sets of photocells, 5 mm above the rod floors and evenly spaced on the front and back walls of the chamber, were activated at the start of each session. The total number of photobeam breakages within the operant chamber was automatically assessed and used as the locomotor activity measure. During self-administration and *in vivo* microdialysis test sessions, a swivel was attached at one end by Tygon tubing to a syringe mounted on a motor-driven syringe pump (Razel, St. Albans, VT) located outside the chamber. At the other end of the swivel, spring-covered tubing (Plastics One, Roanoke, VA) was attached to the animal's catheter inlet to enable drug delivery. Experimental sessions and data collection was programmed and controlled via computer and specialized program software (Med Associates, St. Albans, VT).

Operant Training

Animals were trained in an operant chamber to lever press for a food pellet (45 mg) on a FR1 schedule of reinforcement. Once the animals learned to lever press for food (≈3 days), 10-min food-reinforced operant sessions (FR1) were conducted for the next 6 days without food restriction.

Surgery

After completion of food-reinforced operant training, animals underwent surgery for jugular catheterization and unilateral intracranial cannula implantation under sodium pentobarbital (50 mg/kg i.p.) and chloral hydrate (80 mg/kg i.p.) as previously described [19]. Briefly, a Silastic catheter (0.625 mm o.d.) was implanted in the right external jugular vein. The free end of the tubing was fused with a modified cannula termination (C313G, Plastics One, VT) and exited through a subcutaneous pathway along the side of the neck and out an incision on top of the skull. The intracranial cannula (21 g) was implanted above the NAcc (flat skull; AP +2.0; ML +/− 1.2; DV −2.5) and was secured to the skull with dental cement and four stainless steel screws (Plastics One) along with the catheter termination. A dummy cannula (Plastic One, Roanoke, VA) was inserted inside the guide cannula to prevent debris build-up within the lumen. After the surgery, animals received 0.1 ml of saline containing 67.0 mg/ml of the antibiotic, Timentin and 30 U/ml heparin through their i.v. catheters daily for the next week. Animals continued receiving the same solution daily without the Timentin component through the duration of the experiment to maintain catheter patency [25].

Drug

(+/-) 3,4-methylenedioxymethamphetamine (MDMA) was obtained through NIDA Drug Inventory Supply and Control (National Institute on Drug Abuse, Bethesda MD, USA) and was dissolved in 0.9% saline.

Self-Administration Sessions: Acquisition and Maintenance

One week after the surgery, rats started MDMA self-administration sessions. For these sessions, the MDMA dose was set at 1.0 mg/kg/inj for the first 10 days (Acquisition: Days 1–10) and 0.5 mg/kg/inj for the last 10 days (Maintenance: Days 11–20). We, and others [22,

63,67] have previously shown this procedure results in reliable MDMA self-administration behavior. A Control group (Experienced Control) with identical handling, food training and surgical history were run concurrently with the MDMA self-administration condition (Experienced MDMA). These animals had access to the lever and received saline injections of the same volume of fluid as MDMA self-administering animals (0.1 ml) after each lever response. During daily acquisition sessions, all animals had an initial 30-min habituation period with the house light off and the lever retracted, followed by 2 hrs of MDMA (or saline) availability when the lever became available. A 20-s timeout occurred after each injection, during which time the lever was retracted and MDMA or saline was unavailable. Rectal temperatures were monitored before and after each session using a 7001H model microcomputer thermometer (Physitemp, Clifton, NJ).

Dialysis probes and in vitro recovery

Microdialysis probes were constructed in-house as previously described [55]. To determine recovery rates for each probe, Hamilton syringes were filled with freshly prepared filtered artificial cerebral spinal fluid (ACSF) solution (128.3 mM NaCl, 2.68 mM KCl, 1.35 mM Ca Cl₂ and 2 mM Mg Cl₂; pH = 7.3 using 0.1N NaOH solution) and pumped continuously through the probe at a rate of 1.63 µl/min. Probes were placed in a beaker containing ascorbate (1%), 5 nM DA and 5 nM 5-HT maintained at 37°C. 10-min dialysis samples from each probe were collected, and assayed by high performance liquid chromatography coupled to electrochemical detection (HPLC-EC). Probe recovery was calculated by comparing the peak heights of each dialysate and those from a 25% recovery standard solution. Probe recovery values for DA and 5-HT were in the range of approximately 13–15%.

MDMA Challenge: Groups

In vivo microdialysis test sessions were conducted to determine basal NAcc DA and 5-HT levels before and after a single self-administered MDMA (3.0 mg/kg, i.v.) or saline (0.1 ml) injection. Experienced MDMA and Experienced Control groups were the same rats described above, with history of 20 MDMA or saline self-administration sessions. Naïve MDMA and Naïve Control groups had been handled, trained and undergone surgical procedures exactly as the Experienced groups, but had no MDMA experience.

Microdialysis Probe Implantation

On the day of the last self-administration session (Experienced groups) or at least one week after surgery (Naïve groups), rats were briefly anesthetized with 1.5% isoflurane and a microdialysis probe was lowered into the NAcc through the previously implanted guide cannula. The probe was connected to a 1.0 ml gastight Hamilton syringe mounted on a syringe pump (Razel Scientific instruments, Model A) and freshly prepared ACSF was pumped through the probe. Animals implanted with the probe were placed in a holding chamber overnight containing food, water and the same bedding as in the home cage with the syringe pump speed set at 0.261 µl/min.

MDMA Challenge: Test Session

The following day, one hour prior to the test session, the pump speed was changed to 1.63 µl/min. For the test session the animal was moved from the holding chamber into the operant chamber. As during self-administration sessions, animals did not have access to the lever for the first 30 minutes. At the end of 30 minutes, lights came on and the lever was available to the animal for MDMA or saline self-administration. Unlike the self-administration sessions, only one infusion of MDMA (3.0 mg/kg, i.v.) or saline (0.1 ml) was available. Following the infusion (i.e. after the animal pressed the lever), the lever retracted and the animals had no further opportunity to lever press. All animals elicited a lever response within 3 min after lever

presentation. Three 10-min baseline samples were collected during the first 30-min interval and six additional 10-min samples were collected after the lever response. Locomotor activity counts were assessed in matching 10-min intervals for the entire 90-min session.

Assay of dialysate

The dialysate samples were analyzed for DA and 5-HT content using HPLC-EC, as previously described [26], using a C-18 narrow bore column, ESA Model 5200 A Coulochem II Detector, Model 5020 Guard Cell and 5041 amperometric analytic cell (ESA Laboratories, Chelmsford, MA). The mobile phase contained 150 mM $\text{Na}_2\text{H}_2\text{PO}_4$, 50 μM EDTA disodium salt, 4.76 mM citric acid, 4.5–6.0 mM sodium dodecyl sulfate, 12.5% (v/v) acetonitrile, 12.5% (v/v) methanol (pH = 5.6). The flow rate was set at 0.2 ml/min and 10 μl samples were manually injected. The amount of DA and 5-HT within each 10 μl sample was determined by comparison with standards prepared and analyzed on the day of sample analysis. The limit of detection (signal to noise ratio = 3:1) was 0.36 fg/10 μl for DA, and 0.38 fg/10 μl for 5-HT. Chromatographic data was collected and analyzed using an ESA Model 500 data station.

Histology

After completion of the experiment, animals were euthanized, and microdialysis probes placement into the NAcc confirmed with histological analyses of 60 μm coronal sections stained with cresyl violet (see Fig. 1).

Statistical Analyses

Data were analyzed using repeated measures ANOVAs (one- and two-way) and independent t-tests as indicated, using GB Stat 6.5.4. Data collected from the Experienced MDMA (n=9) and Experienced Control (n=6) groups during Self-Administration Sessions (Acquisition and Maintenance Days) included Lever Responses, MDMA Intake, Core Temperature and Locomotor Activity. For these data, the within subject repeated variable was Day (e.g., 20 daily sessions). Test Session data (Baseline and Test intervals) from the same Experienced MDMA and Experienced Control groups, and additional Naïve MDMA (n=7) and Naïve Control groups (n=6) included NAcc DA, 5-HT and Locomotor Activity levels, and the within subject repeated variable was Time (e.g., 10 min intervals; 3 for Baseline and 6 for Test). DA and 5-HT analyses were performed on pg/ μl concentrations, corrected for probe recovery rates. There were 9 missing dialysate data points (out of a total of 504) due to problems with chromatography or sample collection. Degrees of freedom were adjusted accordingly in GB Stat for final analyses. Posthoc tests (Fishers LSD) were performed to determine group differences at specific test intervals when overall ANOVA analyses revealed significant main effects.

Results

Self-Administration Sessions

Lever Presses—Independent t-tests were performed on the total number of lever presses during Days 1–10 (Acquisition) and Days 11–20 (Maintenance) in the Experienced MDMA and Experienced Saline groups. During Acquisition, non-reinforced lever presses (e.g., Saline) were significantly greater than MDMA-reinforced presses ($t(13) = -3.78$; $p = 0.002$). During the Maintenance phase, MDMA-reinforced presses significantly exceeded Saline group operant responses ($t(13) = 2.27$; $p = 0.04$; see Fig 2).

MDMA Intake—A one-way ANOVA was performed on MDMA intake (mg/kg/day) across the 20 self-administration sessions (Experienced MDMA) showed significant within subject (Day) effects ($F(19,152) = 2.7$; $p = 0.0004$), reflecting the gradual rise in MDMA intake with increasing MDMA exposure. An independent t-test comparing total MDMA intake during

Acquisition and Maintenance sessions showed significantly greater intake during the Maintenance phase ($t(16) = -2.07$; $p = 0.05$; see Fig. 3).

Core Temperature—2-way repeated measures ANOVA performed on Core Temperature difference scores (After Session Core Temperature minus Before Session Core Temperature) in rats self-administering MDMA and saline revealed that the MDMA group had significantly lower core temperatures after self-administration sessions than Controls during the Acquisition interval (Days 1–10) ($F(1,13) = 9.67$; $p = 0.008$). During the Maintenance interval (Days 11–20), no significant group differences in core temperature were observed ($F(1,13) = 0.018$; n.s.; see Fig 4).

Locomotor Activity—Total locomotor activity during each of the 20 self-administration sessions was compared in MDMA and saline self-administering groups. A 2-way repeated measures ANOVA showed significant Group ($F(1,13) = 13.98$; $p = 0.002$), Day ($F(19,247) = 2.17$; $p = 0.003$) and Group X Day interaction effects ($F(19, 247) = 4.30$; $p < 0.0001$). Posthoc analyses revealed MDMA-stimulated locomotor activity was significantly greater than saline level locomotor activity during five sessions in the Acquisition interval and all 10 sessions of Maintenance (see Fig 5).

MDMA Challenge Test Session

Separate 2-way repeated measures ANOVAs were performed on Baseline and Test interval DA, 5-HT and locomotor activity as indicated below:

Extracellular DA—No significant Group or Interaction effects were detected in an overall comparison of baseline levels ($F(3,24) = 0.25$ and $F(6,48) = 0.52$, respectively; both n.s.), though significant Time effects were observed ($F(2,48) = 13.7$; $p = 0.001$). Significant Time ($F(5,120) = 7.33$; $p < 0.0001$) and Group X Time Interaction effects ($F(5,120) = 1.04$; $p = 0.02$) were observed in overall comparisons during the Test Interval. Posthoc analyses revealed that the Experienced MDMA group had significantly greater DA levels than the Experienced Control at every test interval. NAcc DA levels in the Naïve MDMA group were significantly greater than the Naïve Controls for the first 30 min of the test interval. Significant differences between the saline control conditions were not revealed with the exception of the 4th interval post-injection (see Fig 6).

Extracellular 5-HT—No significant group, time or interaction effects were detected in an overall comparison of baseline levels ($F(3,24) = 0.57$, $F(2,48) = 2.08$, and $F(6,48)$, respectively; all n.s.). However, significant group, time and interaction effects were detected in overall comparisons across the Test interval ($F(3,23) = 5.87$; $p = 0.004$; $F(5,115) = 10.19$ and $F(15,115)$; $p < 0.0001$ for both). Posthoc tests revealed MDMA-receiving groups showed significantly greater 5-HT responses than their respective controls, and significantly higher extracellular NAcc 5-HT in the Naïve compared to Experienced MDMA group. No significant differences were detected between the two saline control conditions (see Fig. 7).

Locomotor Activity—No significant group, time or interaction effects were detected in an overall comparison of baseline activity levels ($F(3,24) = 2.44$; $F(2,48) = 1.32$; $F(6,48) = 0.38$, respectively, all n.s.). During the Test interval, significant Group ($F(3,24) = 7.58$; $p = 0.001$), Time ($F(5,120) = 7.99$; $p < 0.0001$) and Interaction effects ($F(15,120) = 2.61$; $p = 0.002$) were detected. Posthoc tests revealed MDMA-receiving groups had comparable levels of increased activity immediately after MDMA administration, which were significantly higher than their respective controls. Locomotor activity in the Experienced MDMA group was significantly higher than Naïve MDMA and Experienced Control groups, but not Naïve Controls (see Fig 8).

Discussion

Ecstasy popularity spreads worldwide among teenagers and young adults, yet the addictive and/or enduring effects of this drug remain unclear. Several studies have reported detrimental effects associated with high doses of experimenter-administered MDMA [5,70,72], but this is the first study to examine effects of self-administered MDMA on body temperature, locomotor activity, drug-seeking behavior, NAcc DA and 5-HT responses in MDMA-experienced and naïve rats. Our findings show progressive changes in behavior and neurochemical responses to MDMA as the result of chronic MDMA experience. Since the observed responses to MDMA were induced by low to moderate doses of self-administered MDMA, these findings have translational relevance to human recreational MDMA use. [8,56].

When MDMA is compared to other drugs of abuse, such as methamphetamine and cocaine, it is regarded as a weaker reinforcer [23,47,80]. Nevertheless, the current study shows that MDMA can reliably support self-administration behavior over several daily sessions, in agreement with previous work [22,63,67]. In particular, the observation that the number of lever responses increased in direct correspondence with decreased MDMA unit dose confirmed that MDMA intake level regulated the rate of behavioral responding. It should also be noted that the Experienced Controls had significantly more total responses during the first 10 sessions (e.g., Acquisition), while the Experienced MDMA group made significantly more responses during the last 10 sessions (e.g., Maintenance). As indicated previously, all animals underwent food-reinforced operant training prior to surgical procedures. Similar to the present study, high rates of non-reinforced responding during initial Acquisition sessions have been previously reported [63] and are not unanticipated under the circumstances, as this is a typical response pattern during extinction of food-reinforced operant training [54].

In the present study, MDMA intake during the last 10 sessions (e.g., Maintenance) was significantly greater than intake during first 10 sessions (e.g., Acquisition), even though animals had to respond twice as many times during Maintenance to receive the same amount of drug. Differences in MDMA experience, physiological responses and drug unit doses between these phases make it difficult for direct drug intake comparisons. Nevertheless, the enhanced level of MDMA self-administration suggests that MDMA reinforcement value was altered in some way as MDMA experience increased. Increased drug intake is characteristic of drug dependence and is often interpreted as an index of tolerance to the positive effects of the drug [50]. Indeed, as mentioned above, experienced MDMA users report decreased pleasurable MDMA effects and will increase the number of pills taken per event, presumably to compensate for diminished effects of the drug [56]. However, alternate explanations for this behavior in animals have been proposed, including sensitization to the rewarding aspects of the drug and/or sensitization to the drug's incentive properties [50,82]. In the present study, whether enhanced lever responding for MDMA was due to tolerance or sensitization processes cannot be reliably determined. Nevertheless, the gradual increase in MDMA intake may reflect experience-dependent neural adaptations that directly influence motivational properties of MDMA.

In addition to MDMA intake, locomotor activity was significantly enhanced over the course of MDMA self-administration sessions. However, since MDMA intake levels varied across sessions, the most conservative estimation is that the increase in locomotor activity was a dose-dependent effect. Yet, locomotor activity observed during the MDMA challenge test suggest that the duration of MDMA-induced hyperactivity may increase with experience. For instance, locomotor activity in the Experienced and Naïve MDMA groups was comparable immediately MDMA injection (3.0 mg/kg, i.v.), but the duration of the enhanced response was longer for the Experienced compared to the Naïve group. On the other hand, since the activity in the Experienced MDMA group was comparable to the Naïve Saline group, it is difficult to argue

that these data are evidence of behavioral sensitization to MDMA, as previously reported by others after repeated MDMA use [2,3,17].

Lethal outcomes due to Ecstasy use alone are rare, but of those previously reported, hyperthermia was a major syndrome associated with immediate death [64]. In rats, changes in core temperature are influenced by the ambient temperature home cage or MDMA-taking environment [10,20,49]. MDMA administration, at ambient temperatures of 22°C or higher, is associated with hyperthermia [10,20,52,53]. However, MDMA administered at room temperature 22°C or lower induce hypothermia [20,21,49,59]. MDMA-induced hyperthermia in human subjects do not appear to be affected by ambient temperature [30]. Therefore, the occurrence and persistence of MDMA thermoregulatory disruption in rats may be more significant to the human condition than the direction of thermal response. Our study, performed in a 22°C (+/- 1.0) environment, resulted in significantly decreased core temperatures after initial MDMA sessions, but a normalization of temperature in the latter sessions. This diminishing influence of MDMA on body temperature with increasing MDMA experience suggests the development of tolerance to the thermal-disrupting effects of MDMA. These findings are in agreement with our previous work [63] and are consistent in principle to experiments showing the thermal response to MDMA (hyperthermia) occurs only after the first weekly injection of a multiple week treatment regimen [16]. MDMA-induced disruptions in thermoregulation have also been shown to persist after repeated MDMA administration [33, 39]. However, different methodologies, such as mode of MDMA administration, dosage and schedule, are likely to account for variations in thermal responses to MDMA across studies.

At the MDMA Challenge test, the Naïve and Experimental groups differed in more aspects than just drug experience. For example, the lever response elicited by the Experienced MDMA group was likely motivated by prior drug experience, while the same response in the Naïve groups may have been motivated by prior food reinforcement experience. In addition, the MDMA Challenge test context and drug experience were novel for the Naïve MDMA group but not for Experienced groups because of their previous non-reinforced and MDMA self-administration sessions. Indeed, Experienced and Naïve Controls showed differences in NAcc DA levels during the test session that may be attributed to environmental habituation in the Experienced Controls and/or novelty effects in the Naïve Controls [45]. However, since the Experienced and Naïve MDMA groups showed comparable increases in NAcc DA immediately after MDMA injection, any variations in motivation, novelty and drug experience did not appear to have a significant impact on the MDMA-stimulated NAcc DA response.

MDMA is a unique compound in the sense that it causes a massive release of 5-HT in several brain regions, in a manner similar to fenfluramine (e.g., a non-exocytotic 5-HT releaser), and also promotes DA enhancement, similar to DA releasers, such as amphetamine. In vitro and in vivo experiments describe MDMA as a more potent releaser of 5-HT compared to DA [14,31,40,69,76], while other studies have shown a greater effect on DA levels [31], or comparable DA and 5-HT effects [41].

In agreement with previous studies [7,43,44], we found that extracellular NAcc levels of DA and 5-HT were significantly increased by MDMA, and that 5-HT response was several fold greater than the DA response immediately after MDMA administration. Also consistent with previous work [6], our findings of a less pronounced NAcc 5-HT response after the MDMA challenge (3.0 mg/kg, i.v.) in Experienced (~5 fold) compared to Naïve animals (~9 fold) suggest the development of tolerance to MDMA's 5-HT effects. It may be noted that a previous study [7] comparing MDMA-evoked responses showed a greater magnitude of 5-HT response to a comparable MDMA injection than the present study (e.g., approx 18-fold versus 9-fold). However, a number of procedural differences between studies, including mode and schedule of drug delivery and variations in microdialysis techniques, could account for discrepancies in

5-HT response magnitude. For example, in the current study, animals self-administered the challenge MDMA injection (3.0 mg/kg), while in the previous study MDMA two injections (1.0 and 3.0 mg/kg) were administered by the experimenter 60-mins apart. This study also collected 20-min interval dialysate samples that passed through a microbore column during analyses. In the present study, dialysate samples were collected in 10-min intervals and utilized a narrow bore column. Under certain circumstances, differences in sampling interval alone impacts on neurotransmitter concentrations. For instance, the use of a longer sampling interval allows dialysate samples to be collected at a slower flow rate through the probe. Since microbore columns require smaller sample volumes, a very low flow rate would be the optimal way to utilize a 20-min sampling interval, as it is well known that decreasing probe flow rates increase the concentration of neurotransmitter within a dialysate sample [74].

While there is overwhelming evidence linking increased DA neurotransmission with drugs of abuse [4,24,46,77], the exact role of 5-HT in drug reinforcement remains unclear. Previous studies have suggested enhanced 5-HT transmission correlates negatively with drug self-administration. For example, pre-treatment with drugs that increase extracellular 5-HT levels are known to reduce amphetamine, methamphetamine and cocaine self-administration [15, 35,51,65]. On the other hand, clinical work has demonstrated that acute 5-HT reductions decrease cue-induced cocaine craving [1,66]. Like other drugs of abuse, long-term MDMA-induced alterations within the mesocorticolimbic pathway might contribute to MDMA dependence. Increased 5-HT activity has been associated with both potentiation and inhibition of MDMA-induced reward [29,75,80]. Our findings revealed that the NAcc 5-HT response to MDMA diminished, but the NAcc DA response did not. Therefore, the higher proportion of NAcc DA to 5-HT after chronic MDMA exposure corresponds with our observation of enhanced MDMA intake behavior by the end of MDMA self-administration sessions. These results are in agreement with previous work showing the development of tolerance to 5-HT responses [6], and also support the notion that the ratio of NAcc DA and 5-HT responses specifically influence drug-induced positive reinforcement [65,80]. The decrease in NAcc 5-HT response to MDMA, as observed in the current Experienced MDMA group, makes it reasonable to speculate that experienced Ecstasy users may suffer from 5-HT depletion. Indeed, it has been suggested [6] that 5-HT depletion induced by MDMA exposure underlies the MDMA dose escalation observed in experienced users [56]. It is conceivable that this effect of repetitive MDMA use may also contribute to deficits in cognition [61,81] and social functioning [18] particular to this drug use population.

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Literature References

1. Aronson SC, Black JE, McDougale CJ, Scanley BE, Jatlow P, Kosten TR, Heninger GR, Price LH. Serotonergic mechanisms of cocaine effects in humans. *Psychopharmacology (Berl)* 1995;1192:179–185. [PubMed: 7659765]
2. Ball KT, Budreau D, Rebec GV. Context-dependent behavioural and neuronal sensitization in striatum to MDMA (ecstasy) administration in rats. *Eur J Neurosci* 2006;241:217–228. [PubMed: 16882018]
3. Ball KT, Wellman CL, Fortenberry E, Rebec GV. Sensitizing Regimens of MDMA (Ecstasy) Elicit Enduring and Differential Structural Alterations in the Brain Motive Circuit of the Rat. *Neuroscience*. 2009
4. Bassareo V, De Luca MA, Di Chiara G. Differential impact of pavlovian drug conditioned stimuli on in vivo dopamine transmission in the rat accumbens shell and core and in the prefrontal cortex. *Psychopharmacology (Berl)* 2007;1913:689–703. [PubMed: 17072592]

5. Battaglia G, Yeh SY, De Souza EB. MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. *Pharmacol Biochem Behav* 1988;292:269–274. [PubMed: 2452449]
6. Baumann MH, Clark RD, Franken FH, Rutter JJ, Rothman RB. Tolerance to 3,4-methylenedioxymethamphetamine in rats exposed to single high-dose binges. *Neuroscience* 2008;1523:773–784. [PubMed: 18313226]
7. Baumann MH, Clark RD, Rothman RB. Locomotor stimulation produced by 3,4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. *Pharmacol Biochem Behav* 2008;902:208–217. [PubMed: 18403002]
8. Baumann MH, Wang X, Rothman RB. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. *Psychopharmacology (Berl)* 2007;1894:407–424. [PubMed: 16541247]
9. Braida D, Iosue S, Pegorini S, Sala M. 3,4 Methylenedioxymethamphetamine-induced conditioned place preference (CPP) is mediated by endocannabinoid system. *Pharmacol Res* 2005;512:177–182. [PubMed: 15629265]
10. Broening HW, Bowyer JF, Slikker W Jr. Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (+/-)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. *J Pharmacol Exp Ther* 1995;2751:325–333. [PubMed: 7562567]
11. Bubar MJ, Pack KM, Frankel PS, Cunningham KA. Effects of dopamine D1- or D2-like receptor antagonists on the hypermotive and discriminative stimulus effects of (+)-MDMA. *Psychopharmacology (Berl)* 2004;1733-4:326–336. [PubMed: 15064911]
12. Cadoni C, Solinas M, Pisanu A, Zernig G, Acquas E, Di Chiara G. Effect of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") on dopamine transmission in the nucleus accumbens shell and core. *Brain Res* 2005;10551-2:143–148. [PubMed: 16098489]
13. Callaway CW, Rempel N, Peng RY, Geyer MA. Serotonin 5-HT1-like receptors mediate hyperactivity in rats induced by 3,4-methylenedioxymethamphetamine. *Neuropsychopharmacology* 1992;72:113–127. [PubMed: 1358088]
14. Callaway CW, Wing LL, Geyer MA. Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. *J Pharmacol Exp Ther* 1990;2542:456–464. [PubMed: 1974635]
15. Carroll ME, Lac ST, Asencio M, Kragh R. Fluoxetine reduces intravenous cocaine self-administration in rats. *Pharmacol Biochem Behav* 1990;351:237–244. [PubMed: 2315363]
16. Clemens KJ, Cornish JL, Hunt GE, McGregor IS. Repeated weekly exposure to MDMA, methamphetamine or their combination Long-term behavioural and neurochemical effects in rats. *Drug Alcohol Depend.* 2006
17. Colussi-Mas J, Schenk S. Acute and sensitized response to 3,4-methylenedioxymethamphetamine in rats: different behavioral profiles reflected in different patterns of Fos expression. *Eur J Neurosci* 2008;289:1895–1910. [PubMed: 18973603]
18. Curran HV, Rees H, Hoare T, Hoshi R, Bond A. Empathy and aggression: two faces of ecstasy? A study of interpretative cognitive bias and mood change in ecstasy users. *Psychopharmacology (Berl)* 2004;1733-4:425–433. [PubMed: 14735288]
19. D'Souza MS, Duvauchelle CL. Certain or uncertain cocaine expectations influence accumbens dopamine responses to self-administered cocaine and non-rewarded operant behavior. *Eur Neuropsychopharmacol* 2008;189:628–638. [PubMed: 18499407]
20. Dafters RI. Effect of ambient temperature on hyperthermia and hyperkinesia induced by 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") in rats. *Psychopharmacology (Berl)* 1994;1143:505–508. [PubMed: 7855209]
21. Dafters RI, Lynch E. Persistent loss of thermoregulation in the rat induced by 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") but not by fenfluramine. *Psychopharmacology (Berl)* 1998;1382:207–212. [PubMed: 9718291]
22. Daniela E, Brennan K, Gittings D, Hely L, Schenk S. Effect of SCH 23390 on (+/-)-3,4-methylenedioxymethamphetamine hyperactivity and self-administration in rats. *Pharmacol Biochem Behav* 2004;774:745–750. [PubMed: 15099919]

23. De La Garza R 2nd, Fabrizio KR, Gupta A. Relevance of rodent models of intravenous MDMA self-administration to human MDMA consumption patterns. *Psychopharmacology (Berl)* 2007;1894:425–434. [PubMed: 16470404]
24. Di Chiara G. A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. *J Psychopharmacol* 1998;121:54–67. [PubMed: 9584969]
25. Emmett-Oglesby MW, Peltier RL, Depoortere RY, Pickering CL, Hooper ML, Gong YH, Lane JD. Tolerance to self-administration of cocaine in rats: time course and dose-response determination using a multi-dose method. *Drug Alcohol Depend* 1993;323:247–256. [PubMed: 8348876]
26. Feduccia AA, Duvauchelle CL. Auditory stimuli enhances MDMA-conditioned reward and MDMA-induced nucleus accumbens dopamine, serotonin and locomotor responses. *Brain Res Bull.* 2008
27. Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biol Psychiatry* 2005;589:751–759. [PubMed: 16098484]
28. Flagel SB, Robinson TE. Quantifying the psychomotor activating effects of cocaine in the rat. *Behav Pharmacol* 2007;184:297–302. [PubMed: 17551322]
29. Fletcher PJ, Korth KM, Robinson SR, Baker GB. Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies on locomotor activity and responding for conditioned reinforcement. *Psychopharmacology (Berl)* 2002;1623:282–291. [PubMed: 12122486]
30. Freedman RR, Johanson CE, Tancer ME. Thermoregulatory effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology (Berl)* 2005;1832:248–256. [PubMed: 16163516]
31. Gough B, Ali SF, Slikker W Jr, Holson RR. Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoamines in rat caudate. *Pharmacol Biochem Behav* 1991;393:619–623. [PubMed: 1723797]
32. Green AR, O'Shea E, Colado MI. A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. *Eur J Pharmacol* 2004;5001-3:3–13. [PubMed: 15464016]
33. Green AR, Sanchez V, O'Shea E, Saadat KS, Elliott JM, Colado MI. Effect of ambient temperature and a prior neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA) on the hyperthermic response of rats to a single or repeated ('binge' ingestion) low dose of MDMA. *Psychopharmacology (Berl)* 2004;1733-4:264–269. [PubMed: 14726996]
34. Herin DV, Liu S, Ullrich T, Rice KC, Cunningham KA. Role of the serotonin 5-HT_{2A} receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine. *Psychopharmacology (Berl)* 2005;1784:505–513. [PubMed: 15765263]
35. Howell LL, Byrd LD. Serotonergic modulation of the behavioral effects of cocaine in the squirrel monkey. *J Pharmacol Exp Ther* 1995;2753:1551–1559. [PubMed: 8531128]
36. Hubner CB, Bird M, Rassnick S, Kornetsky C. The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. *Psychopharmacology (Berl)* 1988;951:49–51. [PubMed: 2898790]
37. Ikegami A, Duvauchelle CL. Nucleus accumbens and medial prefrontal cortex dopaminergic response to self-administered cocaine in naive rats. *Neurosci Lett* 2004;3543:205–208. [PubMed: 14700732]
38. Ikegami A, Olsen CM, D'Souza MS, Duvauchelle CL. Experience-dependent effects of cocaine self-administration/conditioning on prefrontal and accumbens dopamine responses. *Behav Neurosci* 2007;1212:389–400. [PubMed: 17469929]
39. Jaehne EJ, Salem A, Irvine RJ. The effect of long-term repeated exposure to 3,4-methylenedioxymethamphetamine on cardiovascular and thermoregulatory changes. *Psychopharmacology (Berl)* 2008;2012:161–170. [PubMed: 18679656]
40. Johnson MP, Hoffman AJ, Nichols DE. Effects of the enantiomers of MDA, MDMA and related analogues on [3H]serotonin and [3H]dopamine release from superfused rat brain slices. *Eur J Pharmacol* 1986;1322-3:269–276. [PubMed: 2880735]
41. Kankaanpaa A, Meririnne E, Lillsunde P, Seppala T. The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. *Pharmacol Biochem Behav* 1998;594:1003–1009. [PubMed: 9586861]
42. Kelly PH, Iversen SD. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. *Eur J Pharmacol* 1976;401:45–56. [PubMed: 1033072]

43. Koch S, Galloway MP. MDMA induced dopamine release in vivo: role of endogenous serotonin. *J Neural Transm* 1997;1042-3:135–146. [PubMed: 9203077]
44. Kurling S, Kankaanpää A, Seppälä T. Sub-chronic nandrolone treatment modifies neurochemical and behavioral effects of amphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in rats. *Behav Brain Res* 2008;1891:191–201. [PubMed: 18261810]
45. Ladurelle N, Roques BP, Dauge V. The transfer of rats from a familiar to a novel environment prolongs the increase of extracellular dopamine efflux induced by CCK8 in the posterior nucleus accumbens. *J Neurosci* 1995;154:3118–3127. [PubMed: 7722650]
46. Leyton M. Conditioned and sensitized responses to stimulant drugs in humans. *Prog Neuropsychopharmacol Biol Psychiatry* 2007;318:1601–1613. [PubMed: 17888557]
47. Lile JA, Ross JT, Nader MA. A comparison of the reinforcing efficacy of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") with cocaine in rhesus monkeys. *Drug Alcohol Depend* 2005;782:135–140. [PubMed: 15845316]
48. Lin HQ, Jackson DM, Atrens DM, Christie MJ, McGregor IS. Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. *Brain Res* 1997;7442:351–357. [PubMed: 9027397]
49. Malberg JE, Seiden LS. Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. *J Neurosci* 1998;1813:5086–5094. [PubMed: 9634574]
50. Mendelson JH, Sholar M, Mello NK, Teoh SK, Sholar JW. Cocaine tolerance: behavioral, cardiovascular, and neuroendocrine function in men. *Neuropsychopharmacology* 1998;184:263–271. [PubMed: 9509494]
51. Munzar P, Baumann MH, Shoaib M, Goldberg SR. Effects of dopamine and serotonin-releasing agents on methamphetamine discrimination and self-administration in rats. *Psychopharmacology (Berl)* 1999;1413:287–296. [PubMed: 10027510]
52. Nash JF Jr, Meltzer HY, Gudelsky GA. Elevation of serum prolactin and corticosterone concentrations in the rat after the administration of 3,4-methylenedioxymethamphetamine. *J Pharmacol Exp Ther* 1988;2453:873–879. [PubMed: 2898523]
53. O'Shea E, Escobedo I, Orio L, Sanchez V, Navarro M, Green AR, Colado MI. Elevation of ambient room temperature has differential effects on MDMA-induced 5-HT and dopamine release in striatum and nucleus accumbens of rats. *Neuropsychopharmacology* 2005;307:1312–1323. [PubMed: 15688085]
54. Olds ME, Christenson T. Effects of drive and training on extinction after self-stimulation and food reward. *Am J Physiol* 1970;2191:208–213. [PubMed: 5424846]
55. Olsen CM, Duvauchelle CL. Intra-prefrontal cortex injections of SCH 23390 influence nucleus accumbens dopamine levels 24 h post-infusion. *Brain Res* 2001;9221:80–86. [PubMed: 11730704]
56. Parrott AC. Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or Ecstasy. *J Psychopharmacol* 2005;191:71–83. [PubMed: 15671132]
57. The rat brain: in stereotaxic coordinates (atlas & Computer Graphics Files). San Diego: Academic Press, Inc.; 1997. Pages
58. Pierce RC, Kalivas PW. Locomotor behavior. *Curr Protoc Neurosci* 2007;Chapter 8:1–8. [PubMed: 18428664]
59. Piper BJ, Fraiman JB, Meyer JS. Repeated MDMA ("Ecstasy") exposure in adolescent male rats alters temperature regulation, spontaneous motor activity, attention, and serotonin transporter binding. *Dev Psychobiol* 2005;472:145–157. [PubMed: 16136551]
60. Piper BJ, Meyer JS. Increased responsiveness to MDMA in adult rats treated neonatally with MDMA. *Neurotoxicol Teratol* 2006;281:95–102. [PubMed: 16271852]
61. Quednow BB, Jessen F, Kuhn KU, Maier W, Daum I, Wagner M. Memory deficits in abstinent MDMA (ecstasy) users: neuropsychological evidence of frontal dysfunction. *J Psychopharmacol* 2006;203:373–384. [PubMed: 16574711]
62. Ratzenboeck E, Saria A, Kriechbaum N, Zernig G. Reinforcing effects of MDMA ("ecstasy") in drug-naïve and cocaine-trained rats. *Pharmacology* 2001;623:138–144. [PubMed: 11287814]
63. Reveron ME, Maier EY, Duvauchelle CL. Experience-dependent changes in temperature and behavioral activity induced by MDMA. *Physiol Behav* 2006;358–363. [PubMed: 16876209]

64. Rogers G, Elston J, Garside R, Roome C, Taylor R, Younger P, Zawada A, Somerville M. The harmful health effects of recreational ecstasy: a systematic review of observational evidence. *Health Technol Assess* 2009;136:1–315. iii-iv, ix-xii.
65. Rothman RB, Baumann MH. Balance between dopamine and serotonin release modulates behavioral effects of amphetamine-type drugs. *Ann N Y Acad Sci* 2006;1074:245–260. [PubMed: 17105921]
66. Satel SL, Krystal JH, Delgado PL, Kosten TR, Charney DS. Tryptophan depletion and attenuation of cue-induced craving for cocaine. *Am J Psychiatry* 1995;1525:778–783. [PubMed: 7726319]
67. Schenk S, Gittings D, Johnstone M, Daniela E. Development, maintenance and temporal pattern of self-administration maintained by ecstasy (MDMA) in rats. *Psychopharmacology (Berl)* 2003;1691:21–27. [PubMed: 12774185]
68. Schlaepfer TE, Pearlson GD, Wong DF, Marengo S, Dannals RF. PET study of competition between intravenous cocaine and [¹¹C]raclopride at dopamine receptors in human subjects. *Am J Psychiatry* 1997;1549:1209–1213. [PubMed: 9286178]
69. Schmidt CJ, Levin JA, Lovenberg W. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem Pharmacol* 1987;365:747–755. [PubMed: 2881549]
70. Schmidt CJ, Wu L, Lovenberg W. Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. *Eur J Pharmacol* 1986;1241-2:175–178. [PubMed: 2424776]
71. Sprague JE, Moze P, Caden D, Rusyniak DE, Holmes C, Goldstein DS, Mills EM. Carvedilol reverses hyperthermia and attenuates rhabdomyolysis induced by 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) in an animal model. *Crit Care Med* 2005;336:1311–1316. [PubMed: 15942349]
72. Stone DM, Johnson M, Hanson GR, Gibb JW. A comparison of the neurotoxic potential of methylenedioxymethamphetamine (MDA) and its N-methylated and N-ethylated derivatives. *European Journal of Pharmacology* 1987;1342:245–248. [PubMed: 2883012]
73. Tanda G, Pontieri FE, Frau R, Di Chiara G. Contribution of blockade of the noradrenaline carrier to the increase of extracellular dopamine in the rat prefrontal cortex by amphetamine and cocaine. *Eur J Neurosci* 1997;910:2077–2085. [PubMed: 9421168]
74. Tang A, Bungay PM, Gonzales RA. Characterization of probe and tissue factors that influence interpretation of quantitative microdialysis experiments for dopamine. *J Neurosci Methods* 2003;1261:1–11. [PubMed: 12788497]
75. Trigo JM, Renoir T, Lanfumey L, Hamon M, Lesch KP, Robledo P, Maldonado R. 3,4-methylenedioxymethamphetamine self-administration is abolished in serotonin transporter knockout mice. *Biol Psychiatry* 2007;626:669–679. [PubMed: 17306775]
76. Verrico CD, Miller GM, Madras BK. MDMA (Ecstasy) and human dopamine, norepinephrine, and serotonin transporters: implications for MDMA-induced neurotoxicity and treatment. *Psychopharmacology (Berl)* 2007;1894:489–503. [PubMed: 16220332]
77. Volkow ND, Fowler JS, Wang GJ. The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. *Neuropharmacology* 2004;47:3–13. [PubMed: 15464121]
78. Volkow ND, Fowler JS, Wang GJ, Ding YS, Gatley SJ. Role of dopamine in the therapeutic and reinforcing effects of methylphenidate in humans: results from imaging studies. *European Neuro psychopharmacology* 2002;126:557–566.
79. Volkow ND, Fowler JS, Wang GJ, Goldstein RZ. Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. *Neurobiology of Learning & Memory* 2002;783:610–624. [PubMed: 12559839]
80. Wang Z, Woolverton WL. Estimating the relative reinforcing strength of (+/-)-3,4-methylenedioxymethamphetamine (MDMA) and its isomers in rhesus monkeys: comparison to (+)-methamphetamine. *Psychopharmacology (Berl)* 2007;1894:483–488. [PubMed: 17063335]
81. Zakzanis KK, Campbell Z. Memory impairment in now abstinent MDMA users and continued users: a longitudinal follow-up. *Neurology* 2006;665:740–741. [PubMed: 16534114]
82. Zernig G, Ahmed SH, Cardinal RN, Morgan D, Acquas E, Foltin RW, Vezina P, Negus SS, Crespo JA, Stockl P, Grubinger P, Madlung E, Haring C, Kurz M, Saria A. Explaining the escalation of drug use in substance dependence: models and appropriate animal laboratory tests. *Pharmacology* 2007;802-3:65–119. [PubMed: 17570954]

Fig 1. Schematic representations of active dialysis probe membrane regions in the NAcc of MDMA and saline-treated rats completing the dialysis experiment (N=28). Probe locations indicate that dialysate was collected predominantly from both the core and shell NAcc regions of each animal. Numbers depicted next to each brain slice indicate mm anterior to Bregma. The diagram was drawn with the assistance of the atlas of Paxinos and Watson [57].

Fig 2. Self-Administration Sessions: *Lever Responses*. Mean total ($\pm$ SEM) lever responses during MDMA (n=9) and non-reinforced (Saline; n=6) self-administration sessions. During Acquisition, lever responses were highest in rats receiving Saline infusions. During Maintenance, MDMA-reinforced lever presses surpassed non-reinforced responding (*, ** = significant differences between groups @ $p < 0.05$, 0.01).

Fig 3.

Self-Administration Sessions: *MDMA Intake*. Data points represent daily mean ($\pm$ SEM) MDMA intake (mg/kg) during self-administration sessions ($n=9$). Inset shows the mean ($\pm$ SEM) cumulative intake totals for Acquisition (Days 1–10) and Maintenance (Days 11–20) intervals. MDMA intake was significantly greater during the last 10 MDMA sessions (Maintenance) compared to the first 10 sessions (* = significant difference between groups @ $p < 0.05$).

Fig 4. Self-Administration Sessions: *Core Temperature Difference Scores*. Data represent the mean ($\pm$ SEM) of core temperature difference scores (After Session minus Before Session) in rats self-administering MDMA (n=9) and Saline (n=6). MDMA significantly decreased core temperature during Acquisition (Days 1–10), but not during the Maintenance phase (Days 11–20).

Fig 5.

Self-Administration Sessions: *Locomotor Activity*. Data represent the mean ($\pm$ SEM) total activity counts recorded during daily self-administration sessions for MDMA (n=9) and Saline (n=6) groups. Rats self-administering MDMA showed significantly greater locomotor activity counts during daily sessions than non-reinforced rats (*, ** = significant differences @ $p < 0.05$, 0.01).

Fig 6. MDMA Challenge Test Session: *Extracellular NAcc DA*. Data represent mean ($\pm$ SEM) NAcc DA levels (pg/ul; adjusted for probe recovery values) before (Baseline) and after a self-administered injection of MDMA (3.0 mg/kg) or Saline (0.1 ml) in Experienced MDMA and Experienced Saline groups (n=9 and n=6, respectively; same animals that had participated in the reported 20 daily self-administration sessions), and in Naïve MDMA (n=7) and Naïve Saline (n=6) groups (e.g., rats without previous self-administration experience). The NAcc DA response to MDMA was comparable between Experienced and Naïve MDMA groups. Experienced MDMA rats had significantly higher NAcc DA than Experienced Saline groups at every 10-min test interval (6/6) after self-administered injections. NAcc DA levels in the Naïve MDMA was significantly greater than Naïve Saline groups for the first 30 min of the test interval (^, ^^ = significant difference @ $p < 0.05$, 0.01 between MDMA-receiving group and matched control).

Fig 7. MDMA Challenge Test Session: *Extracellular NAcc 5-HT*. Data represent mean ($\pm$ SEM) NAcc 5-HT levels (pg/ul; adjusted for probe recovery values; same animals as reported above) before (Baseline) and after a self-administered injection of MDMA (3.0 mg/kg) or Saline (0.1 ml) in Experienced MDMA (n=9), Experienced Saline (n=6), Naïve MDMA (n=7) and Naïve Saline (n=6) groups. Significant differences in NAcc 5-HT levels between MDMA-receiving groups and their matched controls were observed during the first 2 10-min intervals after injections. In addition, in the first 10-min test interval, 5-HT responses in the Naïve MDMA group were significantly greater than those in the Experienced MDMA group (** = significant difference between Naïve MDMA and Experienced MDMA groups @ $p < 0.01$; ^^ = significant differences @ $p < 0.01$ between MDMA-receiving group and matched control).

Fig 8. MDMA Challenge Test Session: *Locomotor Activity*. Data represent mean ($\pm$ SEM) locomotor activity counts before (Baseline) and after a self-administered injection of MDMA (3.0 mg/kg) or Saline (0.1 ml) in Experienced MDMA (n=9), Experienced Saline (n=6), Naïve MDMA (n=7) and Naïve Saline (n=6) groups (same animals as reported above). Significantly greater activity levels in MDMA-receiving groups were observed at the first 10-min interval post-injection compared to their matched controls. Activity remained significantly higher for the entire test duration in the Experienced MDMA compared to the Experienced Saline Group, and significantly higher than the Naïve MDMA group for 3 out of 6 test intervals. Activity levels in the Naïve MDMA group dropped from significantly greater than the Naïve Saline group to significantly lower during 4/6 of the 10-min test intervals. (**, * = significant difference between Experienced MDMA and Naïve MDMA and groups @ $p < 0.01$; 0.05, respectively; ^^, ^ = significant differences @ $p < 0.01$, 0.05, respectively between MDMA-receiving groups and matched controls).