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Heat increases MDMA-enhanced NAcc 5-HT and body temperature, but not MDMA self-administration

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Abstract

There is concern that hot environments enhance adverse effects of 3,4-methylenedioxymethamphetamine (MDMA or “Ecstasy”). In this study, long-term (4-wks) daily MDMA self-administration sessions and an MDMA challenge test were conducted with rats under normal and high thermal conditions (23° or 32° C). During MDMA self-administration sessions, activity and body temperature were increased by heat or MDMA experience, while MDMA self-administration rates increased with experience, but were comparable between thermal conditions. At the MDMA challenge test (3.0 mg/kg, i.v.), *in vivo* microdialysis showed nucleus accumbens serotonin (NAcc 5-HT) and dopamine (DA) responses were significantly increased in both thermal conditions. In the heated environment, MDMA-stimulated 5-HT responses and core temperature (but not DA) were significantly greater than at room temperature. Though the heated environment did not acutely boost MDMA intake, exaggerated NAcc 5-HT responses to MDMA may result in 5-HT depletion; a condition associated with Ecstasy use escalation and neural dysfunctions altering mood and cognition.

Keywords

3,4-methylenedioxymethamphetamine; MDMA reinforcement; ambient temperature; heat

1. Introduction

The amphetamine derivative, 3,4-methylenedioxymethamphetamine (MDMA), is a major component of Ecstasy, a commonly abused drug that is particularly popular among electronic dance music enthusiasts and club goers. Nightclubs and raves feature loud techno music, laser lights, crowded and hot social environments that attract Ecstasy users. Indeed,

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human subjects report a higher euphoric state when taking the drug in sensory rich environments, as compared to people who take the drug in less stimulating contexts (McElrath et al. 2002; Parrott 2004; Bedi et al. 2006). Though MDMA-induced lethality is rare, the use of MDMA is associated with several negative consequences such as cardiac arrhythmias, renal failure, rhabdomyolysis, cognitive deficits, negative affect and aggressive bias (Kalant 2001; Curran et al. 2004; Hall et al. 2006; Indlekofer et al. 2009). It has been proposed that elevated ambient temperatures, such as those encountered in rave venues, can exacerbate MDMA-induced temperature-increasing effects and the likelihood of adverse drug effects (Parrott 2002; Parrott 2004).

MDMA increases extracellular levels of both serotonin (5-HT) and dopamine (DA) (Gudelsky et al. 1996; Kankaanpää et al. 1998). Several studies show the increase in magnitude is greater for 5-HT (e.g., Verrico et al. 2007; Baumann et al. 2008), though others report greater DA effects (Gough et al. 1991). As the specific receptor subtypes, D1 and 5-HT_{2A} have been shown to influence thermoregulatory responses (Benamar et al. 2008; Shioda et al. 2008), the combined enhancement of 5-HT and DA may contribute to MDMA's unique effects on thermoregulation. In animal studies, MDMA has been reported to induce both hypothermia and hyperthermia, depending on several factors including MDMA dosage, amount of MDMA experience, and environmental ambient temperature (Malberg et al. 1998). High MDMA doses (20 mg/kg, i.p.) reliably produce hyperthermia in rats (Benamar et al. 2008), but a heated environment (e.g., 30° C) can elicit hyperthermia from a lower MDMA dose (10 mg/kg, i.p.) (Hargreaves et al. 2007). In rodents, the magnitude of the hyperthermic response has been tightly correlated with MDMA-induced 5-HT depletion in various brain regions (Broening et al. 1995; Malberg et al. 1998; Sanchez et al. 2004).

MDMA is thought to be of lower reinforcement value than other abused drugs, such as cocaine (Lile et al. 2005), but is reliably self-administered by rodents (e.g., Daniela et al. 2004). We previously reported that initial response rates for MDMA are low, but that MDMA-reinforced responding increases with experience (Reveron et al. 2006; Reveron et al. 2010). These findings are in line with other MDMA effects that are also experience-dependent. For example, with increasing levels of MDMA exposure, MDMA-stimulated activity levels, behavioral sensitization and temperature dysregulation effects are enhanced (Ratzenboeck et al. 2001; Schenk et al. 2003; Daniela et al. 2004; Kalivas et al. 1998; Reveron et al. 2006; Schenk et al. 2007).

In the present study, operant chambers were maintained at either 23° C (e.g., Room Temperature) or 32° C (e.g., High Temperature). During 20 daily 2-hr sessions, operant-trained rats had the opportunity to press a lever that delivered either MDMA or saline. Following completion of the self-administration sessions, a 1-hr MDMA Challenge test was conducted using *in vivo* microdialysis techniques. For this test, animals were placed in the operant chamber under the same thermal conditions as during self-administration sessions and were allowed to elicit a single lever response that resulted in either MDMA- (3.0 mg/kg) or saline (0.1 ml). Dialysate samples collected in 10-min intervals enabled the determination of NAcc 5-HT and DA levels under differing thermal conditions.

2. Experimental Procedures

2.1. Animals

Male Sprague-Dawley rats (5 weeks old, Charles River Laboratories, Inc., Wilmington, MA) were housed in an animal colony (ambient temperature 22 ±1° C) in clear cages with a 12:12 reverse light dark cycle. Laboratory food pellets and water were available *ad libitum*. Rats underwent 2 weeks of handling before the start of the experiment. All

protocols and procedures were 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. Apparatus

2.2.1. Operant Chambers—All experimental sessions were conducted in operant chambers (28 × 22 × 21 cm) located within sound-attenuating compartments (Med Associates, St. Albans, VT). A house light within the chamber and a single retractable lever located on the right wall were activated at the start of each session. During self-administration sessions, the catheter inlet from each rat was connected to spring-covered tubing (Plastics One, Roanoke, VA) attached to a drug swivel mounted on a balancing arm. Tygon tubing attached at the other end of the drug swivel extended to a 10 cc syringe, containing MDMA or saline solution, that was mounted on a motor-driven syringe pump (Razel, St. Albans, VT). Each lever press activated the syringe pump that delivered 0.1 ml of saline or MDMA solution. A stimulus light directly above the lever remained on for the duration of the injection (6 sec). To guard against non-specific lever activation, immediately after each lever response, the lever retracted from the chamber and was not accessible for a 20-sec time-out period. Three sets of photocells, spaced evenly apart across the lower front and back walls of the chamber detected photobeam breakages that were used as an index of locomotor activity. Lever presses and photobeam breakage were recorded during each session by a Med Pentium 100 MHZ computer equipped with Med-PC software.

2.2.2. Thermal Control System—Ceramic infrared heat emitters (Big Apple Herpetological Inc., Holbrook, NY) controlled by a proportional thermostat (Big Apple Herpetological Inc., Holbrook, NY) and digital thermometers (Fisher Scientific, Pittsburgh, PA) were used to regulate and maintain operant chamber temperatures at either 23° or 32° C (+/- 1° C) during experimental sessions.

2.3. Food Training

Rats were trained to lever press using food reward (45 mg sucrose pellets; P.J. Noyes, Lancaster, NH) and a fixed ratio (FR1) schedule of reinforcement. Animals were food restricted (approximately 6 g of laboratory rat chow per day, adjusted to maintain weight) until lever responding was acquired (e.g., 50+ lever responses/session). Food-reinforced operant sessions were 10 min/day for approx 8 days with chambers maintained at room temperature (23 °C (+/- 1° C)).

2.4. Surgical Procedures

For intravenous drug or saline delivery and *in vivo* microdialysis procedures, jugular catheterization and stereotaxic surgery for guide cannula implantation was performed as previously described (Feduccia et al. 2008). During the surgical procedure, rats were anesthetized with 2.5% isoflurane (VetEquip, Pleasanton, CA) vaporized in oxygen at a flow rate of 0.8 L/min. Coordinates for the unilateral guide cannula (21 g; Plastic One, Roanoke, VA) aimed above the NAcc were as follows: AP: + 0.2 mm, ML: +/- 0.12 mm, DV: -2.5 mm. After surgery, Rimadyl (5 mg/kg, s.c.) was administered for prophylactic pain relief. To maintain patency, jugular catheters were flushed daily with 0.1 ml of 0.9% saline containing 1 U/ml heparin and 67 mg/ml Timentin. After one week, the Timentin component was removed from the solution, though daily catheter flushing continued throughout the duration of the experiment.

2.5. MDMA

(+/-) 3,4-methylenedioxymethamphetamine HCl (MDMA) (NIDA Drug Inventory Supply and Control Program; RTI International, Research Triangle Park, NC) was used in this experiment. MDMA was dissolved in isotonic saline solution (0.9 %) in the appropriate dose concentrations according to the weights of the animals.

2.6. Groups and Experimental Procedures

One week after surgery, animals were randomly assigned to one of four groups: (1) MDMA Room Temperature, (2) MDMA High Temperature, (3) Control Room Temperature, or (4) Control High Temperature. During the 30-min habituation and 2-hour self-administration session, Room Temperature groups had daily access to MDMA or Saline in an operant chamber maintained at 23° C (+/- 1°), while sessions for the High Temperature groups were conducted in operant chambers maintained at 32° C (+/- 1°). The core temperature of each animal was assessed immediately before and after every self-administration session using a 7001H model microcomputer thermometer (Physitemp, Clifton, NJ). The thermometer probe was inserted in the rectum to a depth of approximately 4 cm.

2.7. Self-Administration Procedures

To achieve optimal MDMA self-administration behavior, as previously reported (Reveron et al. 2006; Schenk et al. 2007), the unit dose of MDMA was set at 1.0 mg/kg/inj for the first 10 sessions ("Acquisition"), followed by 0.5 mg/kg/inj for the last 10 days ("Maintenance"). Control groups had access to saline injections of the same volume of infusion (0.1 ml/inj) for the entire 20 self-administration sessions (5 days/week, weekends off). When animals were placed in the operant chamber and at the start of the session, the chamber remained dark and the lever was unavailable for a 30-min habituation period. After this interval, the house light illuminated and the lever was inserted into the chamber. Animals then had access to the lever and the opportunity to administer MDMA or saline injections for 2 hr/session.

2.8. Microdialysis Procedures

2.8.1. In vitro recovery calibration—Microdialysis probes were constructed as previously described (Duvauchelle et al. 2000) with an active membrane length of 2.5 mm at the probe tip. For each probe, recovery values were calculated by comparing the peak heights of samples to those from a standard as previously described (Ikegami et al. 2007). The mean ($\pm$ SEM) recovery of probes used in the experiment was $11.55 \pm 0.39\%$ for DA and $10.79 \pm 0.44\%$ for 5-HT.

2.8.2. Probe implantation—At least 12 hours prior to the MDMA Challenge and *in vivo* microdialysis test, animals were briefly anesthetized with isoflurane and implanted with a microdialysis probe through their indwelling guide cannula. Animals were placed in a holding chamber (23° C) overnight until the test session. Probes extended 6.25 mm past the end of the guide for placement within the NAcc. After placement, artificial cerebral spinal fluid (ACSF) was pumped through the probe at a speed of 0.2 μ l/min with a 1.0 ml gastight Hamilton 1000 series syringe mounted on a syringe pump (Razel®, Model A). The pump speed was increased to 1.60 μ l/min one hour before the test session.

2.8.3. MDMA Challenge and Microdialysis Test Session—24 hrs after the last self-administration session (e.g., Day 21) animals were placed within the operant chamber and experienced the 30-min habituation period, thermal and drug group conditions identical to those assigned during self-administration sessions. The only differences in this test session were that (1) animals were allowed only a single operant response resulting in MDMA (3.0 mg/kg) or saline (0.1 ml) and lever was retracted for the remainder of the session, and (2)

brain dialysate samples were collected throughout the session at 10-minute intervals (3 10-min baseline during the 30-min habituation period and 6 10-min post-injection).

2.8.4. Analysis of DA and 5-HT—To determine *in vivo* extracellular DA and 5-HT concentrations, dialysate samples were analyzed by high performance liquid chromatography and electrochemical detection (HPLC-EC; Shizeido Capcell C-18 narrow bore column, ESA model 5200A Coulochem III Detector, Model 5041 cell (oxidizing potential set to +200 mV, sensitivity 100 pA) and a Model 5020 Guard Cell (potential 400 mV); ESA, Inc., Chelmsford, MA). The mobile phase contained 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. An ESA model 420 dual pistons HPLC pump circulated mobile phase through the system at a rate of 0.2 ml/min. An ESA Model 500 data station controlled the programs and data collection. The detection limit of DA was 0.1 nM and 0.12 nM for 5-HT, both with a signal/noise ratio of 3:1. Uncorrected basal concentrations of NAcc DA ranged from 0.443 to 0.479 nM and basal NAcc 5-HT ranged from 0.126 to 0.189 nM.

2.9. Histology

At the conclusion of the experiment, animals were sacrificed and brains were collected, stored in 10% formalin/30% sucrose solution, sectioned (48 μ m) and stained with cresyl violet for histological analysis to confirm placement within the NAcc (see Fig 1).

2.10. Statistical Analyses

Three-way repeated measures ANOVAs (Drug $\times$ Ambient Temperature $\times$ Day) were performed on number of daily lever responses, daily locomotor activity (e.g., photobeam breakages), and daily change in core body temperature (e.g., difference scores) during Acquisition and Maintenance intervals. DA and 5-HT nM concentrations collected during the MDMA Challenge test were converted to percent of baseline values and analyzed using three-way repeated measures (Drug $\times$ Ambient Temperature $\times$ Time). A two-way ANOVA was used to compare baseline values of DA and 5-HT between the MDMA and Control groups to confirm comparable levels between groups prior to testing. One-way ANOVAs were used to compare mean core temperature differences (e.g., core temperature after minus before self-administration sessions) across Acquisition and Maintenance sessions and before and after the MDMA Challenge test. T-tests (Independent Samples) were used to compare MDMA intake (total mg/kg) between MDMA Room and High Temperature groups and between Acquisition and Maintenance intervals (Paired Samples). Pearson's Correlation analyses were performed to determine relationships between the number of lever responses and core temperature changes during self-administration sessions. Post hoc analyses (Fisher's LSD) were used when justified by significant interaction effects.

3. Results

3.1. Operant Sessions: Acquisition and Maintenance Phases

3.1.1. Lever Responses

Acquisition (Session Days 1–10): A three-way repeated measures ANOVA performed on daily lever responses during Acquisition showed significant Day [$F(9,252)=12.86$; $p<0.001$], Drug [$F(1,28)=13.068$; $p<0.001$] and Drug $\times$ Day interaction effects [$F(9, 252)=14.188$; $p<0.001$], but no significant Temperature or additional interaction effects were detected. Post hoc tests revealed Control groups had significantly greater lever responses than MDMA groups on several occasions (see Fig 2 Panel A).

Maintenance (Session Days 11–20): Significant Drug [$F(1,28)=15.388$; $p<0.001$] and Drug $\times$ Day interaction effects [$F(9,252)=3.636$; $p<0.01$] were observed, but no Day, Temperature, or any other interaction effects were detected. Post hoc tests revealed significantly greater lever responses in MDMA Groups compared to Controls during several sessions (see Fig 2 Panel B).

3.1.2. MDMA Intake (Acquisition vs Maintenance)—No significant differences were detected in total MDMA intake (mg/kg) between the two MDMA thermal conditions during Acquisition [$t(14)=-0.879$; *n.s.*] or Maintenance [$t(14)=-0.542$; *n.s.*], therefore MDMA intake data was combined for comparison purposes. Paired samples t-test revealed that the total MDMA intake during Maintenance was significantly greater than during the Acquisition interval [$t(15)=-2.219$; $p<0.05$] (see insert Fig 2).

3.1.3. Core Temperature

Acquisition (Session Days 1–10): A three-way repeated measures ANOVA performed on daily core body temperature changes during Acquisition showed significant Day [$F(9,252) = 3.52$, $p < 0.01$] and Drug $\times$ Day interaction effects [$F(9,252)=2.155$; $p<0.05$], but no significant effects of Drug, Temperature, or any other interaction effects (see Fig 3). A one-way ANOVA performed on mean core temperature difference scores (core temperature after session minus core temperature before session) of all experimental groups showed no significant differences [$F(3,31)=0.993$; *n.s.*] (see subpanel Fig 3A).

Maintenance (Session Days 11–20): A three-way repeated measures ANOVA comparing daily core body temperature changes during Maintenance showed significant effects of Temperature [$F(1,28) = 12.178$, $p < 0.01$], but no Drug, Day or any interaction effects were detected. A one-way ANOVA performed on mean core temperature difference scores detected significant differences [$F(3,31)=5.316$; $p<0.01$]. Post hoc tests revealed the MDMA High Temperature group had significantly greater core temperatures during self-administration sessions than MDMA and Control Room Temperature Groups ($p<0.01$), but not greater than the Control High Temperature group (see subpanel Fig 3B).

3.1.4. Correlation between Total Lever Responses and Core Temperature

Acquisition (Session Days 1–10): Correlation analyses (Pearson's Correlation) performed between lever responses and core temperature differences during Acquisition sessions revealed significant correlations for MDMA High Temperature group [$r=0.780$; $p=0.022$] but not MDMA Room Temperature [$r=0.67$; *n.s.*] or Control conditions [Control Room Temperature: $r=-0.196$; Control High Temperature: $r=-0.492$; both *n.s.*] (see Fig 4A).

Maintenance (Session Days 11–20): A significant correlation between lever responses and change in core temperature was determined for both MDMA groups [MDMA High Temperature: $r= 0.713$; $p=0.047$; MDMA Room Temperature: $r= 0.864$; $p=0.006$] but not Control conditions [Control Room Temperature: $r=-0.083$; Control High Temperature: $r=-0.53$; both *n.s.*] (see Fig 4B).

3.1.5. Locomotor Activity

Acquisition (Session Days 1–10): A three-way ANOVA performed on locomotor activity counts during lever access across Acquisition sessions showed significant Day [$F(9,252)=4.944$; $p<0.0001$] and Temperature $\times$ Day [$F(9,252)=2.348$; $p<0.05$], but no Drug, Temperature or other interaction effects (see Fig 5 Panel A).

Maintenance (Session Days 11–20): A three-way ANOVA detected significant Drug [$F(1, 28)=31.493$; $p<0.001$], but no Temperature, Day or interaction effects (see Fig 5 Panel B).

3.2. Microdialysis Test Session

Dialysate levels of DA and 5-HT in nM concentrations (uncorrected values) were converted to percent of baseline to enable MDMA response magnitude comparisons. Basal concentrations (uncorrected values) of NAcc DA ranged from 0.443 to 0.479 nM and basal NAcc 5-HT ranged from 0.126 to 0.189 nM and did not differ significantly between groups as confirmed by two-way repeated measures ANOVA (Group $\times$ Time) performed on DA nM and 5-HT nM baseline means (no significant Group [$F(3, 28) = 2.875$ and 2.252 , respectively; both n.s.], Time [$F(2, 56) = 0.841$ and 1.367 , respectively; both n.s.], and Group $\times$ Time Interaction effects [$F(6, 56) = 0.936$ and 0.807 , respectively; both n.s.].

3.2.1. NAcc 5-HT—A three-way repeated measure ANOVA (Drug $\times$ Temperature $\times$ Time) showed significant Drug [$F(1,28)=56.553$; $p<0.001$], Time [$F(8,224)=39.393$; $p<0.001$] and Drug $\times$ Time [$F(8,224)=44.641$; $p<0.001$], Drug $\times$ Temperature [$F(1,28)=7.269$; $p<0.05$], Temperature $\times$ Time [$F(8,224)=5.037$; $p<0.05$] and Drug $\times$ Temperature $\times$ Time [$F(8,224)=5.063$; $p<0.05$] interaction effects. Post hoc analysis revealed that both MDMA groups showed significant enhancement of NAcc 5-HT from baseline ($p<0.001$) for all post-injection time points, and that the magnitude of enhanced NAcc 5-HT in the MDMA High Temperature group was significantly greater than all other groups at all post-injection time points ($p<0.01$). Control groups showed no significant variations across the testing period (see Fig 6A).

3.2.2. NAcc DA—A three-way repeated measures ANOVA showed significant Drug [$F(1,28)=22.229$; $p<0.001$], Time [$F(8,224)=12.009$; $p<0.001$], and Drug $\times$ Time interaction effects [$F(8,224)=14.773$; $p<0.001$], but no other significant interaction effects. Post hoc tests revealed that MDMA (3.0 mg/kg, i.v.) on test day resulted in a significant NAcc DA increase from baseline for all post-injection time points ($p<0.01$). Post-injection increases in NAcc DA did not significantly differ between the MDMA Room Temperature and MDMA High Temperature groups. NAcc DA levels in the Control conditions were comparable across the testing interval (see Fig 6B).

3.2.3. Core Temperature—A one-way ANOVA performed on mean core temperature difference scores detected significant effects [$F(3,31)=4.548$, $p<0.01$]. Posthoc testing revealed that core temperature in the MDMA High Temperature group increased to a significantly greater extent than all other groups (see Fig 7).

4. Discussion

Findings from the present experiment indicate that the heated environment did not influence voluntary MDMA intake or MDMA-stimulated locomotor activity, but did increase MDMA-stimulated NAcc 5-HT and core temperature. On the other hand, MDMA experience significantly enhanced MDMA intake, locomotor activity and core temperature. For instance, MDMA intake and locomotor activity were significantly enhanced in the last 10 self-administration sessions (e.g., during Maintenance), but not in the first 10 sessions (e.g., the Acquisition phase). In addition, during the MDMA Challenge test, self-administered MDMA (3.0 mg/kg, i.v.) produced significantly higher NAcc 5-HT and core temperature levels when rats occupied a heated environment (32° C) compared to an identical injection administered under normal ambient temperature conditions (23° C). Although MDMA significantly increased NAcc DA levels, statistical differences in DA responses between thermal conditions were not detected. Control groups showed no

significant variation from basal NAcc DA or 5-HT levels across the entire test period, confirming that thermal effects alone did not impact DA and 5-HT in this region.

Previous work has shown that reliable MDMA self-administration behavior is achieved in operant-trained animals over several sessions in which the MDMA unit dosage in the last 10 sessions (Maintenance = 0.5 mg/kg/inj) is half the dose of the first 10 sessions (Acquisition = 1.0 mg/kg/inj) (Ratzenboeck et al. 2001; Schenk et al. 2003; Daniela et al. 2004; Schenk et al. 2007). It should be noted that a minimum lever response criterion, as has been utilized in previous work (e.g., Daniela et al. 2004), was not required in the present experiment. As a result, lever response rates shown here are lower in comparison due to inclusion of data from all animals in the experiment, not just those that attained high intake levels. In addition, Control groups (e.g., rats receiving saline infusions) showed the highest response rates during Acquisition. As indicated above, all animals in the experiment underwent food-reinforced operant training prior to surgical procedures. High rates of non-reinforced responding during initial Acquisition sessions are not unanticipated under the circumstances as this is a typical response pattern during extinction of food-reinforced training (Olds et al. 1970). We have previously reported the same pattern of responding in Control animals under similar conditions (Reveron et al. 2006). Even though these non-reinforced responses were high for the first session, the number of lever presses dramatically decreased by the next session and continued at low levels for the remaining sessions (see Fig 2). Subsequently, during the Maintenance phase, MDMA-reinforced responding was significantly higher than saline self-administering Controls.

The present findings show that a heated environment does not increase the rate of MDMA intake and appears at odds with a previous study reporting a heat-induced increase in MDMA self-administration (Cornish et al. 2003). However, several methodological details differed between the present and the previous study. In the current study, MDMA self-administration at 32° C proceeded over 20 sessions. In the previous study, rats were tested during a single self-administration session at 30° C after MDMA self-administration responding had stabilized at room temperature (21° C) (though the number of MDMA self-administration sessions were not specified). It is not entirely surprising the different procedures would yield different outcomes and interpretations of the findings. However, in the present study and past work (Reveron et al. 2006), we observed that MDMA-experienced rats increase MDMA intake over time. In addition, novelty has been shown to enhance rewarding effects of an environment (Bevins et al. 2002). Therefore, one possible explanation for enhanced responding reported in the previous study is that the combination of novel stimuli (e.g., higher temperature environment) and increasing MDMA experience may account for increased self-administration behavior during a heated test session. Future studies of this phenomenon could examine whether animals with stable MDMA self-administration rates while in a heated environment will alter response rates when placed in an operant environment set at room temperature. In that way, effects of novelty and drug experience may be addressed.

Progressive changes in core temperature responses during self-administration sessions suggest “experience-dependent” effects of heat and MDMA exposure. For instance, during the Acquisition phase (sessions 1–10), core temperature effects were not significantly altered in any of the experimental conditions. Yet, core temperature readings obtained during the Maintenance phase (sessions 11–20; e.g., after more MDMA experience) showed significant enhancement in the MDMA High Temperature group compared to the MDMA and Control Room Temperature groups, though not when compared to the Control High Temperature group. Correlation analyses revealed that hyperthermic responses to MDMA administration were also progressively enhanced with experience. For example, during Acquisition, the number of MDMA-reinforced lever responses was positively correlated

with increased core temperature in the MDMA High Temperature, but not the MDMA Room Temperature group.

However, during Maintenance, the positive correlation between these factors was significant in both the MDMA High Temperature and Room Temperature groups. It is possible that the thermal response to MDMA could be a conditioned effect elicited by ambient temperature and further enhanced by daily MDMA accumulation. In any event, our results suggest that a heated environment facilitates MDMA-induced disruption of homeostatic thermoregulatory responses, but that repeated exposure to MDMA may also disrupt thermoregulation regardless of ambient temperature (Dafters 1994; Green et al. 2004; Sanchez et al. 2004).

In accordance with previous findings (Gold et al. 1989; Spanos et al. 1989; Bankson et al. 2002), the current study showed locomotor activity significantly increased during MDMA self-administration in both the Room Temperature and High Temperature conditions during Maintenance. Since activity levels in both MDMA thermal conditions were comparable, the data indicate that MDMA-stimulated locomotor activity is unaffected by ambient temperature, as reported by others (Dafters 1994; O'Shea et al. 2005). Locomotor activity increased as sessions proceeded, which is consistent with findings of drug-induced locomotor sensitization, which also been reported by numerous previous studies (Spanos et al. 1989; Kalivas et al. 1998; Reveron et al. 2006). However, since MDMA intake also increased over time, increased activity may be attributable to the increase in MDMA dose rather than an exaggerated response to MDMA.

Elevation of synaptic 5-HT and DA is the primary mechanism of action by which MDMA exerts its major effects (Gough et al. 1991; Rudnick et al. 1992; Gudelsky et al. 1996; McCreary et al. 1999; Gudelsky et al. 2008). Consistent with previous work, in the present study, both groups receiving MDMA had a robust enhancement of NAcc 5-HT and a significant, but less exacerbated release of DA (Koch et al. 1997; Baumann et al. 2008; Kurling et al. 2008). In addition, 5-HT, but not DA, was significantly higher in the MDMA High Temperature condition compared to all other groups. These findings are in partial agreement with previous work showing that MDMA-induced (2.5 and 5 mg/kg, i.p.) NAcc 5-HT responses were enhanced in an elevated temperature condition (30° C) compared to 5-HT responses in a lower temperature environment (20° C) (O'Shea et al. 2005). Also consistent with the present work, this study showed that ambient temperature did not influence locomotor activity, but the heated environment (30° C) enhanced MDMA-induced hyperthermia. Though the previous finding reporting enhanced levels of DA in a heated environment diverged from our statistical findings, a closer evaluation of the current data shows higher mean DA values at all intervals in the heat condition. This observation suggests that a larger sample size would yield significant effects of heat. However, since significant effects of temperature were observed in 5-HT responses with the present sample size, it is difficult to justify the use of additional animals to attain the statistical power needed to obtain another significant, though less dramatic effect. Still, there were also differences between our work and the previous studies that may account for our observation of a less robust differentiation between DA responses. For instance, the previous study utilized drug-naïve rats, while in the current study rats had extensive MDMA experience prior to dialysis testing. In addition, factors that influence the magnitude and duration of drug-induced neurochemical responses also varied, including route and mode of MDMA administration (Battaglia et al. 1988; Spanos et al. 1989; O'Shea et al. 1998). Thus, it is possible that, in the previous study, the combination of higher temperature and differing experimental conditions may have affected DA neurotransmission to a greater extent than in the present report.

Neuroimaging studies have demonstrated reduced 5-HT transporter ligand binding and changes in the 5-HT_{2A} receptor in abstinent recreational users; conditions indicative of serotonin system dysregulation (Semple et al. 1999; Reneman et al. 2002; Cowan et al. 2003). In addition, clinical studies report memory impairment in current and abstinent Ecstasy users is common, and perhaps indicative of neural damage (Quednow et al. 2006; Zakzanis et al. 2006) and decreased 5-HT function (Verkes et al. 2001). As reported here, high ambient temperatures increased MDMA-stimulated 5-HT release and hyperthermia, indicating a greater likelihood of MDMA-induced 5-HT attenuation after drug use in a heated environment. Since the MDMA dosages and intake examined in the present study were at low to moderate levels, these findings hold particular relevance for Ecstasy users whose drug use often occurs in hot, overcrowded environments. Our findings indicate that even with low MDMA intake levels, a heated environment facilitates MDMA-induced hyperthermia.

In conclusion, our findings indicate that ambient temperature does not change the reinforcing efficacy of MDMA, but enhances 5-HT efflux. For human users, these findings suggest that hot environments would not immediately initiate greater levels of MDMA-seeking or administration. However, the heat-induced increase in the magnitude of MDMA-stimulated 5-HT responses could lead to residual, prolonged 5-HT depletion; a condition that has been associated with escalation of drug intake in experienced Ecstasy users (Parrott 2005). In addition, as 5-HT depletion has also been linked with severe effects on cognition (Quednow et al. 2006; Zakzanis et al. 2006), mood and aggressive bias (Curran et al. 2004), the combination of heat and MDMA use poses an insidious threat, extending even to those who consider their drug use as “recreational”.

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Fig 1. Histology. Illustrations depict active membrane regions of dialysis probes in the core and shell of the nucleus accumbens. Illustrations drawn with assistance (Paxinos and Watson 1997). Coronal sections ranged from +2.7 to +1.6 mm anterior to bregma.

Fig 2.

MDMA Self-Administration Sessions: *Lever Responses and MDMA Intake*. Mean daily lever responses and total MDMA intake (mg/kg) (+/- SEM) during **(A) Acquisition** (Session Days 1–10) and **(B) Maintenance** (Session Days 11–20). During Acquisition, MDMA dose was 1.0 mg/kg/inj and 0.5 mg/kg/inj during Maintenance sessions. Groups included: MDMA Room Temperature (n = 8), MDMA High Temperature (n = 8), Control Room Temperature (n = 8), and Control High Temperature (n = 8). Ambient temperature did not influence lever responding in either the MDMA or Control groups across all sessions. **(A)** All groups were trained to lever press for food reinforcement prior to MDMA sessions. The high number of responses for the first 2 sessions in both Control groups is a characteristic food extinction response pattern. Lever responses of the Control groups were significantly greater than MDMA groups during Acquisition (++ = both Control groups significantly greater than both MDMA groups @ $p < 0.01$). **(B)** Lever responses in the MDMA groups were significantly greater than Controls (*, ** = both MDMA groups significantly greater than both Control groups @ $p < 0.05$ and 0.01 , respectively). **Bar Insert:** Total MDMA Intake (both MDMA groups combined) was significantly greater during Maintenance (B) compared to the Acquisition (A) interval.

Fig 3.

MDMA Self-Administration Sessions: *Daily Core Temperature Changes*: Mean core temperature for all animals prior to sessions was 38.1 °C ($\pm$ 0.12). Graph depicts daily core temperature difference scores (Mean core temperatures after session minus before session values, $\pm$ SEM) during (A) **Acquisition** (Session Days 1–10), when daily core temperatures varied significantly over Sessions ($p < 0.01$) and showed Drug $\times$ Session interaction effects ($p < 0.05$), and (B) **Maintenance** (Session Days 11–20), when core temperatures were significantly affected by ambient temperature alone. ***Inset bar graphs:*** Data represent the overall Acquisition and Maintenance mean ($\pm$ SEM) of core temperature difference scores. (A) No significant difference in mean core temperature

change was detected across Acquisition sessions. **(B)** The MDMA High Temperature group (black) showed significantly greater increases in mean core temperature compared to the MDMA Room Temperature (gray) and Control Room Temperature (wide stripes). Mean core temperature changes in the Control High Temperature group (thin stripes) were not significantly different than the MDMA High Temperature group.

4A

4B

Fig 4.

4A and 4B. Self-Administration Sessions: Correlation between lever response totals and increased core temperature (core temperature before session minus core temperature post-session) during (A) **Acquisition** (Session Days 1–10) and (B) **Maintenance** (Session Days 11–20). (A) MDMA High Temperature was the only group to show a significant positive correlation between lever responses and increased core temperature. (B) Significant positive correlations between lever responses and increased core temperature were revealed in both MDMA groups (High and Room Temperature) but not Control conditions.

Fig 5.

MDMA Self-Administration Sessions: *Locomotor Activity*. Mean daily locomotor activity units ($\pm$ SEM) during **(A) Acquisition** (Session Days 1–10) and **(B) Maintenance** (Session Days 11–20). **(A)** Across all Acquisition sessions, locomotor activity was not significantly enhanced in the groups self-administering MDMA, though Day ($p < 0.0001$) and interactions between Temperature and Day ($p < 0.05$) factors influenced activity levels. **(B)** During Maintenance, MDMA groups showed significantly greater levels of locomotor activity than Controls, but ambient temperature did not influence locomotor activity in either condition.

Fig 6.

6A and 6B. MDMA Challenge Test Session: Data represent mean ($\pm$ SEM; expressed as Baseline mean %) (A) NAcc 5-HT, and (B) NAcc DA before (Baseline) and after a self-administered injection of MDMA (3.0 mg/kg) or saline (0.1 ml) in MDMA High Temperature (n=8), MDMA Room Temperature (n=8), Control High Temperature (n=8) and Control Room Temperature groups (n=8; same animals that had participated in the reported 20 daily self-administration sessions). (A) Both MDMA groups showed significant 5-HT enhancement from baseline levels, while Control groups showed no change in 5-HT. The magnitude of MDMA-induced 5-HT response was significantly greater in the MDMA High Temperature group compared to all other groups (^, ^^ = MDMA High Temp significantly

greater than MDMA Room Temp @ $p < 0.01, 0.05$, respectively). **(B)** Both groups self-administering MDMA showed a significant increase in NAcc DA from baseline, and maintained higher levels of DA than Control groups after MDMA infusion. Control groups showed no significant changes in NAcc DA from baseline levels.

Fig 7. MDMA Challenge Test Session: *Core Temperature Difference Scores*. Data represent the mean ($\pm$ SEM) of core temperature difference scores (after session minus before session). The MDMA High Temperature group showed the greatest change in body temperature compared to all other groups on in response to MDMA (3.0 mg/kg/i.v.) (** = MDMA Hi Temp significantly greater increase in core temperature compared to all other conditions @ $p < 0.01$).