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Effects of 3,4-methylenedioxymethamphetamine and related amphetamines on autonomic and behavioral thermoregulation

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Abstract

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') and related amphetamines such as *para*-methoxyamphetamine (PMA) disrupt normal thermoregulation in humans and rats. Behavior, an important component of thermoregulation in mammals, has not been investigated with respect to these drugs. This is surprising as harm minimization depends on appropriate thermoregulatory behavior by drug users. The effects of MDMA (10 mg/kg), PMA (10 mg/kg) and D-amphetamine (2 mg/kg) were therefore studied in Sprague–Dawley rats, with telemetry implants measuring core body temperature (T_C), locomotor activity and heart rate. Rats were administered an amphetamine or saline and confined to an ambient temperature of 21, 30 or 15 °C for 30 min, before being able to choose their preferred temperature (T_P) on a thermally graded runway (11–41 °C). Confinement at 21 °C had little effect on T_C in any group. At 30 °C MDMA and PMA increased T_C compared to saline ($p<0.001$). MDMA treated animals behaviorally overcompensated for this effect ($p<0.01$). Locomotor activity after MDMA treatment was significantly elevated compared with saline ($p<0.01$). In contrast, at 15 °C MDMA administration resulted in a lower T_C than saline ($p<0.001$). MDMA and PMA disrupt autonomic components of thermoregulation, while behavioral components are disrupted to a lesser extent. These results highlight differences in thermoregulatory responses to individual drugs, which were only evident when behavior was measured, and this may be important in assessing their risk.

Keywords: 3,4-Methylenedioxymethamphetamine; Para-methoxyamphetamine; Ecstasy; Behavior; Thermoregulation; Telemetry

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is an amphetamine derivative widely used in rave party and club scenes. Several reports state that the use of MDMA is currently rising around the world, with increases of over 60% in use by young people seen in all Western regions over the last 10 years (Green et al., 2003). Australia has the highest per capita use of ecstasy in the world, about twice the level of both the USA and Europe (United Nations, 2003), with 20% of all 20–29 year olds having ever tried the drug (Australian Institute of Health and Welfare, 2002).

While there are many desirable effects of ecstasy, such as feelings of peacefulness and closeness to others, euphoria, and heightened sensory awareness, there are also several major adverse effects. Although the incidences of these are low, the events are unpredictable and can lead to death or morbidity (Williamson et al., 1997; Gowing et al., 2002). One major adverse effect of MDMA ingestion is hyperthermia, which can lead to death due to cardiac arrhythmias, acute renal failure, rhabdomyolysis and disseminated intravascular coagulation (Screaton et al., 1992; Lyles and Cadet, 2003). Other acute adverse effects include motor and muscular problems and tachycardia (Lyles and Cadet, 2003).

Many experimental studies have illustrated an effect of MDMA on thermoregulation in rats, and shown that

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MDMA affects body temperature control such that core body temperature becomes dependent on ambient temperature. This may explain its increased toxicity in warm, crowded, clubs. Dafters and Lynch (1998) reported that MDMA doses of 10 or 15 mg/kg resulted in significant increases of up to 2.32 °C at ambient temperature 22 °C, and decreases of 2.75 °C at 17 °C. Malberg and Seiden (1998), used higher doses of 20 and 40 mg/kg, and showed hyperthermia compared with saline controls at ambient temperatures of 28 and 30 °C, and hypothermia at 20 and 22 °C.

Normal body temperature control in all mammals involves both autonomic and behavioral responses. Thermal discomfort leads to behavioral responses, which minimizes autonomic thermoregulatory strain (Attia, 1984; Sessler, 1997). Behavioral thermoregulation after administration of MDMA has not previously been studied, which is surprising, as current harm minimization strategies to prevent hyperthermia in clubs involves the provision of 'cool rooms' for people to go to if they feel hot. The effect of another stimulant drug which also alters body temperature, cocaine, has been shown to impair heat perception during progressive heat stress in humans (Crandall et al., 2002), which would be likely to lead to altered behavioral responses to the change in body temperature. Cocaine appears to cause hyperthermia by impairing cutaneous vasodilation through enhancement of constriction pathways (Vongpatanasin et al., 1999; Crandall et al., 2002), an effect shared by MDMA (Pedersen and Blessing, 2001). This indicates that MDMA may too have the potential to affect perception of body temperature and hence impair behavioral responses.

Neurotransmitters thought to be involved in controlling body temperature include serotonin (5-HT) and dopamine (DA). It is the action of MDMA to increase levels of these neurotransmitters in the central nervous system that leads to its effects on body temperature. This has been shown using 5-HT and DA uptake inhibitors and receptor antagonists that prevent MDMA-induced hyperthermia. However, these results are variable, even for similar drugs acting at the same site (Malberg et al., 1996; Mechan et al., 2002), suggesting the exact mechanism is very complex.

Para-methoxyamphetamine (PMA) is a drug related to MDMA, also sometimes found in 'ecstasy' tablets, which has been reported to have greater effects on thermoregulation in humans sometimes resulting in death (Ling et al., 2001; Caldicott et al., 2003). However, investigations on the underlying mechanism have failed to fully explain this apparent difference in toxicity between the two drugs (Daws et al., 2000; Gough et al., 2002). The effect of PMA on behavioral thermoregulation has also not been investigated, which as with MDMA, is surprising.

The doses of drugs used in this study were based on results of previous multiple dose studies in our, and other, laboratories demonstrating reliable changes in body temperature after MDMA and PMA treatment (Dafters and Lynch, 1998; Malpass et al., 1999; Daws et al., 2000). These studies used doses ranging from 2 to 20 mg/kg and measured core temperature and locomotor activity. A dose of 10 mg/kg provides a reliable increase in body temperature at 30° ambient without resulting in fatalities or impairing motor function. It should also be noted that this dose of MDMA, the main drug of interest in this paper, has been shown to result in similar plasma concentrations of the drug as has been reported in human cases of hyperthermia (Colado et al., 1995; Chu et al., 1996; Connor et al., 2000). D-amphetamine (AMPH) was also included to reveal secondary effects on body temperature due to increased activity, a result of the stimulant properties of these drugs. The dose of amphetamine was chosen after dose response curves were constructed for locomotor activity indicated that 2 mg/kg produced a similar locomotor response in rats as 10 mg/kg MDMA.

It is clear that MDMA has a major effect on thermoregulation, and behavior plays a very important part in temperature control. The aim of the study was therefore to measure behavioral responses to the disruption of normal thermoregulation by MDMA and related amphetamines and compare these with previously used physiological measures. We hypothesized that this novel approach would reveal a heterogeneity of pharmacological effects for these drugs, which may explain the variations in toxicity referred to in human case reports.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, aged two months and weighing 280±10 g at the start of experiments, were used for all testing. The rats were housed in groups of 2–4 during the experimental period, with food and water available ad libitum. Ambient temperature of the laboratory was in the range 20–22 °C. All behavioral testing was conducted between 1000 and 1500 hours, when the core body temperature of rats varies little under normal conditions (Gordon, 1990). All experimentation was approved by the University of Adelaide animal ethics committee.

2.2. Equipment

The apparatus used was based on previous studies (Gordon, 1987; Florez-Duquet et al., 2001). It consists of an insulated aluminium floor (120 cm) with an actual runway length of 72 cm divided into five zones with dimensions 14.5 by 30 cm. The runway is split into two 15 cm wide sides so that two rats can be observed simultaneously. The ends and center divide are aluminium (28 cm high), and there are clear plexiglass sidewalls for observations. The confinement areas have dimensions 14.5 by 15 cm, with a lid 14 cm above the floor. At one end of the floor

during experiments is a metal container filled with ice, and at the other end underneath the floor is a heat box set at 60 °C. Thermocouple wires are attached between the under side of the floor and a layer of Styrofoam insulation, at the center of each zone. The equipment was allowed to equilibrate to the required floor temperatures for at least 1 h prior to each experiment. The floor temperature for the five zones were 12, 18, 23, 29 and 40±1 °C, and were measured continuously throughout each experiment.

2.3. Preparation and administration of drugs

All drugs were dissolved in 0.9% saline to give concentrations of 10 mg/ml MDMA and PMA, and 2 mg/ml AMPH. Doses of each drug were administered at 1 ml/kg via i.p. injection. Pilot experiments have shown this dose of AMPH to give an equivalent increase in locomotor activity to 10–15 mg/kg of MDMA, and it acted as a positive control for this behavior. Injection controls received the same dose of saline only. Rats had at least 1 week between administration of MDMA and AMPH, given in a crossover design, to allow sufficient time for each drug to be cleared from their system (Law and Moody, 1994). MDMA and PMA were given as the hydrochloride salts, and AMPH as D-amphetamine sulfate, each obtained from The Australian Government Analytical Laboratories (Sydney, Australia).

2.4. Data acquisition

Rats were surgically implanted with telemetry devices (TA11CTA-F40, Data Sciences International), which measure core body temperature, activity and ECG, as reported previously (Bexis et al., 2004). The implants were placed into the rats' abdominal cavity under anesthesia (sodium pentobarbital, 60 mg/kg). At least 10 days recovery from surgery was allowed before rats underwent any injection treatments. Radio receivers, placed parallel to the floor of the runway, received information from the implants and transferred it to a computer which recorded the data using Dataquest LabPro software (Data Sciences International). Data was recorded every 2 min over the experimental period.

2.5. Experimental protocol

Rats were taken from their home cage, administered either saline, AMPH or MDMA, and placed in to an area at room temperature or at a cool or warm ambient temperature for 30 min. Ambient air temperatures at the center of these zones were 21, 15, and 30±1 °C, respectively. Pilot experiments showed 30 min was an appropriate length of time to elicit a significant but not dangerous change in body temperature after 10 mg/kg MDMA in the warm area. Eight rats were used for each ambient temperature. PMA was administered to a separate group of rats at only 30 °C. At the end of the 30 min (time (t)=0 min), rats were allowed access to the thermal gradient for 1 h to choose their preferred floor temperature. Temperature preference (T_P) was recorded as the zone each rat was in at the end of every 2-min period. Core body temperature (T_C), locomotor activity (LMA) and heart rate (HR) were measured every 2 min remotely as described above.

2.6. Data analysis

All calculations and analysis were done using Graph Pad Prism software. Initial analysis of the raw data was undertaken using repeated measures ANOVA and Tukey's post hoc test. This involved comparing values at the end of confinement (t=0) and the end of the experiment (t=60) to values from the start of the experiment (t=−30) for each individual treatment. For clarity, indications of statistical significance for continuous recordings have not been included in the figures but are indicated in the text. Due to the complex nature of the data sets, subsequent area under the curve (AUC) values for T_P, LMA, HR and change in T_C over time were calculated for time periods −30–0, 0–30 and 30–60 min and analyzed between treatments by repeated measures ANOVA and Tukey's post hoc test. PMA data was compared to MDMA from the earlier experiment and saline control treatment using a one-way ANOVA with Tukey's post hoc test. Significance was set at $p<0.05$.

3. Results

3.1. Response to confinement at room temperature after administration of saline, AMPH and MDMA (Fig. 1)

3.1.1. Core body temperature (Fig. 1A)

At the end of confinement (t=0 min), T_C had increased significantly from the start of the experiment after both saline ($p<0.001$) and AMPH ($p<0.001$) (Fig. 1A). By the end of the experiments (t=60), T_C was still significantly higher than at time of injection in both saline ($p<0.01$) and AMPH ($p<0.001$) treated rats. The peak increase after saline and AMPH was 0.81±0.21 and 0.79±0.16 °C, respectively, while after MDMA T_C fell 0.58±0.24 °C before rising to 0.56±0.24 °C above baseline, towards the end of the experimental period.

Repeated measures ANOVA of AUC of change in T_C showed a significant effect of treatment during confinement ($F(2,14)=11.4$, $p=0.0012$) and the first 30 min ($F(2,14)=27.2$, $p<0.0001$) in the runway. AUC after treatment with MDMA was significantly different in the opposite direction to other treatments during confinement ($p<0.01$) and the first half of time in the runway ($p<0.001$), before increasing towards the same level as saline later in the experimental period.

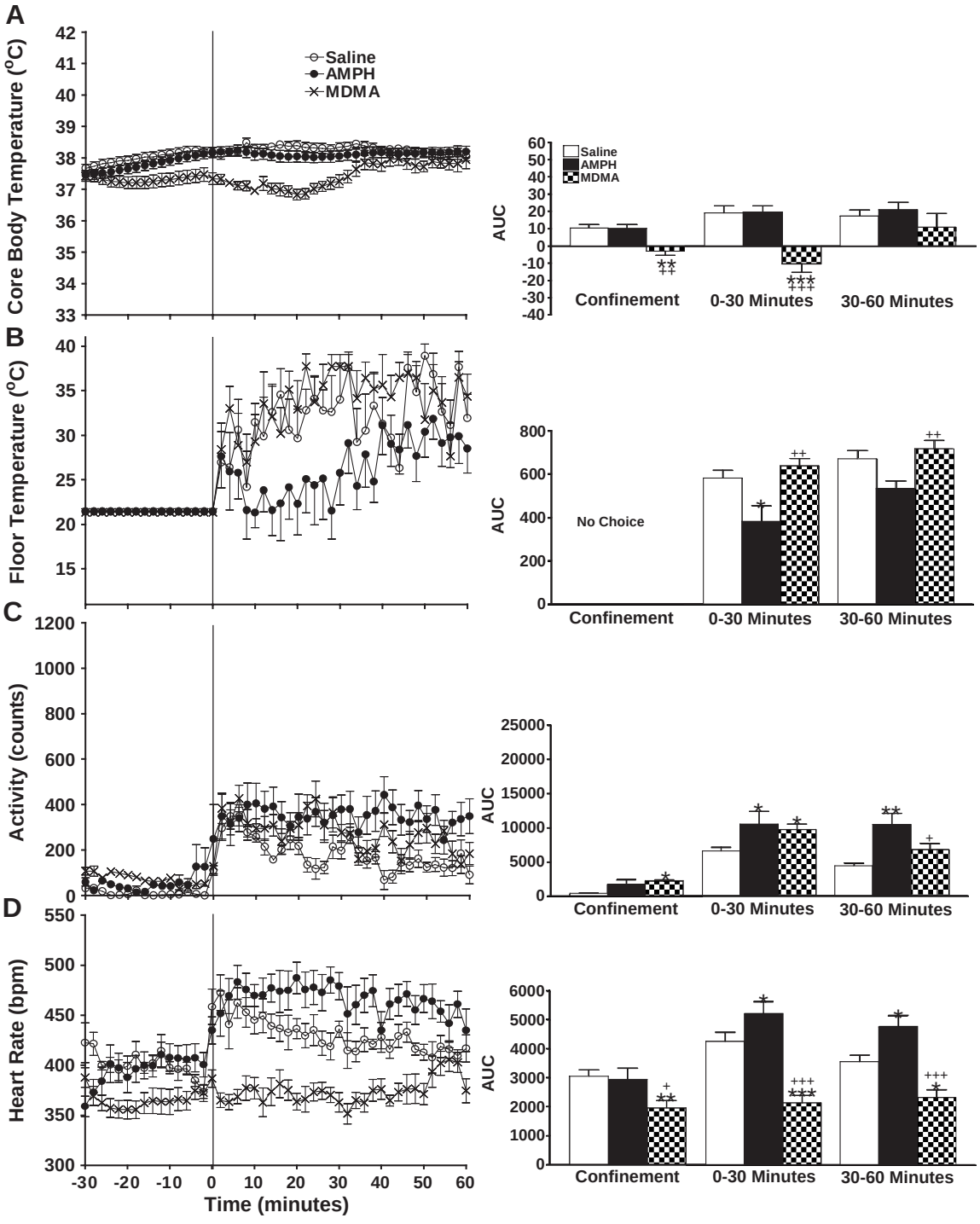

Fig. 1. Effect of confinement to room temperature (21±1 °C) after administration of saline (○), D-amphetamine (2 mg/kg i.p.) (●) or MDMA (10 mg/kg i.p.) (×) on core body temperature (A), preferred temperature (B), locomotor activity (C) and heart rate (D). Column graphs (right) represent the AUC for each 30-min period of the corresponding line graphs (left): open bar saline, closed bar D-amphetamine, checked bar MDMA. All data represent mean±SEM ($n=8$). Drug was given at $t=-30$ min and animals were allowed access to a thermal gradient at $t=0$ (vertical line). *Significant difference from saline, +significant difference from D-amphetamine using a repeated measures ANOVA with Tukey's post hoc test (*$p<0.05$, **$p<0.01$ or ***$p<0.001$).

3.1.2. Temperature preference (Fig. 1B)

Repeated measures ANOVA showed a significant effect of treatment on T_P in both the first and second half of time in the runway ($F(2,14)=10.7, p=0.0015$; $F(2,14)=5.82, p=0.015$, respectively). Rats treated with AMPH had a lower T_P, and hence had a significantly lower AUC in the first half hour of time in the runway than both saline ($p<0.05$) and MDMA ($p<0.01$). The difference between AUC for saline and AMPH was not significant in the second half, but AUC of T_P at this time was still lower than after MDMA ($p<0.01$).

3.1.3. Locomotor activity (Fig. 1C)

Repeated measures ANOVA showed a significant effect of treatment on LMA after all measurement periods ($F(2,14)=6.03$, $p=0.013$; $F(2,14)=5.99$, $p=0.013$; $F(2,14)=10.4$, $p=0.0017$, respectively). LMA was low after all treatments during confinement, although MDMA treated rats had a significantly higher AUC than saline during this period ($p<0.05$). When rats were allowed to move around in the runway, AUC of LMA for saline was significantly lower than both MDMA ($p<0.05$) and AMPH ($p<0.05$) treatments for the first half of the hour but only AUC for AMPH was higher than saline later in the hour ($p<0.01$).

3.1.4. Heart rate (Fig. 1D)

HR rose significantly from time of injection until the end of confinement ($t=0$) in AMPH treated rats ($p<0.001$), and was still significantly increased compared to the first measurement by the end of the experiment ($t=60$) ($p<0.001$) (Fig. 1D).

Repeated measures ANOVA showed a significant effect of treatment on HR after all measurement periods ($F(2,14)=7.84$, $p=0.0052$; $F(2,14)=41.1$, $p<0.0001$; $F(2,14)=21.3$, $p<0.0001$, respectively). AUC of HR after MDMA treatment was significantly lower during confinement than both saline ($p<0.01$) and AMPH ($p<0.05$) treatment. AUC of HR was again significantly lower during the first 30 min of choice than saline ($p<0.001$) and AMPH ($p<0.001$), and also at the end compared with both saline ($p<0.05$) and AMPH ($p<0.001$). AMPH treated rats had the same HR as saline during confinement, which then rose so that AUC was significantly higher during both the first ($p<0.05$) and second half ($p<0.05$) of the hour in the runway.

3.2. Response to confinement in a cold area after administration of saline, AMPH and MDMA (Fig. 2)

3.2.1. Core body temperature (Fig. 2A)

At the end of confinement ($t=0$), T_C had increased significantly after AMPH ($p<0.01$) and decreased after MDMA treatment ($p<0.001$) from the start of experiments (Fig. 2A). By the end of the experiments ($t=60$), T_C was only significantly higher than at time of injection in AMPH treated rats ($p<0.001$). The peak increases after saline and AMPH treatment were 0.44 ± 0.10 and 0.84 ± 0.23 °C, while T_C fell 3.18 ± 0.42 °C after treatment with MDMA.

Repeated measures ANOVA showed a significant effect of treatment on change in T_C after all measurement periods ($F(2,14)=46.8$, $p<0.0001$; $F(2,14)=36.3$, $p<0.0001$; $F(2,14)=6.81$, $p=0.0086$, respectively). The AUC of change in T_C for MDMA displayed a highly significant difference in the opposite direction to the other treatments during confinement ($p<0.001$) and the first half of the time in the runway ($p<0.001$). Body temperature then rose later to be the same as saline, although the AUC for the change from baseline was still significantly lower than AMPH ($p<0.01$).

3.2.2. Temperature preference (Fig. 2B)

Repeated measures ANOVA showed a significant effect of treatment on T_P in both the first and second half of time in the runway ($F(2,14)=21.5$, $p<0.0001$; $F(2,14)=15.8$, $p=0.003$, respectively) (Fig. 2B). Rats treated with AMPH had a lower T_P, and hence had a significantly lower AUC in the first half of time in the runway than both saline ($p<0.001$) and MDMA ($p<0.001$). AUC of T_P was still significantly lower in the final 30 min compared to both saline ($p<0.001$) and MDMA ($p<0.01$).

3.2.3. Locomotor activity (Fig. 2C)

Repeated measures ANOVA showed a significant effect of treatment on LMA after all measurement periods ($F(2,14)=7.87$, $p=0.0051$; $F(2,14)=9.79$, $p=0.0022$; $F(2,14)=8.34$, $p=0.0041$, respectively) (Fig. 2C). AUC of LMA after treatment with saline was significantly lower during confinement than both MDMA ($p<0.01$) and AMPH ($p<0.05$). While LMA for MDMA treated rats tended to be higher than saline in the runway, only AUC for AMPH treated rats was significantly higher during both the first ($p<0.01$) and second half ($p<0.01$) of time in the runway.

3.2.4. Heart rate (Fig. 2D)

HR at the end of confinement ($t=0$) was significantly higher than the start of the experiment after AMPH treatment ($p<0.01$), which was also still the case at the end of the experiment ($t=60$) ($p<0.001$). AUC of HR after MDMA treatment was significantly lower during confinement than both saline ($p<0.001$) and AMPH ($p<0.001$) treatment (Fig. 2D).

Repeated measures ANOVA showed a significant effect of treatment on HR after all measurement periods ($F(2,14)=55.6$, $p<0.0001$; $F(2,14)=51.2$, $p<0.0001$; $F(2,14)=13.5$, $p=0.0005$, respectively). AUC of HR was again significantly lower during the first 30 min of choice than saline ($p<0.001$) and AMPH ($p<0.001$), and at the end compared with only AMPH ($p<0.01$). During confinement, AUC of HR after AMPH was significantly lower than saline ($p<0.01$), although HR after both treatments changed when rats were allowed in the runway so that AMPH was significantly higher for both the first ($p<0.05$) and second half ($p<0.01$) of the hour in the runway.

3.3. Response to confinement in a warm area after administration of saline, AMPH and MDMA (Fig. 3)

3.3.1. Core body temperature (Fig. 3A)

At the end of confinement ($t=0$), T_C had increased significantly after each of saline ($p<0.001$), AMPH ($p<0.001$) and MDMA ($p<0.001$) treatments from the start of experiments (Fig. 3A). By the end of the experiments

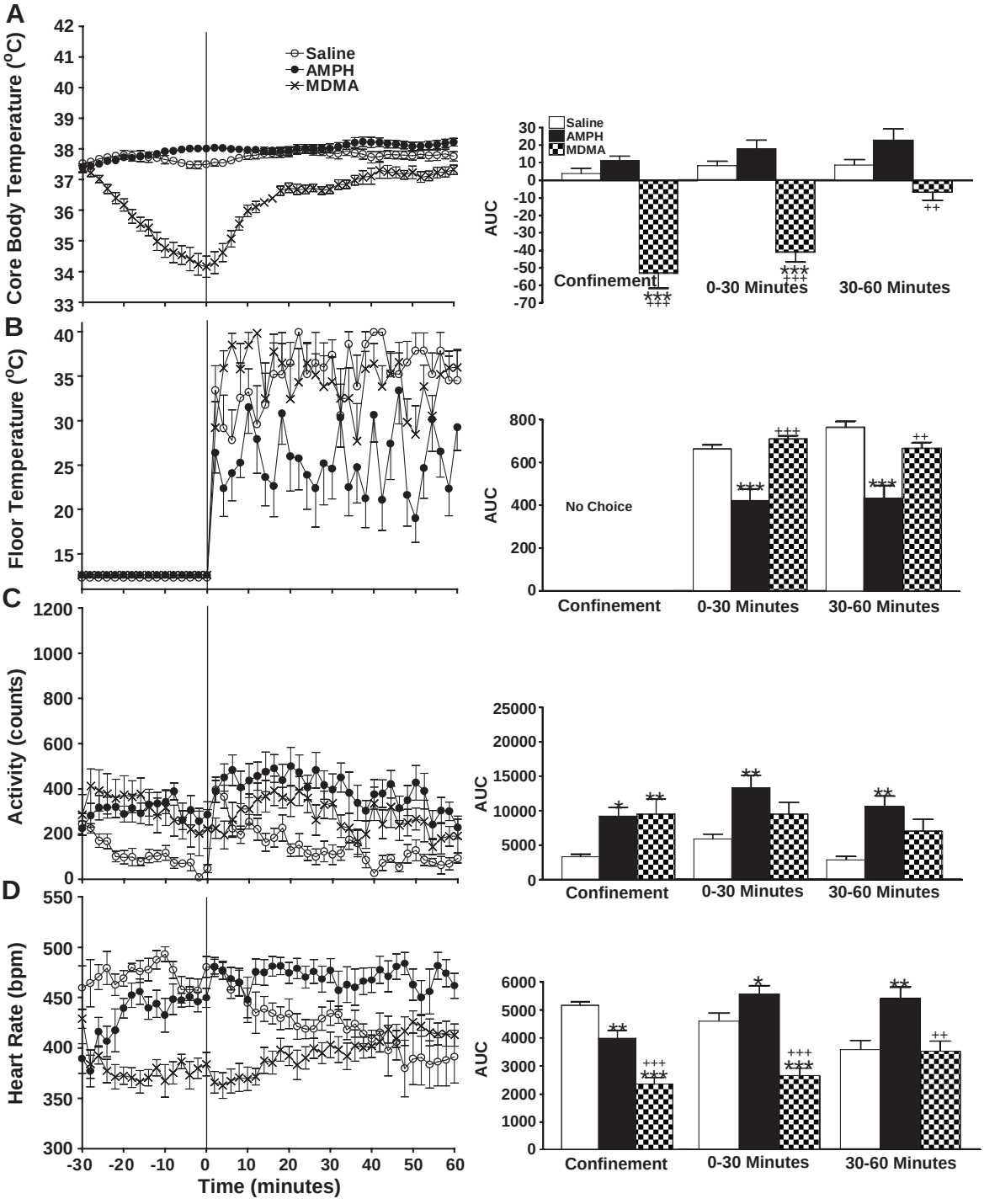

Fig. 2. Effect of confinement to a cold area (15±1 °C) after administration of saline (○), D-amphetamine (2 mg/kg i.p.) (●) or MDMA (10 mg/kg i.p.) (×) on core body temperature (A), preferred temperature (B), locomotor activity (C) and heart rate (D). Column graphs (right) represent the AUC for each 30-min period of the corresponding line graphs (left): open bar saline, closed bar D-amphetamine, checked bar MDMA. All data represent mean±SEM ($n=8$). Drug was given at $t=-30$ min and animals were allowed access to a thermal gradient at $t=0$ (vertical line). *Significant difference from saline, +significant difference from D-amphetamine using a repeated measures ANOVA with Tukey's post hoc test (*$p<0.05$, **$p<0.01$ or ***$p<0.001$).

($t=60$), T_C was only significantly less in MDMA treated rats ($p<0.05$) compared to time of injection. The peak rise in temperature after saline, AMPH and MDMA was 1.94±0.20, 3.44±0.16 and 3.92±0.18 °C, respectively. T_C of MDMA treated rats then fell to 0.83±0.20 °C below baseline.

Repeated measures ANOVA showed a significant effect of treatment on T_C during confinement ($F(2,14)=22.0$,

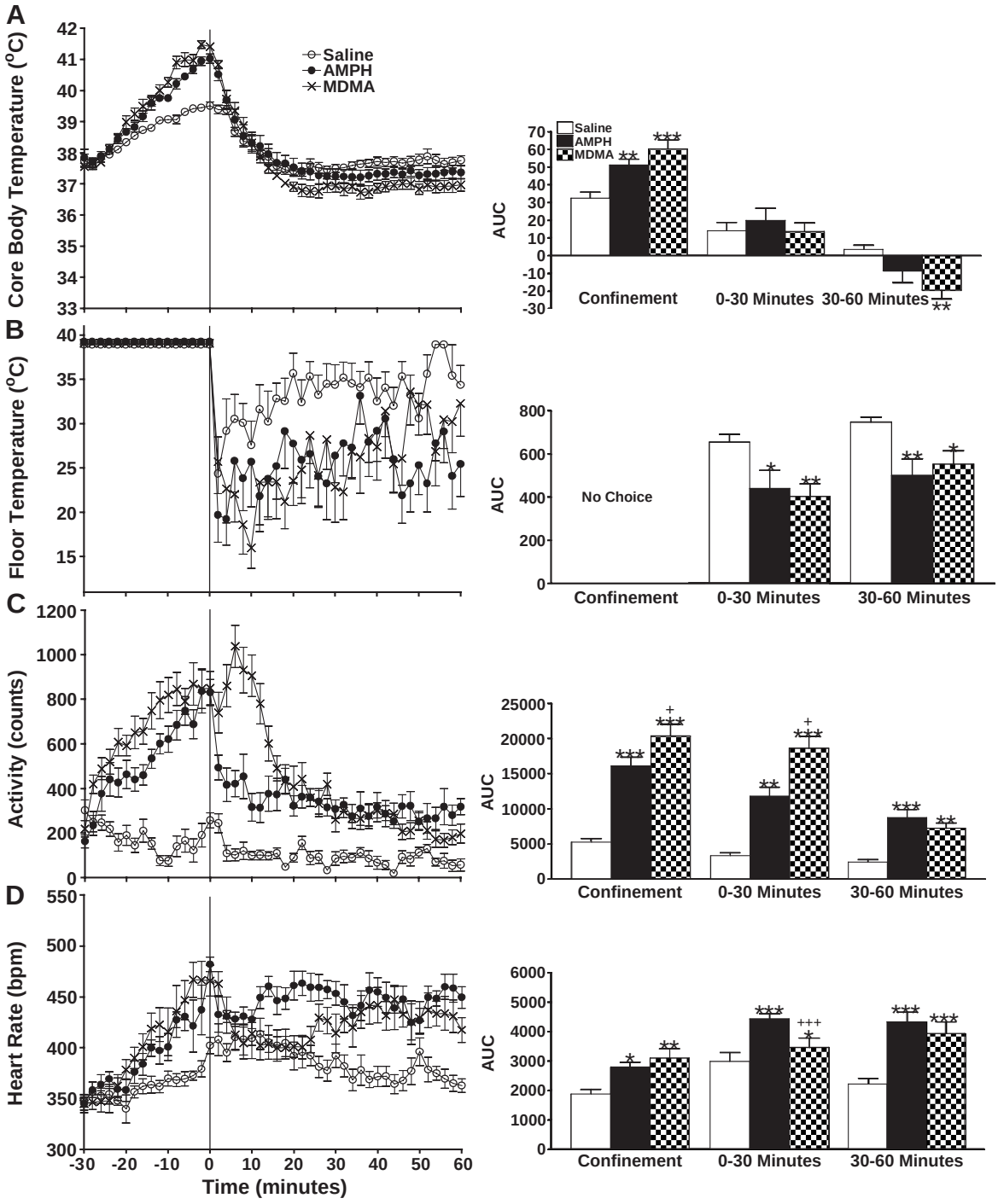

Fig. 3. Effect of confinement to a warm area (30±1 °C) after administration of saline (○), D-amphetamine (2 mg/kg i.p.) (●) or MDMA (10 mg/kg i.p.) (×) on core body temperature (A), preferred temperature (B), locomotor activity (C) and heart rate (D). Column graphs (right) represent the AUC for each 30-min period of the corresponding line graphs (left): open bar saline, closed bar D-amphetamine, checked bar MDMA. All data represent mean±SEM ($n=8$). Drug was given at $t=-30$ min and animals were allowed access to a thermal gradient at $t=0$ (vertical line). *Significant difference from saline, +significant difference from D-amphetamine using a repeated measures ANOVA with Tukey's post hoc test (*$p<0.05$, **$p<0.01$ or ***$p<0.001$).

$p<0.0001$) and the final 30 min ($F(2,14)=10.3$, $p=0.0018$) in the runway. AUC of the change was significantly less after saline treatment than MDMA ($p<0.001$) and AMPH ($p<0.01$) during confinement. T_C then fell during the first half of time in the runway similarly for all treatments, although AUC of T_C change in MDMA treated rats was significantly different in the opposite direction to saline in the final 30 min ($p<0.01$).

3.3.2. Temperature preference (Fig. 3B)

Repeated measures ANOVA showed a significant effect of treatment on T_P in both the first and second half of time

in the runway ($F(2,14)=8.55$, $p=0.0037$; $F(2,14)=9.47$, $p=0.0025$, respectively) (Fig. 3B). Rats treated with saline had a higher T_P, and hence a significantly higher AUC in the first 30 min in the runway than both MDMA ($p<0.01$) and AMPH ($p<0.05$). This was the same in the latter half of the experiment also, with AUC of T_P again higher than both MDMA ($p<0.05$) and AMPH ($p<0.01$).

3.3.3. Locomotor activity (Fig. 3C)

At the end of confinement ($t=0$), LMA was significantly higher in both AMPH ($p<0.001$) and MDMA ($p<0.001$)

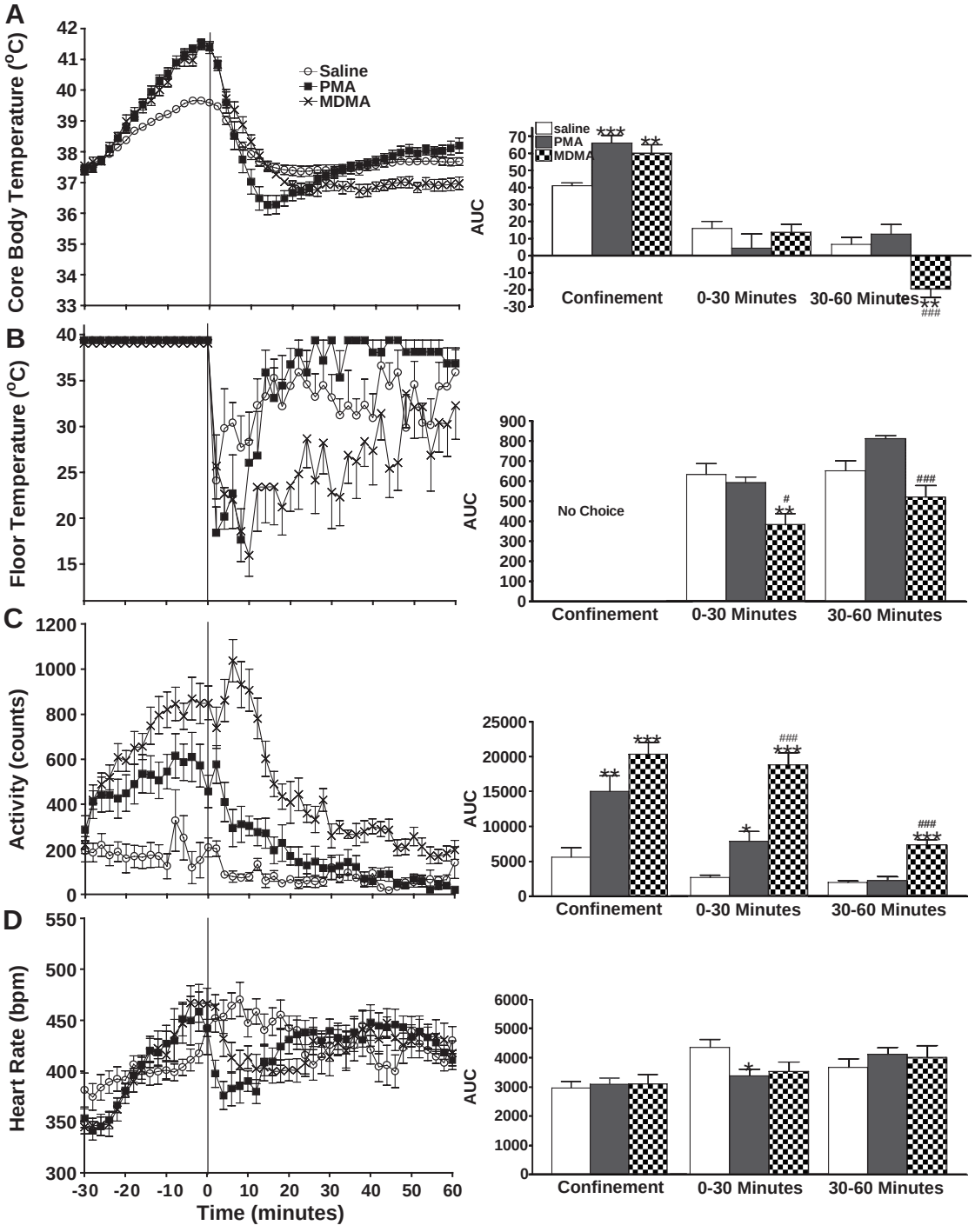

Fig. 4. Effect of confinement to a warm area (30±1 °C) after administration of saline (○) or PMA (10 mg/kg i.p.) (■) on core body temperature (A), preferred temperature (B), locomotor activity (C) and heart rate (D). Column graphs (right) represent the AUC for each 30-min period of the corresponding line graphs (left): open bar saline, dark bar PMA. MDMA data from Fig. 3 is used here for comparison. All data represent mean±SEM ($n=8$). Drug was given at $t=-30$ min and animals were allowed access to a thermal gradient at $t=0$ (vertical line). *Significant difference from saline, #significant difference from PMA using an ANOVA with Tukey's post hoc test (*$p<0.05$, **$p<0.01$ or ***$p<0.001$).

treated rats than at the start of experiments (Fig. 3C). This was still the case at the end ($t=60$) after only AMPH ($p<0.05$), at which time saline treated rats had a LMA significantly lower ($p<0.01$) than time of injection.

Repeated measures ANOVA showed a significant effect of treatment on LMA after all measurement periods ($F(2,14)=53.0$, $p<0.0001$; $F(2,14)=27.6$, $p<0.0001$; $F(2,14)=20.1$, $p<0.0001$, respectively). AUC of LMA after saline treatment was also significantly lower during confinement than both MDMA ($p<0.001$) and AMPH ($p<0.001$). LMA was again lower during the first 30 min of choice than MDMA ($p<0.001$) and AMPH ($p<0.01$), as well as the final 30 min compared to MDMA ($p<0.01$) and AMPH ($p<0.001$). AUC of LMA was also significantly higher in MDMA treated rats than after AMPH treatment in the first half of time in the runway ($p<0.05$), when LMA took more than 10 min to fall to a steady level.

3.3.4. Heart rate (Fig. 3D)

At the end of confinement ($t=0$), HR had increased significantly in saline ($p<0.001$), AMPH ($p<0.001$) and MDMA ($p<0.001$) treated rats from the start of observations (Fig. 3D). It was still increased at the end of experiments ($t=60$) in both AMPH ($p<0.001$) and MDMA ($p<0.001$) treated rats.

Repeated measures ANOVA showed a significant effect of treatment on HR after all measurement periods ($F(2,14)=10.5$, $p=0.0017$; $F(2,14)=34.2$, $p<0.0001$; $F(2,14)=37.7$, $p<0.0001$, respectively). AUC of HR after saline treatment was significantly lower during confinement than both MDMA ($p<0.01$) and AMPH ($p<0.05$) treatment. AUC of HR was again significantly lower during the first 30 min of choice than MDMA ($p<0.05$) and AMPH ($p<0.001$), and also in the last half hour compared with both MDMA ($p<0.001$) and AMPH ($p<0.001$). After confinement, during the first half of time in the runway, HR in MDMA treated rats fell so that AUC was significantly lower than AMPH ($p<0.001$), although rose again to the same level later in the experiments.

3.4. Response to confinement in warm area after administration of PMA (Fig. 4)

3.4.1. Core body temperature (Fig. 4A)

At the end of confinement ($t=0$), T_C of PMA treated rats had risen significantly ($p<0.001$) from the start of the experiment, and at the end ($t=60$) was again significantly different from the first measured value ($p<0.01$) (Fig. 4A). The peak rise was 4.08±0.22 °C, although T_C also fell as low as 1.14±0.38 °C below baseline.

One-way ANOVA showed a significant effect of treatment on T_C during confinement ($F(2,21)=11.1$, $p=0.0005$) and the final 30 min ($F(2,21)=11.9$, $p=0.0004$) in the runway. AUC of T_C in rats treated with PMA was the same during confinement as after earlier experiments with MDMA. T_C appears to fall further, and at a faster rate than MDMA after confinement, although this is not reflected by a significantly different AUC for the change in body temperature. AUC of T_C rises back to the same level as saline in the latter half of time in the runway, contrasting with the effect of MDMA ($p<0.01$).

3.4.2. Temperature preference (Fig. 4B)

One-way ANOVA showed a significant effect of treatment on T_P in both the first and second half of time in the runway ($F(2,21)=7.90$, $p=0.0028$; $F(2,21)=10.5$, $p=0.0007$, respectively) (Fig. 4B). Rats treated with PMA chose warmer zones, and hence had a significantly higher AUC of T_P than those administered MDMA in both the first ($p<0.05$) and second half ($p<0.001$) of the hour allowed in the thermal gradient.

3.4.3. Locomotor activity (Fig. 4C)

One-way ANOVA showed a significant effect of treatment on LMA after all measurement periods ($F(2,21)=17.6$, $p<0.0001$; $F(2,21)=41.6$, $p<0.0001$; $F(2,21)=28.4$, $p<0.0001$, respectively) (Fig. 4C). AUC of LMA after treatment with PMA was much higher than saline treated controls during confinement ($p<0.01$), but fell after rats were allowed in the runway. Although AUC was still significantly higher than saline for the first half ($p<0.05$), it was significantly lower than MDMA for both the first ($p<0.001$) and second half ($p<0.001$) of the hour in the runway.

3.4.4. Heart rate (Fig. 4D)

HR after AUC was higher than time of injection both at the end of confinement ($t=0$) ($p<0.01$) and end of time in the runway ($t=60$) ($p<0.05$) (Fig. 4D).

One-way ANOVA showed a significant effect of treatment on HR for only the first 30 min in the runway ($F(2,21)=3.74$, $p=0.041$). AUC of HR was very similar for all groups in these experiments, with only the AUC for PMA significantly less than saline for the first half of time in the runway ($p<0.05$).

4. Discussion

Exposure to different ambient temperatures had predictable effects on T_C after administration of MDMA. Confinement in normal room temperature had only a small effect on T_C after each treatment, although the change in temperature of MDMA treated rats was opposite to that seen after saline and AMPH treatments. Dafters and Lynch (1998) showed the same dose of MDMA (10 mg/kg) to result in hyperthermia at ambient temperature 22 °C and hypothermia at 17 °C. Ambient temperature of our laboratory was 21 °C, suggesting the limited change in T_C elicited by MDMA may be a reflection of the point the response of the drug changes from causing hyperthermia to hypothermia.

During confinement in the cold area, both saline and AMPH treatment again showed little effect on T_C. A large hypothermia was seen after MDMA administration until these rats were allowed in the gradient and T_C gradually returned to baseline. In contrast to this, after confinement to 30 °C, all treatments resulted in an elevated temperature, close to 3.5 and 4 °C after AMPH and MDMA respectively, but only 2 °C after saline. While T_C fell back to baseline soon after saline and AMPH treated rats were allowed in the runway, T_C of MDMA treated rats fell significantly lower than saline, remaining at this level until the end of the experiment, highlighting differences in the effects of each drug.

MDMA and AMPH likely alter T_C through different mechanisms. Activation of postsynaptic 5-HT_{2A} receptors using (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) causes hyperthermia through cutaneous vasoconstriction in both rats and rabbits (Blessing and Seaman, 2003), while inhibitory presynaptic 5-HT_{1A} receptor activation with 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) leads to vasodilation and a fall in body temperature in rabbits (Ootsuka and Blessing, 2003). MDMA works by this mechanism, leading to release of 5-HT in the CNS and increased cutaneous vasoconstriction accompanied by hyperthermia in both rabbits and rats (Blessing et al., 2003). Increased DA in the central nervous system also appears to have a role in the effects of MDMA on T_C as the D_1 receptor antagonist SCH-23390 decreases MDMA-induced hyperthermia in rats (Mechan et al., 2002). In contrast, AMPH releases mostly DA, leading to the changes in T_C observed. Attenuation of the effects of AMPH on DA through pharmacological means can reduce AMPH induced hyperthermia (Ulus et al., 1975) or even lead to hypothermia (Sabol and Seiden, 1998). The stimulant effects of each drug are believed to result from different mechanisms. MDMA leads to increased LMA through 5-HT–DA interactions (Geyer, 1996; Bubar et al., 2004), while AMPH does so through releasing DA only (Geyer, 1996). This reinforces the idea that the two drugs may also alter T_C through different mechanisms. Differences in the effect on T_C can therefore likely be explained by the different neurotransmitters released after administration of each drug.

PMA treatment led to the same increase in T_C as MDMA. Although T_C tended to fall below that of saline and MDMA treated rats immediately after confinement, it rose again quickly to the same level as saline, remaining here until the end of the experiment. PMA leads to increases in mostly 5-HT in the central nervous system (Daws et al., 2000) and would therefore be expected to show effects more like MDMA than AMPH, such as decreased T_C during confinement to cold areas.

Behavioral observations gave interesting results, which have not been reported previously. After confinement to each of the different ambient temperatures, saline and AMPH treated rats were consistent in choosing warm and room temperature areas on the runway, respectively. The T_P of saline treated rats in these experiments was close to 35 °C, higher than reported in another study on behavioral thermoregulation in Sprague–Dawley rats using a similar thermal gradient (Gordon, 1987). This may be because temperature was measured above the floor, whereas our experiment measured actual floor temperature, as did Florez-Duquet et al. (2001) who found a higher T_P in Long–Evans rats than Gordon (1987). MDMA treated rats chose the same warm areas as after saline treatment following room temperature and cold confinement in our experiments, when they were hypothermic compared to other treatments. AMPH treated rats chose cooler areas than saline controls, which is consistent with a previous study where T_P of mice was reduced from 30 to 25 °C after AMPH treatment (Bushnell and Gordon, 1987). After room temperature and cold confinement in our experiments, T_C was actually the same as saline controls though. The contrasting behavior may have therefore been a response to prevent T_C from rising due to AMPH treatment.

MDMA treated rats moved to cooler areas after being in the warm ambient temperature and becoming hyperthermic. This was similar to AMPH treated animals. The only inappropriate response after MDMA treatment appears to be remaining in cooler areas of the runway compared with saline when T_C falls below baseline. The delay in rats responding to this may be a residual feeling of the greatly increased T_C, or possibly a result of the normal range of T_C increasing, which requires further investigation to determine.

PMA treated rats chose warm areas, not significantly different to saline, which would appear to be inappropriate as T_C increased to the same extent as after MDMA treatment in the warm environment. However, immediately upon release into the gradient, both T_C and T_P were actually very low for the first few minutes. Rats then chose the warmer areas, possibly to prevent T_C staying low like MDMA treated rats, or even continuing to fall further. It is therefore clear that there are differences in the effects of MDMA and PMA on thermoregulation, which are not apparent unless thermoregulatory behavior is examined as well as physiological measures. These differences, if they occur in humans, may contribute to the greater incidence of severe hyperthermia reported after PMA ingestion. For example, if individuals with increased core temperature after PMA administration only choose cool environments briefly this may explain why they are more likely to develop sustained hyperthermia with clinical manifestations than MDMA user who remain in cooler areas for longer.

At room temperature and cold ambient temperature, similar moderate increases in LMA were seen with MDMA and amphetamine. In contrast, large increases in LMA were seen during confinement to the warm area and immediately after rats were allowed in the runway. Activity of AMPH treated rats fell from the high activity in confinement to a steady level immediately. LMA of MDMA treated rats remained elevated for a further 10 min before falling to the

same steady level, an effect not seen after confinement to any other ambient temperature. This was not expected as MDMA has been shown to have no altered effect on LMA in different ambient temperatures (Dafters, 1994). The persistent high activity corresponds to the same period the HR of MDMA treated rats fell significantly lower than AMPH, again suggesting T_C is lowered through different mechanisms. PMA treatment resulted in a moderate but significantly higher LMA than saline during confinement, but fell quickly to a very low level in the latter half of the time in the runway. This is consistent with previous data in which it is shown that PMA has less stimulant properties than MDMA (Daws et al., 2000).

Important responses to changes in ambient temperature in mammals also include cardiovascular adaptations. When core temperature is being maintained at a set point, cardiovascular responses to cold ambient temperatures are closely related to the mechanism for heat production in rats (Chambers et al., 2000), which increases in line with HR. Microdialysis studies in the preoptic anterior hypothalamus, an important brain region involved in both autonomic and behavioral thermoregulation (Humphreys et al., 1976), have also indicated a correlation between heart rate and body temperature (Ishiwata et al., 2002). This relationship has also been shown in non-human primates where disruption of the preoptic anterior hypothalamus by direct cooling leads to high heart rate and T_C, while warming leads to low HR and T_C (Morishima and Gale, 1972). HR may therefore indicate levels of heat production and disrupted autonomic thermoregulation, as can LMA.

Heart rate was unaltered after MDMA treatment at room temperature and at 15 °C, which contrasted with the increases seen in AMPH and saline treated rats. This was unexpected as most studies in rat (Gordon et al., 1991; Bexis et al., 2004) and humans (Lester et al., 2000) show tachycardia However, rats which are hypothermic normally have a depressed HR (Sabharwal et al., 2004). HR in our experiments corresponds to times when T_C was lower than saline, suggesting a low heat production, which may indicate disruption of autonomic thermoregulation in MDMA treated rats. Increased heat loss may also play a role but this was not measured in these experiments.

As this was an initial study there are many issues which need to be addressed in future work. Although we chose doses of drugs which we had shown in previous studies did not cause motor impairment as measured by locomotor activity or stereotypy this should be further examined in more sensitive models of behavior. Furthermore, in this study we could not determine if the animals had difficulty in recognizing increased body temperature or were unwilling to respond with the appropriate behavior. This could be an important factor considering that MDMA is known to reduce motivation to work for food reward (Frederick and Paule, 1997) and lower the threshold for cranial self-stimulation (Hubner et al., 1988; Reid et al., 1996).

In conclusion, we confirmed previous reports showing that autonomic thermoregulation is disrupted by MDMA, AMPH and PMA. In addition, this is the first study to also look at behavioral thermoregulation after MDMA or PMA challenge. Mostly appropriate behavioral responses to initial large changes in body temperature were seen. If this translates into the human situation, it suggests people will have the ability to thermoregulate behaviorally if they become hot. However, the subsequent effects of the individual drugs on core temperature appear dependent on both behavior and intrinsic thermoregulatory mechanisms and these responses may vary with the individual drugs administered. Poly-drug use and distractions of social interactions may also prevent appropriate behavioral thermoregulation, contributing to the unpredictable adverse effects seen in human users. This initial study needs to be extended to accommodate a range of doses, drugs and conditions that more closely resemble human conditions of drug use.

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