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ORIGINAL INVESTIGATION

Acute concomitant effects of MDMA binge dosing on extracellular 5-HT, locomotion and body temperature and the long-term effect on novel object discrimination in rats

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Abstract
Rationale 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) produces an acute release of 5-HT in the brain, together with increased locomotion and hyperthermia.
Objective This study examined whether the acute functional changes of locomotor activity and body temperature are related to enhanced 5-HT release induced by MDMA.
Methods We concomitantly measured changes in extraneuronal 5-HT by in vivo brain microdialysis and used radiotelemetry to measure locomotion and body temperature to establish whether any positive correlations occur between these three parameters. 'Binge-type' repeated administration of low doses of MDMA (3 and 6 mg/kg given at 2-h intervals three times) were given to provide drug exposure similar to that experienced by recreational drug users.
Results MDMA induced acute hyperactivity, changes in core body temperature (both hypothermia and hyperthermia) and elevation of hippocampal 5-HT overflow, all of which were dependent on the dose of MDMA administered. The change in locomotor activity and the magnitude of the hyperthermia appeared to be unrelated both to each other and to the magnitude of MDMA-induced 5-HT release. The study also found evidence of long-term disruption of novel object discrimination 2 weeks following "binge-type" repeated MDMA administration.
Conclusions MDMA-induced 5-HT release in the brain was not responsible for either the hyperthermia or increased locomotor activity that occurred. Since neither dose schedule of MDMA induced a neurotoxic loss of brain 5-HT 2 weeks after its administration, the impairment of recognition memory found in novel object discrimination probably results from other long-term changes yet to be established.

Keywords 3,4-methylenedioxymethamphetamine · 5-HT · Body temperature · Locomotor activity · Novel object discrimination · Radiotelemetry · In vivo microdialysis

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Introduction

Over the last decade, the pattern of 3,4-methylenedioxymethamphetamine (MDMA) ingestion by humans has often involved repeated low-dose drug administration over a single short time period which is referred to as 'binge use' (Hammersley et al. 1999; Parrott 2005; Topp et al. 1999; Winstock et al. 2001). It is claimed that binge use of MDMA boosts its subjective effects and sustains the actions of the drug over time (Parrott 2005). The predominant acute adverse event following MDMA ingestion is hyperthermia and this can lead to other associated clinical problems, including rhabdomyolysis, intravascular coagulation, acute renal failure and even death (see Green et al. 2003). In animal studies, Green et al. (2004) reported a dose-dependent MDMA-induced hyperthermia following repeated MDMA (2, 4 and 6 mg/kg×3 every 3 h) administration to rats and Baumann et al. (2008a) similarly showed a marked and sustained increase of body temperature following MDMA (7.5 mg/kg×3 every 2 h). In addition to acute hyperthermia, Kindlundh-Hogberg et al. (2007) reported hyperactivity caused by repeated MDMA (5 mg/kg×3 every 3 h) and showed that this pattern of

administration enhanced activity in the centre of the open field arena, indicating reduced anxiety or enhanced impulsivity which is known to be associated with altered 5-hydroxytryptamine (serotonin; 5-HT) neuronal activity.

No previous study has concomitantly measured the in vivo changes in extraneuronal neurotransmitter release which accompany the acute functional changes in locomotor activity and body temperature to enable any positive likely association to be made. Therefore, the present study combined the techniques of radiotelemetry and in vivo brain microdialysis to allow, for the first time, the simultaneous measurement of changes in locomotor activity, body temperature and extracellular 5-HT overflow following 'binge-type' repeated administration of low doses of MDMA in the same animal.

As we have previously emphasised, it is important to ensure that preclinical studies in rats are conducted using doses of MDMA that allow translation to human recreational use (Green et al. 2009). Irvine et al. (2006) reported that blood samples taken from recreational users of MDMA at a dance party revealed a mean plasma MDMA concentration of 310 ng/ml (n=27) but with some subjects having a concentration above 750 ng/ml. In rats, a dose of MDMA of 3 mg/kg has recently been reported to produce a peak plasma concentration of 330 ng/ml. Starr et al. (2008) and Goñi-Allo et al. (2008) showed that 5 mg/kg given three times at 2-h intervals resulted in a plasma concentration of around 700 ng/ml with no accumulation on repeated dosing. Therefore, we chose to administer both 3 mg/kg×3 every 2 h and 6 mg/kg×3 every 2 h to produce drug exposure in rats which was similar to that previously observed in recreational users.

MDMA administration produces long-term cognitive deficits in rats as measured in the Morris water maze, Cincinnati water maze, operant delayed non-match to place and novel object recognition tasks (Able et al. 2006; Marston et al. 1999; McGregor et al. 2003; Morley et al. 2001; Sprague et al. 2003), but all these studies [except (Sprague et al. 2003) who measured behaviour only 7 days after MDMA] used total cumulative MDMA doses of between 40 and 60 mg/kg, which are higher than those used in the current study and markedly exceed those ingested by humans. Although many preclinical studies have previously demonstrated long-term neurotoxicity of 5-HT nerve endings and changes in behaviour following administration of MDMA, changes in learning and memory and anxiety-related behaviour may occur following exposure to lower doses of MDMA than those inducing 5-HT neurotoxicity in the rat (Broening et al. 2001; Bull et al. 2004; Fone et al. 2002; Morley et al. 2001). Therefore, in addition to examining the acute effects of 'binge type' repeated administration of MDMA, this study also investigated the long-term consequences of this dosage regime on brain tissue levels of 5-HT and dopamine together with recognition memory using the novel object discrimination task.

Materials and methods

Animals and drugs

Male Lister-hooded rats (Biomedical Services Unit, University of Nottingham, UK) were used in all experiments. This strain, having a pigmented iris, has good visual acuity required for the novel object discrimination task and their use permitted results obtained to be compared with several of our previous studies. Rats were housed at a constant ambient temperature (21±2°C) and humidity (45–65%) on a 12-h light/dark cycle (light on at 07.00 h) with free access to food and water. All experiments were performed in accordance with the UK animals (Scientific Procedures) Act, 1986 and approval of the local ethical committee. All experiments were performed by an observer who was unaware of drug treatment given.

(±)-MDMA was synthesised by the Department of Chemistry, University College Dublin, Ireland.

The acute effects of repeated administration of low doses of MDMA on locomotor activity, body temperature and 5-HT release in the hippocampus

Rats (100–130 g) were implanted with a radio-transmitter in the peritoneal cavity under isoflurane anaesthesia followed by recovery in an individual cage for 1 week. Each cage (41 cm×25 cm with wire lids) was then placed over a receiver plate and normal activity and body temperature of the rat monitored continuously for 1 week. Food and water were freely available. Fourteen days after radio-transmitter implantation, a microdialysis probe was implanted into the hippocampus during isoflurane anaesthesia and again allowed to recover overnight. At this stage in the protocol, the mean body weight of the rats was 260–300 g, equivalent to that of other rats (see below) when measurement was made in subsequent long-term studies.

The following day each rat received either MDMA (3 or 6 mg/kg i.p.) or saline (1 ml/kg i.p.) every 2 h over 6 h. Microdialysis samples were collected 1 h before the first injection and until 2 h after the last injection and samples were subsequently analysed for 5-HT content using high-performance liquid chromatography (HPLC)-with electrochemical detection (ECD). Body temperature and locomotor activity were simultaneously recorded during treatment using radiotelemetry (Fig. 1).

Radiotelemetry

Radiotelemetry was used to continuously and simultaneously monitor core body temperature and horizontal

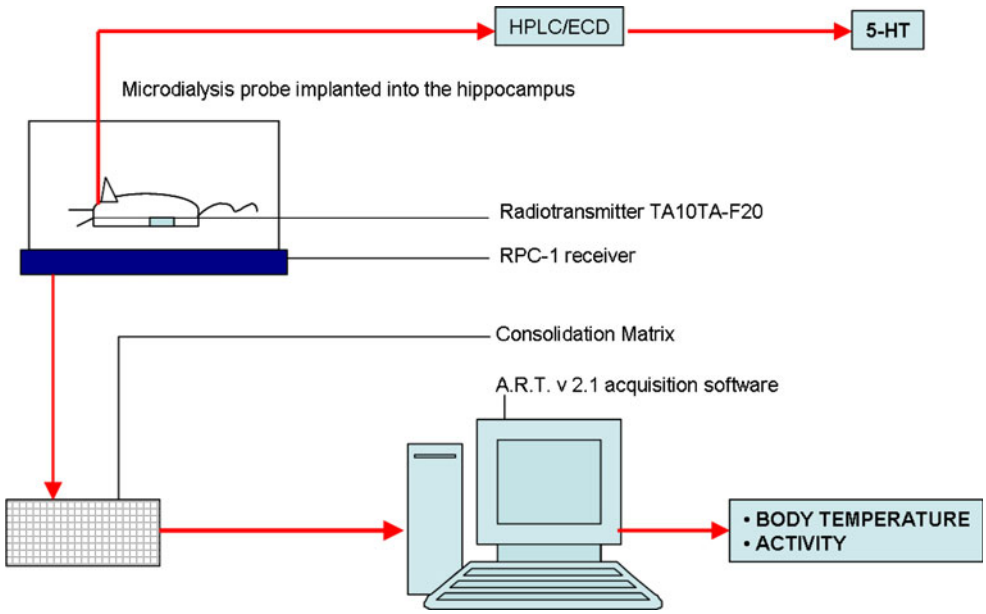

Fig. 1 Schematic diagram showing the simultaneous measurement of the acute effects of repeated low doses of MDMA on locomotor activity, core body temperature and 5-HT release in the hippocampus measured by simultaneous use of radiotelemetry and microdialysis. The telemetric system (DataScience International, St Paul, MN, USA) consisted of a radiotransmitter (TA10TA-F20) implanted into the peritoneal cavity of the rat, an RPC-1 receiver, a consolidation matrix and A.R.T. v2.1 acquisition software. The microdialysis probe was implanted to the rat hippocampus under anaesthesia 14 days after the radiotransmitter implantation. Microdialysis samples were collected every 20 min on the treatment day and analysed for 5-HT content using HPLC-ECD

ambulation at an ambient room temperature of 21±2°C. The telemetric system (DataScience International, St Paul, MN, USA) consisted of an implantable radio-transmitter (Model TA10TA-F20), RPC-1 receiver, consolidation matrix and computer with A.R.T.v2.1 acquisition software. The sterile radio-transmitter was implanted into the peritoneal cavity under isoflurane anaesthesia. Each rat recovered in the home cage for 1 week before recording. Locomotor activity and body temperature were sampled for 10 s every 2 min and results calculated as activity (counts/min) and body temperature (°C) in 20 min time epochs for individual rats.

Brain microdialysis

Intra-hippocampal microdialysis was performed in rats implanted with telemetric devices to monitor synaptic 5-HT overflow. Although this brain area is not a primary regulator of either MDMA-induced locomotor activity or hyperthermia it would have been technically very difficult to perform microdialysis in multiple areas, such as striatum, hippocampus, and hypothalamus which may be more directly linked to the behavioural parameters recorded, and even then probe damage to the latter small region could affect the functional response observed. Since systemic MDMA administration to rats induces a rapid release of 5-HT in all forebrain regions, including the striatum, prefrontal cortex, nucleus accumbens, hippocampus and hypothalamus it is likely that changes in the hippocampus mirror those produced in other brain regions. Microdialysis probes (4 mm long, 220 μm o.d., 180 μm i.d., made of reconstituted cellulose with a 20,000 molecular weight cut off) were connected to a microinfusion pump (Harvard Scientific, USA) via a liquid swivel system (Harvard Scientific, USA) to allow unrestricted animal movement. Artificial cerebrospinal fluid (in mM: NaCl 125.0, $NaHCO_3$ 27.0. KCl 2.5, NaH_2PO_4 0.5 Na_2HPO_4 1.2, $NaSO_4$ 0.5, $MgCl_2$ 1.0, $CaCl_2.2H_2O$ 1.0 adjusted to pH 7.4 with phosphoric acid) was perfused through the probe at 1.6 μl/min.

The microdialysis probe was implanted into the right hippocampus (AP −5.6, ML +4.6 relative to bregma and DV −8.0 below the dura, according to Paxinos and Watson; 1998) during isoflurane anaesthesia and rats allowed to recover overnight.

On the treatment day, microdialysis samples were collected in 0.1 ml HPLC-insert vials every 20 min until 2 h after the final dose of MDMA and the perfusate rapidly frozen in liquid nitrogen. The first three dialysis samples before treatment were averaged to calculate the baseline value for each rat. Probe placement was confirmed by using light microscopy in 20 μm brain sections by perfusion of pontamine sky blue at the end of the experiment and only those located in the hippocampus were used for subsequent 5-HT analysis. The average recovery of 5-HT through the probe measured in vitro was 8%. The ‘dead space’ in the tubing between probe and

sample collection point was approximately 15 μl, causing a small, 8–9 min delay in the microdialysate reaching the collection point. This value has not been deleted from the time-response data shown.

Analysis of 5-HT in microdialysis samples

5-HT was separated on a 75×2.1 mm TARGA C18, 3-μm column (Higgins Analytical, CA, USA) using a mobile phase (0.05 M KH_2PO_4, 0.1 mM EDTA, 120 mg/l octane sulfonic acid disodium, 8 mM KCl, 15% *v*/*v* methanol adjusted to pH 3.0 with o-phosphoric acid), circulated at 0.3 ml/min (Dionex P680 HPLC pump) and an autosampler (series 200, PerkinElmer, USA). Quantification was achieved using Antec VT-03 cell (Antec Leyden, Zoeterwoude, The Netherlands) with a glassy carbon working electrode potential of +0.70 V against an ISAAC reference electrode. Column and electrochemical cell were kept in a constant temperature at 32.5°C. Indoleamine content of samples (15 μl) was determined from standards and the minimum level of detection of 5-HT was 7.5 fmol per sample.

Long-term effects of repeated administration of low doses of MDMA on novel object discrimination and brain 5-HT and dopamine

A further group of rats (300–350 g, Charles River, UK) were housed in groups of four for 1 week before treatment. On the treatment day, each rat was placed in an individual infrared activity monitor chamber (38×23.5 cm) with wire mesh lids of similar dimensions to those used in the radiotelemetry experiments. Rats were given MDMA (3 mg/kg or 6 mg/kg i.p.) or saline (1 ml/kg i.p.) three times every 2 h (n=8 per group). Two weeks, later rats were examined in the novel object discrimination task and killed immediately afterwards. The brain was removed, the frontal cortex, hippocampus and striatum dissected out on ice and kept at −80°C for later analysis of 5-HT and dopamine content.

Novel object discrimination

Novel object discrimination was used to assess recognition memory using a modification of the protocol of Ennaceur and Delacour (1988), as described by Bianchi et al. (2006). The arena comprised a clear Perspex box (39×23.5 cm with 30 cm high walls) in which the two objects to be discriminated were plastic bottles (8 cm high×5 cm diameter) covered in white masking tape alone (familiar object) or white with three horizontal black masking tape rings (novel object). Objects were cleaned with 20% *v*/*v* ethanol prior to each experiment to remove any olfactory cues, and each bottle was filled with water inverted and secured 5 cm from the side and 10 cm from the end walls in opposite corners (front left and back right) of the arena. Experiments were performed in constant light (200 lx at floor level in the arena) between 10:00 and 14:00 h by an observer who was blind to treatment condition.

The experiment comprised of two consecutive 3 min trials; a familiarisation trial and a choice trial. Twenty-four hours prior to testing, each rat was habituated to the individual test arena for 60 min in the absence of any object. On the test day, each rat was habituated in the same arena for 3 min in the absence of any object before being returned to its own home cage for 1 min. In the familiarisation trial rats, were exposed to the two identical objects followed by a 2 h inter-trial interval. Lister hooded rats can discriminate novel and familiar objects with inter-trial intervals of up to 3 h (King et al. 2004). In the choice trial, one object (chosen using a pseudorandom protocol balanced across treatment groups) was replaced with a novel object. During both trials, the object exploration (defined as the time spent sniffing, licking, chewing or touching the object or directed attention with the nose within 1 cm and active vibrissae) was recorded for each object. Sitting on or chewing the object was not regarded as exploratory activity and rarely occurred but resulted in the data from one rat in the 3 mg/kg group being excluded from analysis. To further analyse object discrimination during the choice trial the discrimination ratio (novel/(novel + familiar)) was calculated for each rat using the individual object exploration times recorded.

Analysis of 5-HT and dopamine in tissue samples

Brain tissue samples were weighed, homogenised (1 ml ice cold 0.1 M perchloric acid containing 0.1% *w*/*v* sodium metabisulphite and 0.01% *w*/*v* EDTA), spun in a centrifuge (17,500 g for 20 min at 4°C) and the supernatant stored at −80°C for subsequent analysis by HPLC-ECD.

Monoamines were separated on a SphereClone column (C18, 4.6×150 mm, 5 μm; Phenomenex, Macclesfield, GB) using a mobile phase (0.05 M KH_2PO_4, 0.1 mM EDTA, 0.16 mM octane sulphonic acid disodium and 13.5% *v*/*v* methanol pH adjusted to 3.0 with o-phosphoric acid) with a 1 ml/min flow rate (Jasco PU-980 pump) and measured (against a range of standards) using an Antec Cu-04 controller and Antec VT-03 cell with a glassy working carbon electrode (Antec Leyden, Zoeterwoude, The Netherlands) set at +0.75 V versus a Ag/AgCl reference electrode. The minimum level of detection of 5-HT and dopamine was 0.1 pmol per injection.

Statistical analysis

Locomotor activity and body temperature data were calculated as mean ± SEM in 20 min time epochs and analysed using repeated, two-way ANOVA with treatment

and time as main factors, followed by Bonferroni post hoc test. The mean 5-HT level from the three samples obtained before treatment was used to determine the baseline value and calculate any subsequent change for each rat as a percentage of this baseline value. Two-way ANOVA with time and treatment as main factors was used to compare changes in 5-HT levels followed by Bonferroni post hoc comparison.

For novel object discrimination, two-way ANOVA with objects and treatment as main factor was used for comparisons of time spent exploring the novel versus the familiar object during the choice trial, followed by Bonferroni post hoc test and ANOVA was performed on the resultant calculated discrimination ratio (see "Materials and methods" section) and the total time spent exploring both objects during each trial to assess any non-specific effects and habituation during the task.

For brain tissue 5-HT and dopamine levels, a between-group comparison of 5-HT and dopamine concentration (pmol/mg of tissue) was made using one-way ANOVA followed by Tukey's post hoc test. For all measurements, significant difference was considered as $p<0.05$.

Results

The acute effects of repeated administration of low doses of MDMA on locomotor activity, body temperature, and 5-HT release in the hippocampus

Locomotor activity The average horizontal locomotor activity count per minute recorded over each consecutive 20 min time epoch was recorded by radiotelemetry and is presented in Fig. 2a. Repeated measures two-way ANOVA revealed an overall main effect of time ($F_{(26,364)}=4.00$, $p<0.0001$), treatment ($F_{(2,364)}=16.64$, $p=0.0002$) and a time × treatment interaction ($F_{(52,364)}=3.89$, $p<0.0001$) in the locomotor activity counts. As expected, there was no significant difference in the basal locomotor activity of each treatment group during the 1 h before drug administration. The lower dose of MDMA (3 mg/kg) did not significantly alter locomotor activity compared to that in saline controls at any time point (Fig. 2a) following any of the three injections. In contrast, the higher dose of MDMA (6 mg/kg) produced a significant hyperactivity following each of the three injections compared with that in the saline control and (at several similar time points) from that in rats treated with the lower dose of MDMA. The peak of the hyperactivity occurred 40 min after the first ($p<0.05$), second ($p<0.01$) and third ($p<0.001$) injections of MDMA (6 mg/kg) but, unlike the response to the first two injections, the hyperactivity remained elevated after the third injection of MDMA (6 mg/kg) ($p<0.001$ at $t=280$–360 compared to saline controls) rather than decreasing back to baseline 2 h after the injection (Fig. 2a).

Body temperature Analysis of the average core body temperature recorded in 20 min time bins (Fig. 2b) by repeated measures two-way ANOVA revealed an overall main effect of time ($F_{(26,390)}=5.67$, $p<0.0001$) and treat-

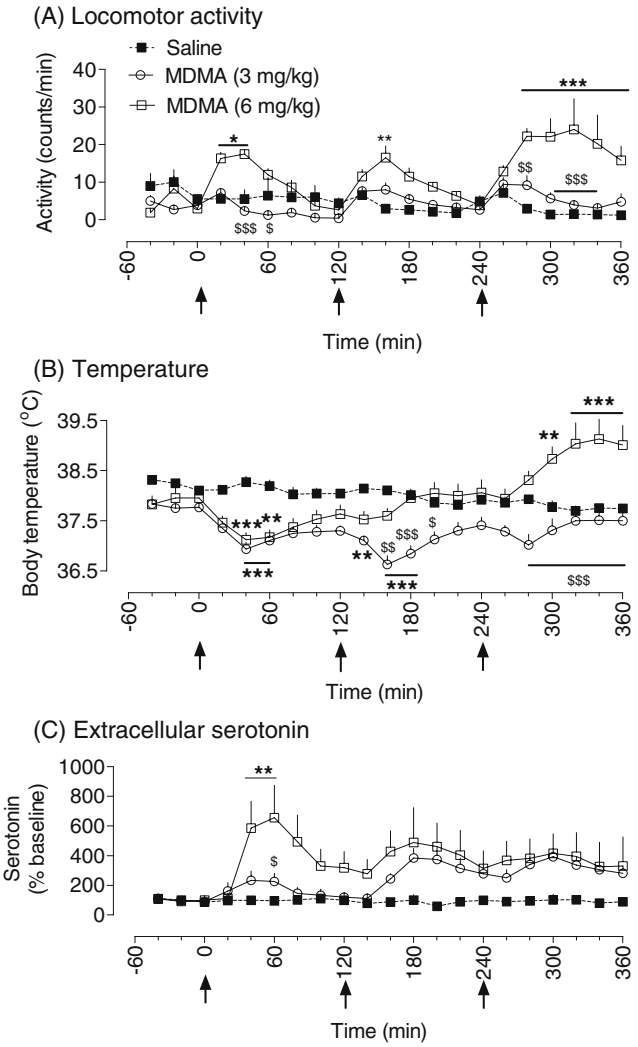

Fig. 2 Effect of repeated administration of MDMA (3 and 6 mg/kgi.p.) or saline (1 mlkg^{-1}i.p.), given as three injections at 2 h intervals as indicated by *arrows* on **a** locomotor activity and **b** body temperature using radiotelemetry and **c** extracellular 5-HT overflow in the hippocampus measured by microdialysis in the conscious rat. Data are presented as mean ± SEM **a** locomotor activity (counts min^{-1}), **b** body temperature (°C) recorded over consecutive 20 min time epochs and **c** percentage change from the mean of the three initial basal 5-HT concentrations (drug injections are indicated by arrows, $n=5$-6 per treatment). *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to saline; $^{\$}p<0.05$, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ compared to MDMA (3×6 mg/kg) Bonferroni post hoc test following ANOVA. Note the lack of any temporal association between the magnitude of change of 5-HT with either locomotor activity or body temperature change over the course of the injections

ment ($F_{(2,390)}$=20.16, p<0.0001) and a time × treatment interaction ($F_{(52,390)}$=7.91, p<0.0001). Prior to drug injection, there was no significant difference in body temperature between the three treatment groups. Saline-treated rats exhibited a small (less than 1°C) progressive decrease of body temperature across the 7 h recording period (Fig. 2b), which was comparable to the normal diurnal body temperature variation recorded during the 1 week acclimatisation period (data not shown). In contrast, MDMA (3 mg/kg) significantly decreased body temperature after each of the three consecutive injections compared to that recorded in saline controls (p<0.001 at t=40, 60, 160, 180, p<0.01 at t=140, p<0.05 at t=280) and the temperature only returned back to that in controls 60–80 min after the final injection. MDMA (6 mg/kg) also significantly decreased body temperature after the first injection (p< 0.001 at t=40, p<0.01 at t=60) in a very similar manner to that seen with the lowest dose of MDMA. In contrast, body temperature progressively increased after the second injection such that there was a marked significant (p<0.01 at t= 300, p<0.001 at t=320–360, Fig. 2b) and sustained hyperthermia reaching almost 2°C above that in saline controls following the third injection of the highest dose of MDMA.

To further analyse the effect of MDMA on thermoregulation, the maximum change in body temperature was calculated by subtraction of the body temperature of an individual rat from the mean value in the 1 h basal period before treatment, thereby providing the mean ± SEM of maximum change for each treatment group in each 2 h interval following each injection. This normalised data showed that both doses of MDMA (3 and 6 mg/kg) produced a maximal decrease of body temperature of −0.9±0.1 and −0.8±0.3°C, respectively, following the first injection. The lower dose of MDMA (3 mg/kg) further decreased body temperature by −1.2±0.2 and −0.4±0.4°C after the second and the third injections, respectively, while MDMA (6 mg/kgi.p.) continuously increased body temperature following the second and third injections reaching a maximum of +1.3±0.5°C after the last injection.

Extracellular 5-HT in the hippocampus The basal extracellular 5-HT concentration in the hippocampus was comparable in all three treatment groups ($F_{(2,13)}$=1.334, p=0.303; one-way ANOVA), being 0.73±0.11 fmol/μl (pre-saline, n =4), 0.84±0.22 fmol/μl (pre-MDMA 3 mg/kg, n=5) and 0.48±0.11 fmol/μl (pre-MDMA 6 mg/kg, n=5). In order to evaluate the impact of drug injection on hippocampal 5-HT release changes were expressed as a percentage from the mean basal value for each rat (see "Materials and methods" section). Two-way ANOVA revealed a main effect of both time ($F_{(21,225)}$=1.87, p=0.0139) and treatment ($F_{(2,225)}$= 39.75, p<0.0001) on extracellular 5-HT levels (Fig. 2c) but no interaction. The higher dose of MDMA (6 mg/kg) produced a marked and significant increase in extracellular 5-HT levels 40 and 60 min after the first injection (being 485%, 555% and 393%, respectively, above the basal level) while the lower dose of MDMA (3 mg/kg) produced a more transient increase in 5-HT with the peak being 134% (compared to baseline) at 40 min after the first injection. The second injection of the highest dose of MDMA (6 mg/kg) produced a smaller maximal increase (389% above baseline) than that seen with the first injection, while the lower dose MDMA (3 mg/kg) produced a slightly larger increase in 5-HT (284% above baseline at t=180) 60 min after the second injection. After the third injection, the magnitude of the peak 5-HT increase produced by 3 mg/kg and 6 mg/kg MDMA (292% and 315%, respectively, above basal at t=300, 60 min after the final injection) was comparable (Fig. 2c).

Long-term effects of repeated administration of low doses of MDMA on novel object discrimination and brain 5-HT and dopamine

Novel object discrimination In a separate set of rats from those used for microdialysis, but receiving an identical triple MDMA injection protocol, novel object discrimination was recorded 2 weeks after drug treatment and post-mortem brain tissue content of 5-HT examined (see below). In the familiarisation trial, when rats explored two identical objects, all three groups of rats spent a comparable time exploring each of the two objects (Fig. 3b) and no group showed any preferential exploration of either object dependent on its location in the arena (data not shown). Following a 2-h inter trial interval, in the choice trial two-way ANOVA revealed a significant main effect of treatment ($F_{(2,21)}$=4.88, p=0.0181) and object ($F_{(1,21)}$=12.54, p= 0.0019) on the time spent exploring the novel versus the familiar object. Rats treated with either saline or the lower dose of MDMA (3×3 mg/kg) spent significantly more time exploring the novel object than the familiar object (p<0.05) while in contrast after the higher dose of MDMA (3×6 mg/kg) rats failed to discriminate the novel from the familiar object (Fig. 3a), spending an equal time exploring both objects. The selective impairment of preferential exploration of the novel object in the choice trial observed with the highest dose of MDMA was further supported by analysis of the discrimination ratio (Fig. 3c), which showed a main effect of treatment (ANOVA $F_{(2,22)}$=5.502, P=0.0125) such that the ratio with the highest dose of MDMA was significantly less (P<0.05) than that in the lower MDMA treatment group. Interestingly, although rats receiving the highest dose of MDMA spent an equivalent total time exploring both objects in the first trial to that of either of the other groups they spent

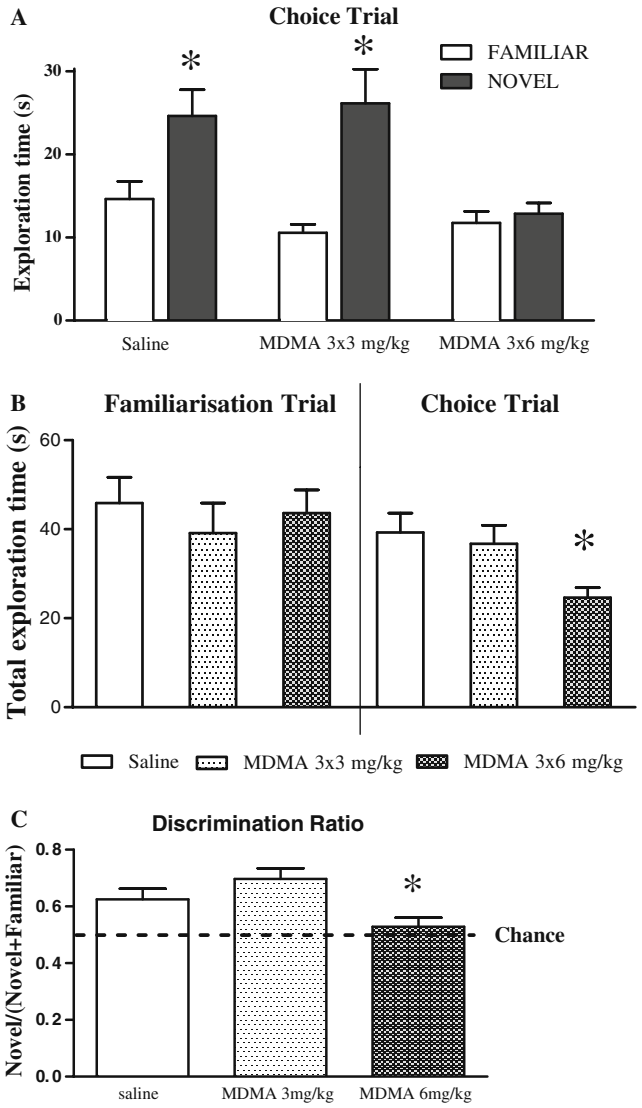

Fig. 3 Effect of MDMA (mean±SEM) on **a** the time spent (s), exploring the novel (*open columns*) and the familiar (*closed columns*) objects during the second choice trial, **b** the total time exploring both objects during the first familiarisation and second choice trial 2 h later and **c** the discrimination ratio (novel/(novel+familiar exploration time)) during the choice trial. Rats received either MDMA (3 or 6 mg/kg i.p.) or saline (1 ml kg^{-1} i.p.) every 2 h to a total of three doses (n=7–8 per treatment) and the novel object discrimination task was performed 14 days after treatment. * $p<0.05$ Bonferroni post hoc test compared with time spent at the novel object in the same treatment group in **a**, or the total exploration time during trial 2 in **b**, or the 3 mg/kg treatment group in **c**

significantly less total time exploring both objects than the saline controls during the choice trial (Fig. 3b). Thus, in addition to being unable to differentiate the novel and familiar objects during the choice trial, they also spent less time attending both objects specifically during the second trial, suggesting that a change in motivation and/or attention span might contribute to the impairment in recognition memory.

5-HT and dopamine concentration in various brain regions Repeated injection of either doses of MDMA (3×3 or 3×6 mg/kg) had no significant effect on the post-mortem 5-HT concentration in the hippocampus, striatum or frontal cortex (Table 1). While the higher dosage regimen of MDMA appeared to produce an increase in striatal dopamine content, this effect failed to reach significance (Table 1).

Discussion

The current study showed that the 'binge-type' administration of MDMA, at doses which produce similar plasma concentrations to those reported with recreational use in man (see the "Introduction" section) produced locomotor hyperactivity, changes in body temperature and an elevation in hippocampal 5-HT release, but that there were no obvious relationships between each other in the magnitude of these changes, suggesting no direct functional association between these parameters and the extracellular 5-HT concentration. It should be noted that the peak extracellular 5-HT concentration occurred approximately 60 min (or 50 min if the small delay in microdialysate collection from the point of release is included) after drug administration, which is coincident with the known peak MDMA concentration measured from hippocampal microdialysates in the rat brain following systemic MDMA administration (Esteban et al. 2001). It is acknowledged at the outset that 5-HT release was measured in the hippocampus and it is unlikely that this brain area is responsible for regulating either locomotor activity or hyperthermia. However, it would have been a difficult technical challenge to perform microdialysis with multiple probes in areas such as the striatum, nucleus accumbens and hypothalamus which may be more directly linked to the parameters recorded. Furthermore, probe damage to the latter small region is likely to limit the value

Table 1 Lack of long-term effect of repeated low doses MDMA administration (3 or 6 mg/kg i.p. given at 2-h intervals to a total of three doses) on the 5-HT concentration in the hippocampus, frontal cortex and striatum and the dopamine concentration in the striatum 2 weeks after treatment

Treatment	Hippocampus	Frontal cortex	Striatum	
	5-HT	5-HT	5-HT	Dopamine
Saline	1.92±0.13	2.80±0.32	3.81±0.29	27.27±4.57
MDMA (3×3 mg/kg)	1.82±0.08	2.90±0.23	3.60±0.32	31.34±3.26
MDMA (3×6 mg/kg)	1.78±0.11	2.74±0.29	3.62±0.32	37.64±4.97

Data are presented as mean±SEM (n=8 per treatment) of 5-HT and dopamine levels (pmol/mg of tissue)

of this approach. Although Benamar et al. (2008) have recently used microdialysis to report that hypothalamic dopamine release may correlate with hyperthermia produced by systemic administration of very high doses of MDMA, they failed to demonstrate that the temperature rise could be prevented by administration of dopamine antagonists in the microdialysate, which would have been stronger proof of a direct functional correlation. In addition, there is no evidence to suggest that MDMA has brain region specific effects in terms of its ability to release monoamines, as systemic MDMA administration to rats induces acute release of 5-HT in all forebrain regions, including the striatum, prefrontal cortex, nucleus accumbens, hippocampus and hypothalamus (Green et al. 2003; Baumann et al. 2008b; Mechan et al. 2002; Stanley et al. 2007; Benamar et al. 2008). It is likely therefore that any 5-HT elevation in the hippocampus mirrors changes produced in other regions. Furthermore, any correlation between neurotransmitter release and a behaviour cannot confirm a direct causal relationship if similar biochemical changes are simultaneously occurring in other brain regions.

A previous microdialysis study (Mechan et al. 2002) showed increased hippocampal 5-HT release occurred following a single dose of MDMA (15 mg/kg). The present study demonstrates that an increase in extracellular 5-HT also occurs in the hippocampus when lower doses of MDMA (3 and 6 mg/kg) are administered. The higher dose of MDMA (6 mg/kg) produced a greater release of 5-HT in the hippocampus after the first injection than the lower dose of MDMA (3 mg/kg) while the magnitude of 5-HT release was similar after the second and third administration of both doses. An attenuation of 5-HT release seen following repeated administration of the higher dose of MDMA might be due to inhibition of tryptophan hydroxylase activity which occurs in various brain regions, including the hippocampus (Che et al. 1995; Johnson et al. 1992; O'shea et al. 2006; Stone et al. 1987) as soon as 15 min after MDMA administration (Stone et al. 1987). In addition, depletion of 5-HT from synaptic vesicles may also have occurred with the higher MDMA (6 mg/kg) dose as it produced a greater release of 5-HT than the low dose on the first injection. Inhibition of 5-HT synthesis and depletion of 5-HT storage caused by the higher dose of MDMA (6 mg/kg) may thus account for the consequent decrease in magnitude of 5-HT release with repeated dosing.

Acute administration of these relatively low doses of MDMA induced locomotor hyperactivity, consistent with many previous reports published over the last 20 years (Capela et al. 2009; Green et al. 2003; Kindlundh-Hogberg et al. 2007). It has been claimed that the increased ambulation is associated with MDMA-induced 5-HT release in the brain (Callaway et al.. 1990; Baumann et al. 2008b) and while this relationship does appear to occur when examining the effect of a single dose of MDMA, our data using binge-type dosing cast doubt on the apparent relationship, since the third dose of 6 mg/kg MDMA produced a marked increase in locomotion but no concomitant increase in extracellular 5-HT.

The observations on the effect of MDMA on body temperature are complex, reflecting the fact that several factors influence the effect of this drug on rat temperature (Docherty and Green 2010). Most studies report that MDMA induces a dose-dependent hyperthermia (for example O'shea et al. 1998) when rats are housed at 'normal' ambient (around 21°C) or raised room temperatures. In contrast, when rats are housed at temperatures below approximately 17°C acute administration of MDMA produces hypothermia (Dafters 1995; Green et al. 2004; Malberg and Seiden 1998). The pharmacological mechanisms underlying this dichotomy of effect on thermoregulation also differ (Green et al. 2005; Mechan et al. 2002). Even when rats are housed in normal ambient room temperature, a low dose of MDMA (2.5 mg/kg) will induce a brief hyperthermia (O'shea et al. 2005), while repeated administration of low doses (4 mg/kg) results in an attenuation of the second response compared to the initial hyperthermic response (O'shea et al. 1998). However, all these studies were carried out in rats placed in a group within the cage whereas the present study measured temperature, locomotor activity (and microdialysis) in singly housed rats, which was required to prevent another rat from causing damage to the cannula or collection tube during microdialysis and therefore this housing was replicated for radiotelemetry. The latter technique offers the advantage of continuous direct behavioural measurement without the confounding impact of handling on locomotion or body temperature. However, the housing condition also influences MDMA-induced changes in body temperature since amphetamines, including MDMA, produce more marked behavioural effects and more profound hyperthermia in animals that are grouped rather than housed singly; an effect called 'aggregation toxicity' (Chance 1947; Fantegrossi et al. 2003; Morton et al. 2001). In the current study the two MDMA dose regimes produced markedly different temporal changes in core body temperature. The lower doses of MDMA (3 mg/kg) produced hypothermia after each subsequent injection, while the higher MDMA doses (6 mg/kg) produced hypothermia after the first injection transforming into hyperthermia after the last injection. These different effects therefore reflect both the dose schedules, which are notably lower than most previous studies, and the housing conditions which also differ by necessity from earlier studies.

Of particular note is the absence of any apparent association between the magnitude of 5-HT release and the change in body temperature. This finding is consistent with the fact that fluoxetine blocks 5-HT release but not

hyperthermia, leading Mechan et al. (2002) to conclude that MDMA-induced hyperthermia in the rat is not functionally related to 5-HT release in the brain. Instead, other studies on MDMA and its simpler congener amphetamines, have pointed to dopamine playing a major role in any resultant body temperature change (Green et al. 2005; Docherty and Green 2010; Yehuda and Wurtman. 1972a; 1972b).

While microdialysis studies have failed to indicate that the hyperthermic effect of MDMA is the direct result of the acute release of 5-HT by the drug, there is substantial evidence to suggest that 5-HT receptor sub-type function can modulate the temperature response, primarily through an alteration of dopamine function (see Docherty and Green 2010). Similarly, evidence suggests that MDMA-induced hyperlocomotion also involves activation of multiple 5-HT receptors again by an interaction of dopamine and 5-HT (Bankson and Cunningham 2002; Green et al. 2003). It was therefore surprising that no apparent relationship existed between extracellular 5-HT concentration and locomotion or body temperature change.

It is important to point out that the temperature response of both monkeys and humans differs in one crucial respect from that seen in rats in that MDMA administration to monkeys (von Huben et al. 2007) and humans (Freedman et al. 2005) always results in hyperthermia even in low ambient temperature conditions (see Docherty and Green 2010).

The current results also allow us to conclude that the hyperactivity is not functionally related to 5-HT release and support previous studies which found no association between MDMA-induced hyperactivity and hyperthermia (Dafters 1994, 1995; O'shea et al. 2005). However, these previous studies examined whether there was an association by placing the animals in either low (Dafters 1994) or high (Dafters 1995; O'shea et al. 2005) ambient room temperatures which might have confounded any relationship. In contrast, we examined whether any relationship occurred when the animals were housed in normal ambient temperature conditions.

The current study also found that binge-type dosing with MDMA also induced a long-term change in recognition memory which occurred without any evidence of simultaneous neurotoxicity of 5-HT nerve endings. The current study showed that 2 weeks after administration of the higher dose of MDMA (3×6 mg/kg) there was a specific impairment in ability to discriminate a novel from a familiar object in a non-spatial recognition memory task. Although this treatment had no effect on the total time exploring both objects during the first familiarisation trial, it was also associated with a reduction in total object exploration during the choice trial rather than a just redistribution of attention from the novel to the familiar object. This specific reduction in exploration during the choice trial suggests that the rats may have reduced attention duration but is consistent with a change in learning and memory rather than a non-specific effect, since there was no change in their exploratory behaviour during the first familiarisation trial.

The long-term effect of MDMA administration on novel object recognition seems to vary across studies. McGregor et al. (2003) showed disruption of novel object recognition using a 1 h inter-trial interval and recording 10–12 weeks following repeated MDMA administration (4×5 mg/kg for 2 days) accompanied by a similar decrease in total object exploration in the choice trial to that reported herein, although due to the additional complexity of measurement at different temperatures this did not reach significance. Furthermore, the same group (Morley et al. 2001) reported a significant deleterious effect on novel object recognition with a 15 min but not a 1-h inter-trial interval when behaviour was monitored 14 weeks after short-term MDMA administration (4×5 mg/kg for 2 days). A repeated 'binge-type' MDMA dosage regimen (2×10 mg/kg MDMA every 5 days for 6 weeks) also reduced the novel object discrimination 7 days after the last injection in Sprague-Dawley rats (Piper and Meyer 2004). Although the statistical analysis was not reported by this group, the total object exploration in the familiarisation trial was reduced by 10 s in the MDMA-treated group, similar to that reported herein. On the other hand, Able et al. (2006) and Skelton et al. (2008), who gave higher doses of MDMA (4×15 mg/kg) which produced neurotoxicity in rats, found no impairment of novel object recognition 4–6 weeks after treatment using a 1-h inter-trial interval. The findings are difficult to compare with the present study as there were differences in the doses used, dosage regimen, the experimental protocol and the rat strain.

While the long-term depletion of brain 5-HT following MDMA administration has been well documented, especially after single or repeated administration of high doses of MDMA in rats (see review Capela et al. 2009; Green et al. 2003), neither of the two low doses administered in the current study produced any loss of 5-HT in hippocampus, striatum and frontal cortex measured 2 weeks following repeated MDMA administration on a single day. Nevertheless, there was disruption of novel object recognition in rats subjected to the higher dose schedule. The evidence suggests that 'binge-type' repeated MDMA treatment in the rat can lead to long-term disruption of this learning and memory paradigm indicating that there is an impairment of declarative recognition memory following repeated MDMA treatment in the rat. In addition, and importantly, this impairment of learning and memory can occur in the absence of any marked 5-HT neurotoxicity and is thus presumably mediated by a mechanism independent to loss of 5-HT nerve terminals in the rat. In a previous study, King et al. (2009) we showed that depletion of the 5-HT

projection from the median raphe nucleus by discrete injection of the neurotoxin, 5, 7-dihydroxytryptamine, prevented the reversal of a time delay-induced impairment of novel object recognition normally produced by systemic administration of the 5-HT_6 receptor antagonist, Ro 04-6790, showing that this behaviour is dependent on intact 5-HT neuronal function. Interestingly, following a similar 'binge-type' protocol to that used in the current study (3×1 or 5 mg/kg i.p. injections at 3 h intervals every 7 days for 3 weeks) 5-HT_6 mRNA increased in the prefrontal cortex and amygdala, while 5-HT_{1B} mRNA was increased in the nucleus accumbens and hypothalamus and 5-HT_{2A} reduced in cortical areas. In two previous studies, Bull et al. (2004, 2006) we also found a reduction in behaviour and altered 5-HT_{2A} receptor agonist evoked local cerebral glucose utilisation in the nucleus accumbens, respectively, 8 weeks after repeated MDMA administration (4×5 mg/kg on two consecutive days in Wistar rats), consistent with the suggestion that these sub-neurotoxic doses of MDMA produce long-term changes in serotonergic receptor expression and function which could be responsible for the observed changes in learning and memory.

In humans, there have been a number of studies showing impairment of working memory following MDMA use, especially in heavy and chronic recreational users (see review Gouzoulis-Mayfrank et al. 2000) and the present study suggests that there is also a risk of impairment of working memory following binge use of MDMA.

In summary, the present study provides evidence that low, clinically relevant binge dosing of MDMA induces hyperthermia (at the higher dose used) and ambulatory hyperactivity. These changes do not appear to be related to increased extracellular 5-HT release, at least in the hippocampus. However, care should be taken in extrapolating any data from rats to humans. In rats, there is a linear relationship between the dose and plasma concentration of the drug (Green et al. 2009). In contrast, in humans there is an increased gradient in the slope with a fourfold increase in plasma MDMA concentration after only a twofold increase in dose from 1 to 2 mg/kg because MDMA inhibits CYP2D6, one of its major metabolising enzymes (Tucker et al. 1994) within 1 h (Yang et al. 2006). Assuming that it is exposure to MDMA that produces the acute adverse event of hyperthermia, then binge dosing will merely cause a linear increase in the peak effect in rats, as has previously been shown (Green et al. 2004), but will markedly potentiate the acute hyperthermic effect in humans. Finally, the present study also provides evidence of long-term disruption of novel object discrimination following "binge-type" repeated MDMA administration. However, this impairment of recognition and working memory is not directly related to any neurotoxic loss of 5-HT neurons, since brain 5-HT content was unaltered and further studies are required to establish the mechanism underlying of this change.

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Conflict of interest The authors declare no conflict of interest.

References

Able JA, Gudelsky GA, Vorhees CV, Williams MT (2006) 3, 4-Methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. Biol Psychiatry 59:1219–1226

Bankson MG, Cunningham KA (2002) Pharmacological studies of the acute effects of (+)-3, 4-methylenedioxymethamphetamine on locomotor activity: role of 5-HT(1B/1D) and 5-HT(2) receptors. Neuropsychopharmacology 26:40–52

Baumann MH, Clark RD, Franken FH, Rutter JJ, Rothman RB (2008a) Tolerance to 3, 4-methylenedioxymethamphetamine in rats exposed to single high-dose binges. Neuroscience 152:773–784

Baumann MH, Clark RD, Rothman RB (2008b) Locomotor stimulation produced by 3, 4-methylenedioxymethamphetamine (MDMA) is correlated with dialysate levels of serotonin and dopamine in rat brain. Pharmacol Biochem Behav 90:208–217

Benamar K, Geller EB, Adler MW (2008) A new brain area affected by 3, 4-methylenedioxymethamphetamine: a microdialysis-biotelemetry study. Eur J Pharmacol 596:84–88

Bianchi M, Fone KCF, Azmi N, Heidbreder CA, Hagan JJ, Marsden CA (2006) Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. Eur J Neurosci 24:2894–2902

Broening HW, Morford LL, Inman-Wood SL, Fukumura M, Vorhees CV (2001) 3, 4-methylenedioxymethamphetamine (ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. J Neurosci 21:3228–3235

Bull EJ, Hutson PH, Fone KCF (2004) Decreased social behaviour following 3, 4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT2A receptor responsivity. Neuropharmacology 46:202–210

Bull EJ, Porkess V, Rigby M, Hutson PH, Fone KC (2006) Pre-treatment with 3, 4-methylenedioxymethamphetamine (MDMA) causes long-lasting changes in 5-HT2A receptor-mediated glucose utilization in the rat brain. J Psychopharmacol 20:272–280

Callaway CW, Wing LL, Geyer MA (1990) Serotonin release contributes to the locomotorstimulant effects of 3, 4-methylenedioxymethamphetamine in rats. J Pharmacol Exp Ther 254:456–464

Capela JP, Carmo H, Remiao F, Bastos ML, Meisel A, Carvalho F (2009) Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. Mol Neurobiol 39:210–271

Chance M (1947) Aggregation as a factor influencing the toxicity of sympathomimetic amines in mice. J Pharmacol Exp Ther 87:214–219

Che S, Johnson M, Hanson GR, Gibb JW (1995) Body temperature effect on methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity. Eur J Pharmacol 293:447–453

Dafters RI (1994) Effect of ambient temperature on hyperthermia and hyperkinesis induced by 3, 4-methylenedioxymethamphetamine (MDMA or "ecstasy") in rats. Psychopharmacology 114:505–508

Dafters RI (1995) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. Physiol Behav 58:877–882

Docherty JD, Green AR (2010). The role of monoamines in the changes in body temperature induced by 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) and derivatives. Br J Pharmacol (in press).

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav Brain Res 31:47–59

Esteban B, O'shea E, Camarero J, Sanchez V, Green AR, Colado MI (2001) 3, 4-methylenedioxymethamphetamine induces monoamine release but not toxicity when administered centrally at a concentration occurring following a peripherally injected neurotoxic dose. Psychopharmacology 154:251–260

Fantegrossi WE, Godlewski T, Karabenick RL, Stephens JM, Ullrich T, Rice KC et al (2003) Pharmacological characterization of the effects of 3, 4-methylenedioxymethamphetamine ("ecstasy") and its enantiomers on lethality, core temperature, and locomotor activity in singly housed and crowded mice. Psychopharmacology 166:202–211

Fone KCF, Beckett SR, Topham IA, Swettenham J, Ball M, Maddocks L (2002) Long-term changes in social interaction and reward following repeated MDMA administration to adolescent rats without accompanying serotonergic neurotoxicity. Psychopharmacology 159:437–444

Freedman RR, Johanson CE, Tancer ME (2005) Thermoregulatory effects of 3, 4-methylenedioxymethamphetamine (MDMA) in humans. Psychopharmacology 183:248–256

Goñi-Allo B, Mathúna BÓ, Segura M, Puerta E, Lasheras B, de la Torre R, Aguirre N (2008) The relationship between core body temperature and 3, 4-methylenedioxymethamphetamine metabolism in rats: implications for neurotoxicity. Psychopharmacology 197:263–278

Gouzoulis-Mayfrank E, Daumann J, Tuchtenhagen F, Pelz S, Becker S, Kunert HJ et al (2000) Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). J Neurol Neurosurg Psychiatry 68:719–725

Green AR, Mechan AO, Elliott JM, O'shea E, Colado MI (2003) The pharmacology and clinical pharmacology of 3, 4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev 55:463–508

Green AR, Sanchez V, O'shea E, Saadat KS, Elliott JM, Colado MI (2004) 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 173:264–269

Green AR, O'shea E, Saadat KS, Elliott JM, Colado MI (2005) Studies on the effect of MDMA ('ecstasy') on the body temperature of rats housed at different ambient room temperatures. Br J Pharmacol 146:306–312

Green AR, Gabrielsson J, Marsden CA, Fone KCF (2009) MDMA: On the translation from rodent to human dosing. Psychopharmacology 204:375–378

Hammersley R, Ditton J, Smith I, Short E (1999) Patterns of ecstasy use by drug users. Br J Criminol 39:625–647

Irvine RJ, Keane M, Felgate P, McCann UD, Callaghan PD, White JM (2006) Plasma drug concentrations and physiological measures in 'dance party' participants. Neuropsychopharmacology 31:424–430

Johnson M, Mitros K, Stone DM, Zobrist R, Hanson GR, Gibb JW (1992) Effect of flunarizine and nimodipine on the decrease in tryptophan hydroxylase activity induced by methamphetamine and 3, 4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 261:586–591

Kindlundh-Hogberg AM, Schioth HB, Svenningsson P (2007) Repeated intermittent MDMA binges reduce DAT density in mice and SERT density in rats in reward regions of the adolescent brain. Neurotoxicology 28:1158–1169

King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KCF (2004) 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation–an effect sensitive to NMDA receptor antagonism. Neuropharmacology 47:195–204

King MV, Spicer C, Sleight AJ, Marsden CA, Fone KCF (2009) Impact of regional 5-HT depletion on the cognitive enhancing effects of a typical 5-ht(6) receptor antagonist, Ro 04-6790, in the Novel Object Discrimination task. Psychopharmacology 202:111–123

Malberg JE, Seiden LS (1998) 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 18:5086–5094

Marston HM, Reid ME, Lawrence JA, Olverman HJ, Butcher SP (1999) Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat. Psychopharmacology 144:67–76

McGregor IS, Gurtman CG, Morley KC, Clemens KJ, Blokland A, Li KM et al (2003) Increased anxiety and "depressive" symptoms months after MDMA ("ecstasy") in rats: drug-induced hyperthermia does not predict long-term outcomes. Psychopharmacology 168:465–474

Mechan AO, Esteban B, O'shea E, Elliott JM, Colado MI, Green AR (2002) The pharmacology of the acute hyperthermic response that follows administration of 3, 4-methylenedioxymethamphetamine (MDMA, 'ecstasy') to rats. Br J Pharmacol 135:170–180

Morley KC, Gallate JE, Hunt GE, Mallet PE, McGregor IS (2001) Increased anxiety and impaired memory in rats 3 months after administration of 3, 4-methylenedioxymethamphetamine ("ecstasy"). Eur J Pharmacol 433:91–99

Morton AJ, Hickey MA, Dean LC (2001) Methamphetamine toxicity in mice is potentiated by exposure to loud music. NeuroReport 12:3277–3281

O'Shea E, Granados R, Esteban B, Colado MI, Green AR (1998) The relationship between the degree of neurodegeneration of rat brain 5-HT nerve terminals and the dose and frequency of administration of MDMA ('ecstasy'). Neuropharmacology 37:919–926

O'Shea E, Escobedo I, Orio L, Sanchez V, Navarro M, Green AR et al (2005) Elevation of ambient room temperature has differential effects on MDMA-induced 5-HT and dopamine release in striatum and nucleus accumbens of rats. Neuropsychopharmacology 30:1312–1323

O'Shea E, Orio L, Escobedo I, Sanchez V, Camarero J, Green AR et al (2006) MDMA-induced neurotoxicity: long-term effects on 5-HT biosynthesis and the influence of ambient temperature. Br J Pharmacol 148:778–785

Parrott AC (2005) Chronic tolerance to recreational MDMA (3, 4-methylenedioxymethamphetamine) or Ecstasy. J Psychopharmacol 19:71–83

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edition, 4th edn. Academic, London

Piper BJ, Meyer JS (2004) Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. Pharmacol Biochem Behav 79:723–731

Skelton MR, Able JA, Grace CE, Herring NR, Schaefer TL, Gudelsky GA et al (2008) (+/−)-3, 4-Methylenedioxymethamphetamine treatment in adult rats impairs path integration learning: a comparison of single vs once per week treatment for 5 weeks. Neuropharmacology 55:1121–1130

Sprague JE, Preston AS, Leifheit M, Woodside B (2003) Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. Physiol Behav 79:281–287

Stanley N, Salem A, Irvine RJ (2007) The effects of co-administration of 3, 4-methylenedioxymethamphetamine ("ecstasy") or para-methoxyamphetamine and moclobemide at elevated ambient

temperatures on striatal 5-HT, body temperature and behavior in rats. Neuroscience 146:321–329

Starr MA, Page ME, Waterhouse BD (2008) MDMA (3,4 methylene-dioxymethamphetamine)-mediated distortion of somatosensory signal transmission and neurotransmitter efflux in the ventral posteriormedial thalamus. J Pharmacol Exp Ther 327:20–31

Stone DM, Merchant KM, Hanson GR, Gibb JW (1987) Immediate and long-term effects of 3, 4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. Neuropharmacology 26:1677–1683

Topp L, Hando J, Dillon P, Roche A, Solowij N (1999) Ecstasy use in Australia: patterns of use and associated harm. Drug Alcohol Depend 55:105–115

Tucker GT, Lennard MS, Ellis SW, Woods HF, Cho AK, Lin LY et al (1994) The demethylenation of methylenedioxymethamphetamine ("ecstasy") by debrisoquine hydroxylase (CYP2D6). Biochem Pharmacol 47:1151–1156

Von Huben SN, Lay CC, Crean RD, Davis SA, Katner SN, Taffe MA (2007) Impact of ambient temperature on hyperthermia induced by (+/−)3, 4-methylenedioxymethamphetamine in rhesus macaques. Neuropsychopharmacology 32:673–681

Winstock AR, Griffiths P, Stewart D (2001) Drugs and the dance music scene: a survey of current drug use patterns among a sample of dance music enthusiasts in the UK. Drug Alcohol Depend 64:9–17

Yang JS, Jamei M, Heydari A, Yeo KR, de la Torre R, Farre M et al (2006) Implications of mechanism-based inhibition of CYP2D6 for the pharmacokinetics and toxicity of MDMA. J Psychopharmacol 20:842–849

Yehuda S, Wurtman RJ (1972a) Effects of d-amphetamine and related drugs on colonic temperatures of rats kept at various ambient temperatures. Life Sci Physiol Pharmacol 11:851–859

Yehuda S, Wurtman RJ (1972b) Release of brain dopamine as probable mechanism for hypothermic effect of d-amphetamine. Nature 240:477–478