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Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine (“Ecstasy”)

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Abstract

Male Wistar rats were administered either (a) a high dose regime of 3,4-methylenedioxymethamphetamine (MDMA) (4 × 5 mg/kg, i.p. over 4 h on each of 2 consecutive days), (b) a moderate dose regime of MDMA (1 × 5 mg/kg on each of 2 consecutive days), (c) D-amphetamine (4 × 1 mg/kg over 4 h on each of 2 days), or (d) vehicle injections. The high MDMA dose regime and the amphetamine treatment both produced acute hyperactivity and hyperthermia. Twelve weeks later, all rats were tested in the drug-free state on a battery of anxiety tests (elevated plus maze, emergence and social interaction tests). A further 2 weeks later they were tested on a novel object recognition memory task. Rats previously given the neurotoxic dose of MDMA showed greater anxiety-like behaviour on all three anxiety tests relative to both controls and D-amphetamine-treated rats. Rats given the moderate MDMA dose regime also showed increased anxiety-like behaviour on all three tests, although to a lesser extent than rats in the high dose group. In the object recognition task, rats given the high MDMA dose regime showed impaired memory relative to all other groups when tested at a 15-min delay but not at a 60-min delay. Rats previously exposed to amphetamine did not differ from saline controls in the anxiety or memory tests. These data suggest that moderate to heavy MDMA exposure over 48 h may lead to increased anxiety and memory impairment 3 months later, possibly through a neurotoxic effect on brain serotonin systems.

Keywords: MDMA (3,4-methylenedioxymethamphetamine); Ecstasy; Anxiety; (Rat); Neurotoxicity; Memory

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ‘Ecstasy’) is an increasingly popular recreational drug in many countries of the world. Concern continues to mount about the long-term neurotoxic effects of the drug and its possible deleterious effects in humans (Boot et al., 2000). In animals, MDMA markedly decreases regional brain serotonin (5-HT) content and produces 5-HT nerve terminal degeneration in forebrain areas of rats (Battaglia et al., 1987; Molliver et al., 1990; Ricaurte et al., 1992) and primates (Fischer et al., 1995). Human MDMA users also show several possible markers of 5-HT depletion such as blunted endocrine responses to D-fenfluramine challenges, decreased cerebrospinal 5-hydroxy-indolacetic acid (5-HIAA) concenrations and a reduced density of brain 5-HT transporter sites (Gerra et al., 2000; McCann et al., 1999; Semple et al., 1999).

Recent studies suggest that heavy MDMA users may suffer from both long-term cognitive deficits (Reneman et al., 2000; Verkes et al., 2001; Wareing et al., 2000) and a number of psychiatric sequale such as anxiety and depression (Parrott et al., 2000; Verkes et al., 2001; Wareing et al., 2000). Although these studies provide increasing evidence of clinical problems associated with long-term ecstasy use, they suffer from certain difficulties of interpretation (Boot et al., 2000). It is not possible for such studies to control for the amount of MDMA used, the purity of the MDMA consumed and the polydrug use of human subjects. In addition, pre-existing abnormalities in 5-HT systems and in cognitive and emotional function cannot be ruled out in human MDMA users. It is therefore likely that preclinical studies will be useful in allowing a definitive account of the long-term behavioural and emotional effects of MDMA.

Surprisingly, there has been a relative paucity of such preclinical studies particularly in relation to anxiety and

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memory. Slikker et al. (1989) found a trend towards increased anxiety-like behaviours in rats given neurotoxic doses of MDMA 2–4 weeks previously. With respect to memory, Marston et al. (1999) documented impairment in a delayed matching to position working memory task in rats at up to 19 days following MDMA treatment. However, Ricaurte et al. (1993) found that the choice accuracy of rats in a T-maze delayed alternation task was generally unaffected following exposure to neurotoxic doses of MDMA.

The present study aimed to further investigate the long-term effects of MDMA on anxiety and memory in rats using a range of behavioural paradigms. A battery of three different anxiety tests were employed for the first paradigm (see Morley and McGregor, 2000), which are thought to reflect different anxiety states in humans (File, 1995). These consisted of: the emergence test (Crawley and Goodwin, 1980), the elevated plus-maze test (Pellow et al., 1985) and the social interaction test (File, 1980). To assess memory, the novel object recognition test was used. This model is thought to measure non-spatial working memory in rats (Blanchard et al., 1970; Aggleton, 1985, 1993).

In addition to a vehicle control group, the present study also utilized a control group given D-amphetamine. Although D-amphetamine has a similar chemical structure to MDMA and similar stimulant and hyperthermic properties, it is not generally thought to be neurotoxic except at very high doses (e.g. Linder et al., 1995; Ryan et al., 1990). Thus, use of this treatment group therefore allowed additional control for any long-term effects of stimulant administration on behaviour (cf. Robinson and Becker, 1986) that are unrelated to neurotoxicity.

2. Materials and methods

2.1. Subjects

A total of 64 inbred male albino Wistar rats (Concord Hospital, Sydney, Australia) were used in the experiments, aged between 75 and 95 days. The rats weighed an average of 273 g at the start of treatment and 360 g at the start of behavioural testing 12 weeks later. The rats were housed in large plastic tubs in groups of 8 per cage in a temperature-controlled environment (average temperature 22 °C). A 12-h reversed light cycle was in operation (lights off at 8:30 a.m.) and all testing took place in the dark cycle. Food and water were freely available. All the experiments were approved by the University of Sydney Animal Ethics Committee.

2.2. Apparatus and procedure

2.2.1. Locomotor activity test

Locomotor activity during acute drug administration was measured in standard operant chambers (30 × 50 × 25.5 cm) with an aluminium side and back walls and Perspex front wall (McGregor, 1996). The floor of the chamber consisted of 16 metal bars. A single count occurred when the rat changed its position on any one bar relative to the others. Counts were recorded by a Macintosh computer running ''Workbench-Mac'' data acquisition software (McGregor, 1996).

2.2.2. Body temperature

Body temperature during acute drug administration was measured using a Braun Thermoscan Instant Thermometer (IRT 1020). The nozzle of the thermometer was placed facing the ear drum and held for 3 s. This method gives a rapid, accurate and reproducible reading of core temperature in rats that is highly correlated with rectal temperature.

2.2.3. Emergence test

The emergence test involves conflict between the desire to explore and the desire to avoid the anxiogenic stimuli of open space (Crawley and Goodwin, 1980). This model is considered to be a measure of generalized anxiety since agents used to alleviate generalized anxiety disorder symptoms modify the behaviours evoked by the procedure (Handley and McBlane, 1993; Hascoet and Bourin, 1998).

The apparatus consisted of a white Perspex walled rectangular arena (96 × 100 × 40 cm). The floor was divided into 16 marked squares and a black wooden hide box (24 × 40 × 15 cm) was placed in the top left corner of the arena. The open field was illuminated with red light (40 W). A miniature video camera feeding a video recorder was used to record all emergence tests which was subsequently scored using the ODLog scoring software package from Macropod Software (www.macropodsoftware.com).

Approximately 12 weeks following drug treatment, rats were tested on a single day in the emergence and plus-maze tests. The rats were placed in the hide box of the emergence apparatus and their behaviour was recorded for 5 min. A 'blind' observer scored emergence latency, emergence frequency, duration of time spent in open field, risk assessment (defined as when front paws, head and back were protruding from the hide box) and number of rears. In between each test session the emergence apparatus was thoroughly wiped down with a damp cloth containing an ethanol solution.

2.2.4. Elevated plus-maze test

The elevated plus-maze test involves conflict between the desire to explore and the desire to avoid the anxiogenic stimuli of open and high spaces (Lister, 1990). The test is considered to be a measure of generalized anxiety since agents used to alleviate generalized anxiety disorder symptoms modify defensive behaviours evoked by the model (Pellow et al., 1985).

The apparatus consisted of a white wooden laminate structure with two open arms (50 × 10 cm) and two opposite closed arms (50 × 10 cm) that had 50 cm high walls. The open and closed arms were connected by a central square (10 × 10 cm). The maze was elevated to a height of 59 cm. Photocell detectors (two infra-red transmitters and two receivers) were placed at the far end of each of the four

arms with output being directed to a Macintosh computer running ''WorkbenchMac'' data acquisition software (McGregor, 1996). Placement of photocells allowed for the determination of the amount of time spent in, and the number of entries to, the closed and open arms and the central square. The testing room was illuminated by a red light (40 W).

Immediately following emergence testing, rats were placed in the centre part of the elevated plus maze, with their head facing a closed arm. Testing continued for 5 min during which open and closed arm times and number of open and closed entries were recorded by computer. In between each test session the maze was thoroughly wiped down with a damp cloth containing an ethanol solution.

2.2.5. Social interaction test

The social interaction model involves the anxiety rats display towards an unfamiliar conspecific (File, 1980). The test has been extensively validated with different classes of drugs, demonstrating the model is effective for demonstrating both anxiogenic and anxiolytic effects (File, 1980, 1985). The test conditions can be manipulated by changing the light level and/or the rats' familiarity with the arena. Here, the long-term effects of MDMA were investigated in a condition (low light, unfamiliar arena) which allows detection of both anxiolytic and anxiogenic effects (File, 1980).

The social interaction test arena was a square clear Perspex box ($52 \times 52 \times 40$ cm) dimly lit with red light (40 W). The floor was marked into quarters. A miniature video camera was placed above the box and a second video camera was placed adjacent to the box. These cameras fed into a video recorder and monitor in a neighbouring room where the interactions of the rats were recorded onto tape for later analysis. This analysis was done by one 'blind' observer using the ODLog Software from Macropod Software (www.macropodsoftware.com).

Twenty-four hours after the emergence and plus-maze tests, rats were tested in the social interaction test. Rats were allocated to pairs so that each pair had received the same drug treatment, were of approximately equal weight and had never been caged together. There were eight pairs for each treatment condition. Rats were placed in the test arena for a 10-min session. The total duration of social interaction was recorded for each pair, as described by File (1980). Aggressive-type behaviours (e.g. kicking away, aggressive groom, see Guy and Gardner, 1985) were also scored. These were treated as a separate entity because such behaviours are modulated by different pharmacological agents than social behaviours (Miczek and Winslow, 1987). The arena was wiped down with an ethanol solution between each test session.

2.2.6. Object recognition test for memory

The object recognition test measures non-spatial working memory in the rat and takes advantage of the rat's unprompted nature to explore its surroundings (Blanchard et al., 1970; Aggleton, 1985). This model is advantageous as it does not require punishment or reward and is quick and simple to implement. Rats are first exposed to two identical objects and then, following a specific delay, the rat is presented with one of the familiar objects and also a novel object. When the subject 'remembers' the previous exposure to the familiar object, the rat will explore the novel object to a greater degree than that of the familiar one (Blanchard et al., 1970; Aggleton, 1985). Rats have been shown to demonstrate a robust working memory of the familiar object compared to the novel object after delays of 1, 15 or 60 min (Bartolini et al., 1996; Morrow et al., 2000).

Testing took place in a square clear Perspex box ($52 \times 52 \times 40$ cm), dimly lit with red light (40 W). A miniature video camera was placed above the box. This camera fed pictures to a video recorder and monitor in a neighbouring room where behaviour was recorded onto tape for later analysis. Analysis was again accomplished by a 'blind' observer using ODLog Software from Macropod Software (www.macropodsoftware.com). The duration of exploration was measured where the rat had to be facing the object with its nose approximately within 2 cm of the object and/or touching it with the nose to be classified as investigation.

Two weeks following the anxiety-test battery, rats were habituated to the memory testing arena for 2 min. At 24 h later, the rats were exposed to two identical objects in the arena for 10 min. After a delay, the rats were placed back in the arena for a further 10 min with one object having been replaced by a new object. Rats were tested with two delays (15 and 60 min) with 7 days between each test.

Two sets of objects were used during each test session and the objects used as the novel object were counterbalanced within and across groups to avoid potential bias. For the 60-min delay, two identical coffee mugs ($7.5 \times 7.5 \times 9$ cm) and coca-cola bottles ($5 \times 5 \times 21$ cm + 2.5×2.5 cm top) were used and for the 15 min delay, identical glass beakers ($9.5 \times 9.5 \times 12$ cm) and metal lids (two taped together: $5 \times 5 \times 8$ cm) were utilized. All objects and the arena were thoroughly cleaned with an ethanol solution and dried before each use.

2.3. Drugs and drug administration

(+/−)3,4-Methylenedioxymethamphetamine hydrochloride (MDMA) and D-amphetamine sulphate were obtained from the Australian Government Analytical Laboratories (Pymble, NSW). The drugs were dissolved in 0.9% saline and injected intraperitoneally at a volume of 1 ml/kg.

Rats were randomly assigned to 1 of 4 groups ($n = 16$/group). Rats in the high dose treatment group were administered 5 mg/kg of MDMA for every 4 h on each of 2 h consecutive days ($2 \times 4 \times 5$ mg/kg). Rats in the amphetamine group were given repeated doses of 1 mg/kg of D-amphetamine using the same regimen ($2 \times 4 \times 1$ mg/kg). Rats in the moderate MDMA dose treatment group were given 5 mg/kg of MDMA for the first hour and then vehicle for the following 3 h on each of the 2 consecutive treatment

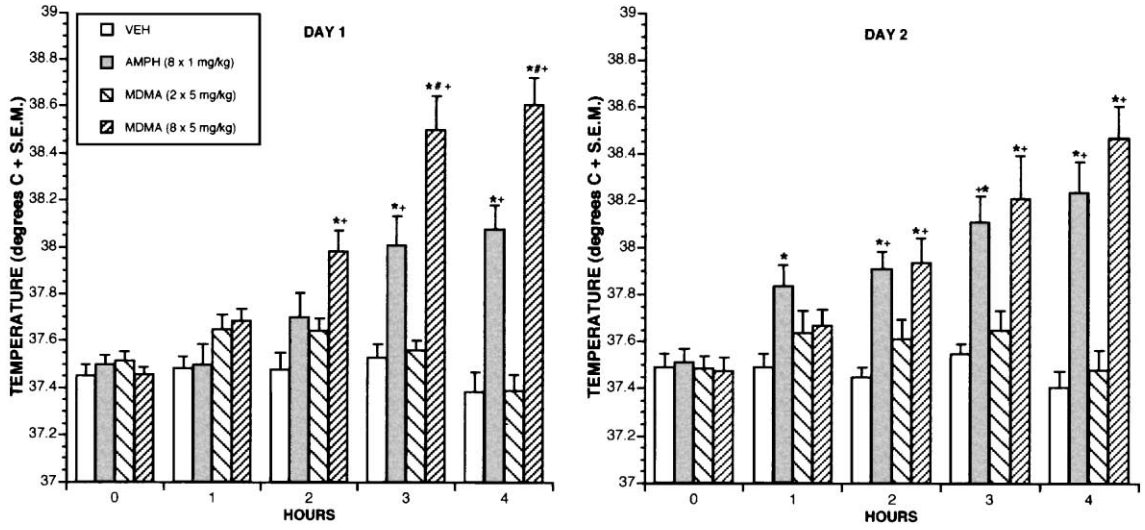

Fig. 1. Mean body temperature (±S.E.M.) on day 1 and day 2 of drug treatment. * $P<0.05$, relative to vehicle within each h block; # $P<0.05$ relative to amphetamine control; + $P<0.05$ relative to moderate MDMA group, Tukey–Kramer post-hoc tests.

days ($2 \times 1 \times 5$ mg/kg). Control rats received equivalent injections of vehicle.

Human MDMA users typically consume 1–2 tablets of MDMA in a single session giving an estimated oral dose of 1–4 mg/kg (Boot et al., 2000). Allowing for interspecies scaling effects and differences in route of administration (Boot et al., 2000), it is estimated that the moderate MDMA dose regime used here may be considered slightly above the dose typically used by humans. On the other hand, the high MDMA dose regime used here is more akin to extreme MDMA "bingeing" that is sometimes seen in human populations (Topp et al., 1999).

Immediately following drug administration, the individual rats were placed in the locomotor chambers for 4 h as described above. The ambient temperature in the room where locomotor activity was measured was set at 28 °C. Previous studies have indicated that moderate to high ambient temperatures enhance the neurotoxic properties of MDMA (Malberg and Seiden, 1998).

On both days locomotor activity was measured for each min of testing. Every 60 min, the rats were taken out of the locomotor activity testing cages for measurement of core body temperature and to receive their next drug injection. At the end of each 4-h session, the rats were returned in their home cages to the colony.

2.4. Statistical analysis

Data from the anxiety tests were analysed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer

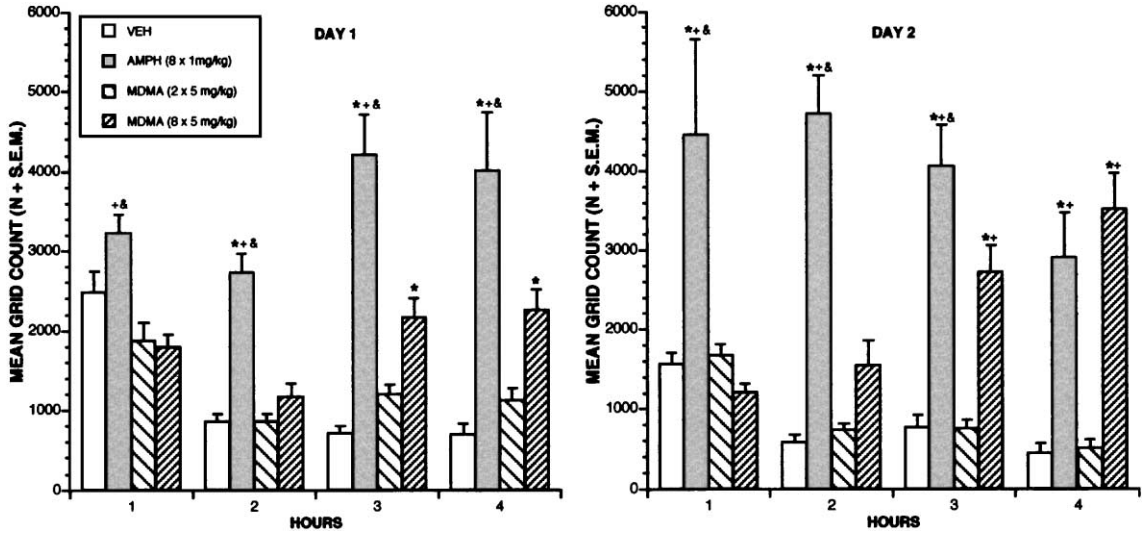

Fig. 2. Mean activity count (±S.E.M.) across 1 h bins for day 1 and day 2 of drug treatment. * $P<0.05$, relative to vehicle within each h block; # $P<0.05$ relative to amphetamine control; + $P<0.05$ relative to moderate MDMA group; & $P<0.05$ relative to high MDMA group, Tukey–Kramer post-hoc tests.

Table 1
Behaviour in the emergence test 12 weeks following treatment with vehicle, amphetamine or MDMA

Groups	Emergence latency (s)	Time in open field (s)	Emergence frequency (*n*)	Rearing (s)	Risk assessment (s)
Vehicle	81.50 ± 18.13	94.79 ± 14.21	4.75 ± 0.42	16.99 ± 3.11	24.53 ± 4.07
Amphetamine	88.53 ± 17.36	83.37 ± 13.11	3.19 ± 0.39	11.46 ± 3.10	29.07 ± 3.84
Moderate MDMA	150.33 ± 23.91	69.32 ± 12.42	2.94 ± 0.44[a]	8.84 ± 1.92	29.29 ± 4.93
High MDMA	178.96 ± 27.90[a,b]	30.97 ± 10.90[a,b]	1.81 ± 0.49[a]	2.94 ± 1.21[a,b]	26.49 ± 4.51

Data represent mean ± S.E.M. Amphetamine (8 × 1 mg/kg); moderate MDMA (2 × 5 mg/kg); high MDMA (8 × 5 mg/kg).
Maximum emergence latency = 300 s due to 5-min test.
[a] $P < 0.05$, relative to vehicle treatment, Tukey–Kramer post-hoc.
[b] $P < 0.05$, relative to amphetamine treatment, Tukey–Kramer post-hoc.

post-hoc tests. A probability level of 0.05 was used to test for statistical significance.

Temperature and locomotor activity measurements from each 1 h block on drug administration day 1 and day 2 were compared across groups using a one-way ANOVA followed by Tukey–Kramer post-hoc tests.

The duration of exploration for both the novel and familiar objects during the test session were measured for each treatment group. The time spent investigating the novel and familiar objects was expressed as a percentage (100 × (novel time/(novel + familiar time))) and compared across groups using an overall ANOVA followed by Tukey–Kramer post-hoc tests. ANOVA was also used to test for differences in overall investigation time in the test session.

3. Results

3.1. Temperature

Body temperatures on the 2 days of drug administration are depicted in Fig. 1. For day 1, ANOVA showed no significant group effect for h 0 ($F < 1$), or h 1 ($F(3, 60) = 2.69$, $P = 0.054$), but revealed a significant group difference for h 2 ($F(3, 60) = 6.45$, $P < 0.001$), h 3 ($F(3, 60) = 20.00$, $P < 0.0001$) and h 4 ($F(3, 60) = 38.64$, $P < 0.0001$). For day 2, ANOVA showed no significant effect for h 0 ($F < 1$), but revealed significant differences for h 1 ($F(3, 60) = 3.26$, $P < 0.05$), h 2 ($F(3, 60) = 8.80$, $P < 0.0001$), h 3 ($F(3, 60) = 8.16$, $P < 0.0001$) and h 4 ($F(3, 60) = 23.99$, $P < 0.0001$). For day 1, post-hoc analysis revealed that there were significant increases in core body temperature for h 2, 3 and 4 in the high MDMA group relative to vehicle and the moderate MDMA group. For h 3 and 4, the amphetamine group was also significantly higher than vehicle and the moderate MDMA groups. For day 2, post-hoc analysis revealed that there were significant increases in core body temperature for h 2, 3 and 4 in the high MDMA group relative to the vehicle and the moderate MDMA groups. The amphetamine control group had significantly higher temperature than the vehicle group for h 1–4, and was hyperthermic relative to the moderate MDMA group for h 2, 3 and 4.

3.2. Locomotor activity

Locomotor activity on the 2 drug administration days are depicted in Fig. 2. For day 1, ANOVA showed a significant group effect for h 1 ($F(3, 60) = 9.06$, $P < 0.0001$), h 2 ($F(3, 60) = 30.21$, $P < 0.0001$), h 3 ($F(3, 60) = 28.76$, $P < 0.0001$) and h 4 ($F(3, 60) = 13.46$, $P < 0.0001$). For day 2, ANOVA showed a significant effect for h 1 ($F(3, 60) = 6.03$, $P < 0.01$), h 2 ($F(3, 60) = 42.83$, $P < 0.0001$), h 3 ($F(3, 60) = 20.49$, $P < 0.0001$) and h 4 ($F(3, 60) = 15.34$, $P < 0.0001$). For day 1, post-hoc analysis revealed that there was significantly greater activity in the amphetamine group relative to both MDMA groups for all 4 h. The amphetamine group also displayed significantly higher activity compared to vehicle for h 2, 3 and 4. The high dose MDMA group displayed significantly higher activity than the vehicle group for h 3 and 4. For day 2, the amphetamine group displayed significantly higher activity than all other groups for h 1, 2 and 3. For h 4, the amphetamine group was

Table 2
Behaviour in the elevated plus-maze test 12 weeks following treatment with vehicle, amphetamine or MDMA

Groups	Open arm time (s)	Closed arm time (s)	Open arm entries (*n*)	Closed arm entries (*n*)
Vehicle	96.84 ± 10.34	199.27 ± 10.18	5.69 ± 0.56	10.50 ± 0.72
Amphetamine	86.20 ± 11.22	209.54 ± 10.98	5.37 ± 0.73	10.25 ± 0.80
Moderate MDMA	34.24 ± 8.72[a,b]	262.80 ± 8.70[a,b]	2.25 ± 0.47[a,b]	12.56 ± 0.86
High MDMA	19.94 ± 8.72[a,b]	275.50 ± 6.98[a,b]	1.63 ± 0.46[a,b]	13.81 ± 1.33

Data represent mean ± S.E.M. Amphetamine (8 × 1 mg/kg); moderate MDMA (2 × 5 mg/kg); high MDMA (8 × 5 mg/kg).
Maximum open/closed arm time = 300 s due to 5-min test.
[a] $P < 0.05$, relative to vehicle treatment, Tukey–Kramer post-hoc.
[b] $P < 0.05$, relative to amphetamine treatment, Tukey–Kramer post-hoc.

Table 3
Behaviour in the social interaction test 12 weeks following treatment with vehicle, amphetamine or MDMA

Groups	Time in social interaction (s)	Time in aggressive behaviours (s)	Squares crossed (*n*)
Vehicle	166.79 ± 11.41	4.38 ± 2.39	108.25 ± 4.75
Amphetamine	152.94 ± 7.23	1.10 ± 0.70	103.63 ± 7.31
Moderate MDMA	132.35 ± 7.38[a]	5.28 ± 4.16	100.38 ± 5.60
High MDMA	107.83 ± 8.33[a,b]	9.90 ± 6.54	82.88 ± 5.27[a]

Data represent mean ± S.E.M. Amphetamine (8 × 1 mg/kg); moderate MDMA (2 × 5 mg/kg); high MDMA (8 × 5 mg/kg).
[a] $P < 0.05$, relative to vehicle treatment, Tukey–Kramer post-hoc.
[b] $P < 0.05$, relative to amphetamine treatment, Tukey–Kramer post-hoc.

significantly higher than vehicle and the moderate MDMA group. The high MDMA group displayed significantly higher activity than vehicle and the moderate MDMA group for h 3 and 4.

3.3. Emergence test

The results for the emergence test are shown in Table 1. One-way ANOVA revealed an overall significant effect of treatment on emergence latency ($F(3,60) = 4.56$, $P < 0.01$), open field time ($F(3, 64) = 4.78$, $P < 0.01$), emergence frequency ($F(3,60) = 7.70$, $P < 0.001$), rear duration ($F(3,60) = 5.58$, $P < 0.01$) but not for risk assessment ($F(3,60) = 0.27$, $P = 0.86$). Post-hoc analysis showed that the high MDMA group had longer emergence latency, less open field time and less rear time than the vehicle and the amphetamine groups. Both the high and moderate dose MDMA groups showed significant less emergence frequency than vehicle group rats.

3.4. Elevated plus-maze test

The results for the elevated plus-maze test are shown in Table 2. One-way ANOVA showed a significant group effect for open arm time ($F(3,60) = 16.40$, $P < 0.001$), closed arm time ($F(3,60) = 16.54$, $P < 0.001$), open arm entries ($F(3,60) = 13.59$, $P < 0.001$), closed arm entries ($F(3,60) = 3.153$, $P < 0.05$), but not for centre time ($F(3,60) = 0.16$, $P = 0.92$). Post-hoc analysis revealed that the high MDMA group showed decreased time in the open arms and number of open arm entries compared to vehicle or amphetamine groups. The high MDMA group also displayed a significant increase in the amount of time spent in the closed arms compared to vehicle and the amphetamine treatments. The moderate dose MDMA group also showed decreased time in the open arms and number of open arm entries relative to the vehicle and amphetamine groups and a significant increase in the amount of time spent in the closed arms compared these groups.

3.5. Social interaction test

The results for the social interaction test are shown in Table 3. One-way ANOVA revealed a significant overall group effect for the duration of social interaction ($F(3,28) = 8.16$, $P < 0.001$), the number of social interactions ($F(3,28) = 3.28$, $P < 0.05$), the number of rears ($F(3,28) = 3.72$, $P < 0.05$) and the number of squares crossed ($F(3,28) = 3.64$, $P < 0.05$) but not for the duration of aggression scores ($F(3,28) = 0.80$, $P = 0.51$), the number of aggression scores ($F(3,28) = 0.26$, $P = 0.86$) or the duration of rearing ($F(3,28) = 2.35$, $P = 0.09$). Post-hoc analysis revealed that the high dose MDMA group displayed a decrease in the total duration of interaction time, the number of interactions, the number of rears and for the number of squares crossed relative to the vehicle group. The high dose MDMA group also displayed significant decreases in social interaction relative to the amphetamine control group. The moderate MDMA group also showed significant decreases in the duration of social interaction compared to the vehicle group.

3.6. Object recognition test for memory

Results for the object recognition task are presented in Table 4. At the 60-min delay, one-way ANOVA showed no overall group effect on the percentage difference scores ($F(3,60)=2.03$, $P=0.12$). For the 15-min delay, one-way ANOVA showed a significant overall treatment effect ($F(3,60)=4.92$, $P<0.01$). Post-hoc analysis showed that, the high dose MDMA group displayed significantly less percentage time investigating the novel object than the vehicle and amphetamine groups. There were no significant group differences in overall investigation time for both the 60- and 15-min delays ($F(3,60) = 0.72$, $P = 0.54$; $F(3,60) = 0.81$, $P = 0.50$).

Table 4
Performance on the object recognition test 14 weeks following vehicle, amphetamine or MDMA treatment

Groups	% Preference for novel object at 15-min delay	% Preference for novel object at 60-min delay
Vehicle	70.19 ± 2.02	65.10 ± 2.03
Amphetamine	70.53 ± 2.15	64.23 ± 2.18
Moderate MDMA	65.71 ± 2.11	64.05 ± 1.85
High MDMA	60.57 ± 2.15[a,b]	58.64 ± 2.20

Data represent mean ± S.E.M. Amphetamine (8 × 1 mg/kg); moderate MDMA (2 × 5 mg/kg); high MDMA (8 × 5 mg/kg).
[a] $P < 0.05$, relative to vehicle treatment, Tukey–Kramer post-hoc.
[b] $P < 0.05$, relative to amphetamine treatment, Tukey–Kramer post-hoc.

4. Discussion

The results of the present study indicate that exposure to MDMA can produce pronounced long-term effects on anxiety-like behaviours, social interaction and memory in rats. No long lasting effects were seen after exposure with another chemically similar stimulant D-amphetamine, suggesting that these behavioural effects are relatively specific to MDMA.

Acute hyperactivity was observed in both MDMA and D-amphetamine groups, as previously demonstrated (Gold et al., 1989; Callaway et al., 1990; Clausing et al., 1995; Kalivas et al., 1998; Bradley and Meisel, 2001). A greater hyperactivity was seen with a cumulative 20 mg/kg dose of MDMA (4×5 mg/kg) than with a single 5 mg/kg dose, which is similar to previous reports showing greater activity for single injections of 20 mg/kg compared to 5 mg/kg (Stephenson et al., 1999). An initial trend towards hypoactivity with MDMA on day 1 of administration may reflect a suppression of investigatory behaviour in a novel environment that is sometimes seen with the drug (Krebs and Geyer, 1993).

Hyperthermia was also seen with both MDMA and D-amphetamine, as has been previously demonstrated at similar ambient temperatures and with similar dose regimens to those used here (Gordon et al., 1991; Dafters, 1995; Malberg and Seiden, 1998; Clausing and Bowyer, 1999). Hyperthermia, however, was not observed in the moderate MDMA group, although thermic effects have sometimes been demonstrated with lower MDMA doses in the past (Dafters, 1994). It is particularly striking that in the present study D-amphetamine produced an MDMA-like effect on both core body temperature and locomotor activity, but did not cause the subsequent long-term changes in behaviour that were obtained with MDMA.

The results from the anxiety battery were particularly consistent, with increased anxiety-like responses seen in all three tests in rats receiving the moderate or high dose MDMA treatment regime 3 months earlier. In the emergence test, these rats showed a marked increase in the time taken to emerge from the hide box. A large number of rats treated with high dose MDMA (6/16) did not emerge for the entire 5-min session. Similarly, in the elevated plus-maze test, MDMA-treated rats in both MDMA groups showed less time in the open arm and fewer entries into the open arm.

Decreased social interaction with a novel conspecific was also seen in both MDMA-treated groups. This suggests that MDMA produced an increased anxiety-type response to such interaction (File, 1980). However, this result might also reflect blunted exploration in the environment in which social interaction was tested. Indeed, rats in the high dose (but not the moderate dose) MDMA group showed an overall decrease in ambulation during this test. In previous work (Morley and McGregor, 2000) we have demonstrated that when given acutely, MDMA actually increases social interaction in rats whilst having an anxiogenic effect in the elevated plus maze and emergence tests. It is therefore very striking that the long-term effects of MDMA on social interaction seen in the present study are opposite to those seen when the drug is given acutely.

What is perhaps most remarkable here is the effect in rats receiving the moderate MDMA dose. These rats had only received 5 mg/kg MDMA on 2 consecutive days and yet on all anxiety measures they showed a similar profile to the rats given the high dose MDMA treatment. These results are reminiscent of those of Hall et al. (1999) who showed that rats given relatively low doses of the serotonergic neurotoxin, 5,7-D-hydroxytryptamine (5,7-DHT) displayed decreased open arm time on the elevated plus maze similar to that seen in rats given higher 5,7-DHT doses.

The present study is the first demonstration to our knowledge of long-lasting anxiety-like behaviour as a result of MDMA administration in rats. Tendencies towards this effect have been shown in one previous experiment using the open field test where rats were tested at 2–4 weeks after MDMA administration (Slikker et al., 1989). The implication of this finding is that MDMA-induced neural damage or perhaps even adaptation to an initial neurotoxic effect causes long-lasting and perhaps permanent behavioural sequelae in rats.

These results are interesting in light of the recent studies suggesting recreational Ecstasy consumption correlates with elevation of anxiety states in humans. Although many studies suggest that occasional to moderate MDMA use is generally not associated with elevated anxiety (Parrott et al., 2000; Verkes et al., 2001), heavier patterns of use are increasingly being associated with persistent anxiety states (Gamma et al., 2000; Parrott et al., 2000; Verkes et al., 2001). In one of the more alarming recent studies, heavy MDMA users displayed higher anxiety levels than non-users even after prolonged periods of abstinence (Wareing et al., 2000). The current study helps in clarifying the complex cause–effect complications present in human MDMA research and suggests that reports of elevated anxiety in human users are, in fact, a consequence of moderate to heavy MDMA exposure.

In the present study, MDMA treatment also had a significant deleterious effect on object recognition memory measured 14 weeks after drug administration. In the object recognition task, novel objects were investigated for longer periods of time in all treatment groups both 15 and 60 min following exposure to the objects. However, when the delay was 15 min, the high dose MDMA group devoted a significantly smaller proportion of investigation time to the novel objects relative to the familiar object. This suggests that memory for the familiar object at this delay interval was not as strong than that of the vehicle and amphetamine-treated rats (Aggleton, 1985). There was also a tendency towards such an effect in rats given the moderate MDMA treatment. At the 60-min delay, overall performance in all rats was worse than at the 15-min delay making it more difficult to isolate an effect of drug treatment due to a

floor effect. Nonetheless, there was still a clear tendency towards the high dose MDMA group showing poorer performance at this longer delay.

These results are in agreement with the subtle cognitive deficits demonstrated by Marston et al. (1999) who showed impaired delayed non-matching to position performance at longer delays (up to 30 s) in rats. In this study, MDMA-treated rats failed to improve their performance at longer delays over 19 days of training while showing normal performance at shorter delays. These delay-dependent impairments in accuracy were attributed to perturbations in short-term memory.

These results in rats parallel the plethora of recent studies associating MDMA use with cognitive impairments in humans (reviewed in Morgan, 2000). Impaired memory and/or learning has been demonstrated in studies with an average lifetime tablet consumption of 100 (Reneman et al., 2000) to 1200 (Wareing et al., 2000). Further, studies have found such deficits after drug-free periods ranging from 1 (Verkes et al., 2001) to 46 weeks (Wareing et al., 2000). These human studies along with the current results with rats are clearly implicating that moderate to high use of MDMA may indeed lead to a number of cognitive problems, even following relatively prolonged periods of abstinence.

The main limitation of the present study is clearly the lack of neurochemical analysis of the brains of rats given the moderate and high dose MDMA treatments. While it is hypothesised that the observed effects on anxiety and memory are related to the neurotoxic action of MDMA on brain 5-HT systems, definitive proof of this is lacking. It would clearly be of particularly interest to determine whether the rats given the moderate dose regime of MDMA have experienced MDMA-induced neurotoxicity. Previous studies suggest that these rats were given a dose regime that is on the borderline of what normally causes a neurotoxic effect (Colado et al., 1995; Dafters et al., 1997). However, the use of a high ambient temperature during MDMA administration may have made a neurotoxic effect more likely in the present study (Malberg and Seiden, 1998).

Future studies from this laboratory aim to replicate the results obtained in the present study with the addition of neurochemical analysis of 5-HT systems. Notwithstanding the lack of a neurochemical analysis, the results of the present experiment are surprisingly clear. Rats given exposure to MDMA 3 months previously show increased anxiety-like behaviours and poorer memory relative to untreated controls and amphetamine-treated rats. These results add to mounting evidence that human MDMA users risk long-term adverse effects on emotion and cognition.

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