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Increased anxiety in rats after 3,4-methylenedioxymethamphetamine: association with serotonin depletion

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Abstract

The long-term behavioural and neurotoxic effects of 3,4-methlyenedioxymethampthetamine (MDMA, ''Ecstasy'') were examined in rats. Rats were given MDMA (5 mg/kg i.p. once per hour for 4 h) or vehicle injections on each of two consecutive days at an ambient temparature of 28 °C. MDMA caused acute hyperthermia and locomotor hyperactivity on both days. Four and six weeks after drug administration the rats previously treated with MDMA showed elevated levels of anxiety-like behaviour in the emergence and social interaction tests, respectively. At 9 weeks post-MDMA, the rats displayed an increase in anxiety on the elevated plus-maze test relative to controls. Ten weeks following treatment the rats were killed and their brains dissected and neurotramitter content analysed using High Performance Liquid Chromotography (HPLC). Rats previously given MDMA showed significantly decreased 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the amygdala, hippocampus and striatum relative to controls. This 5-HT depletion may have a causal role in producing increased anxiety-like behaviours in MDMA-treated rats. These results are consistent with human studies suggesting that exposure to high doses of MDMA may predispose to long-term psychological problems such as anxiety and depression.

Keywords: MDMA (3,4-methylenedioxymethampthetamine); 5-HT (5-hydroxytryptamine, serotonin); Ecstasy; Anxiety; (Rat); Neurotoxicity

1. Introduction

3,4-Methlyenedioxymethampthetamine (MDMA: ''Ecstasy'') is an illicit drug of growing popularity in many countries. Concerns continue to be raised about the possible long-term deleterious psychological and physical effects of the drug, particularly among heavy users. Acutely, MDMA administration causes a massive increase in synaptic 5-hydroxytryptamine (5-HT) and a moderate increase in dopamine levels (Schmidt, 1987). Administration of high doses of MDMA to laboratory animals causes degeneration of 5-HT terminals, decreases in brain 5-HT concentrations and decreased density of 5-HT transporter sites (Ricaurte et al., 2000). There is mounting evidence that similar neurotoxicity may be evident in humans who are heavy MDMA users (Boot et al., 2000; Reneman et al., 2001; Ricaurte et al., 2000). Although neurotoxicity arising from MDMA is increasingly well-established, knowledge of the behavioural, emotional and cognitive consequences of this neurotoxicity is still at a formative stage.

Recent case studies, surveys and laboratory studies have reported an association between MDMA use and psychological dysfunction in humans (McGuire, 2000; Morgan, 2000). MDMA use, particularly heavy use, has been associated with memory impairment (Bolla et al., 1998; Gouzoulis-Mayfrank et al., 2000; Morgan, 1999; Verkes et al., 2001), depression (McGuire, 2000; Parrott et al., 2000; Schifano et al., 1998) and elevated anxiety (Gamma et al., 2000; McGuire, 2000; Parrott et al., 2000; Schifano et al., 1998; Verkes et al., 2001; Wareing et al., 2000). However, the generality of these effects among MDMA users and the causal relationship between MDMA and psychological dysfunction is at present uncertain. Given the complexity of drawing inferences from the human literature it has been suggested that animal models may provide an important means of assessing the long-term consequences of MDMA neurotoxicity (Boot et al., 2000).

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Preclinical information regarding the long-term effects of MDMA is only starting to emerge. More than a decade ago a trend towards an increase in anxiety-like behaviours in rats treated 2–4 weeks previously with MDMA was reported (Slikker et al., 1989). More recently, work from our own laboratory has shown that Wistar rats treated 3 months previously with MDMA show significantly greater anxiety-like behaviours than controls in the emergence, elevated plus-maze and social interaction tests (Morley et al., 2001). Similar effects have also been recently reported in Lister rats given MDMA during adolescence and tested in the social interaction test 12–29 days later (Fone et al., 2002). Decreases in open field behaviour have also recently been reported in rats 4 weeks after administration of neurotoxic doses of the closely related drug methylenedioxyamphetamine (MDA) (Harkin et al., 2001). However, another recent study has reported an apparent decrease in anxiety-related behaviours in the open field and plus-maze in Dark Agouti rats at 73–80 days following a single high dose of MDMA (Mechan et al., 2002b).

The present study aimed to replicate and extend our previous study (Morley et al., 2001). A major limitation of this previous study was the lack of neurochemical analysis of brain regions of rats given MDMA. The neurotoxic effects of MDMA may critically depend upon the dosing regime used (O'Shea et al., 1998), the ambient temperature at the time of dosing (Malberg and Seiden, 1998) and the time that has elapsed between dosing and neurochemical analysis (Battaglia et al., 1988; Hatzidimitriou et al., 1999; Lew et al., 1996; Sabol et al., 1996). Thus, it is not clear that the increase in anxiety seen in MDMA-treated rats is necessarily associated with 5-HT depletion. Indeed, the results of a recent study suggest that long-term increases in anxiety-like behaviour may emerge in MDMA pre-treated rats without any significant neurotoxicity being present (Fone et al., 2002). However, close scrutiny of this study shows a clear trend towards 5-HT depletion in the hippocampus of MDMA-treated rats that may well have been significant had a larger sample size been employed. Further, these authors did not examine 5-HT depletion in other relevant brain structures such as the amygdala. We therefore sought here to demonstrate further the long-term anxiogenic properties of MDMA and to relate them to 5-HT depletion in the hippocampus and amygdala.

2. Materials and methods

2.1. Subjects

The subjects were 26 inbred male albino Wistar rats (Concord Hospital breeding facility) aged 3 months at the beginning of the experiment and weighing an average of 374 g. The rats were housed in groups of six or seven per cage for the duration of the experiment with food and water freely available. Temperature in the colony room was controlled at 22 °C and a 12-h reverse light cycle was in operation. All behavioural testing was conducted during the dark cycle. All experimentation was approved by the University of Sydney animal ethics committee in accordance with the *Australian code of practice for the care and use of animals for scientific purposes* (NH&MRC, 1997).

2.2. Drug administration

(+/−)3,4-Methylenedioxymethamphetamine was supplied by the Australian Government Analytical Laboratories (Pymble, NSW). It was diluted in 0.9% saline and injected at a volume of 5 mg/ml.

Rats in the MDMA group ($n = 13$) were given 5 mg/kg of MDMA i.p. every hour for 4 h on each of two consecutive days to give a cumulative total dose of 40 mg/kg (20 mg/kg/day). Equivalent injections of 0.9% saline were administered to control rats ($n = 13$). This dosing regime was chosen as it has been shown to be a reliable neurotoxic dose (O'Hearn et al., 1988; O'Shea et al., 1998) and is identical to the one used by Morley et al. (2001).

2.3. Method

2.3.1. Locomotor activity and body temperature measurement

Locomotor activity and body temperature were recorded during acute MDMA administration, in the same way as reported by Morley et al. (2001). Briefly, rats were dosed with MDMA or vehicle and were then immediately placed in standard operant chambers ($30 \times 50 \times 25.5$ cm) with aluminium side and back walls and Perspex front wall and a metal grid floor. The walls of the chambers were fitted with two passive infrared detectors that were triggered movements of the head and body of the rats as well as gross locomotion. Activity counts were recorded by a Macintosh computer running "WorkbenchMac" data acquisition software (McGregor, 1996) and the test chamber was placed inside a wooden sound attenuation box which provided darkness and masking fan noise during testing.

After each hour of the 4-h tests, the rats were briefly removed from the test chambers to administer their next injection of MDMA or vehicle and to measure body temperature. Temperature was measured using a Braun Thermoscan Instant thermometer (IRT 1020). This device is inserted into the ear of the rat with a reading provided within 3 s (see (Morley et al., 2001; O'Loinsigh et al., 2001)).

2.3.2. Emergence test

Four weeks after acute dosing with MDMA or vehicle, rats were tested in the emergence test. The apparatus consists of a white Perspex walled rectangular arena ($96 \times 100 \times 40$ cm) with a black wooden hide box ($24 \times 40 \times 15$ cm) placed in the top left corner of the arena. The open part of the arena was illuminated with red

light (40 W) and a video camera was mounted above the arena and connected to a video recorder.

Rats were initially placed inside the wooden hide box (which had a hinged lid through which the rat could be placed inside the box). Testing continued for 5 min during which time the experimenter remained outside the test room.

Subsequent video analysis by an experimenter blind to group assignment scored the latency of rats to emerge from the hide box and the duration of time spent in the open field. "Risk assessment" behavior was also scored which refers to the time rats spent with their head poking out of the hide box but with the majority of the body remaining inside the box. Analysis was accomplished using ODLog data logging software from Macropod software (http://www.macropod-software.com).

After each test session the apparatus was thoroughly wiped down with a damp cloth containing 10% ethanol.

2.3.3. Social interaction test

Two weeks following the emergence test, pairs of rats were assessed in the social interaction test. The apparatus was a square clear Perspex box (52 × 52 × 40 cm) dimly lit with red light (40 W). A miniature video camera was placed vertically above the box. This camera sent its signal to a video recorder and monitor in a neighbouring room where the interactions of the rats were recorded onto tape. The experimenter remained outside the test room during testing and the test arena was wiped down with 10% ethanol in-between each test session.

Testing was performed across three consecutive days with each rat tested with a different partner on the first two days. A small number of rats were tested with a third partner on the third day. Partners were selected so as to be of approximately equal body weight and from the same treatment condition (MDMA or vehicle) but from a different home cage. Data for a total of 14 MDMA pairs and 16 vehicle pairs were obtained.

Each social interaction session lasted for 10 min. The total duration of social interaction and number of interactions during this 10 min period was scored from video by an observer using ODLog software (http://www.macropodsoftware.com). Behaviours that were recorded as social interaction included sniffing, adjacent lying, following, crawling over/under and mutual grooming (File, 1980).

2.3.4. Elevated plus-maze test

Three weeks following the social interaction test (and a total of 9 weeks following MDMA treatment) rats were tested on the elevated plus-maze in a 5-min test. The plus-maze apparatus was made of white Perspex and consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 cm). The closed arms 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. A miniature video camera was mounted to the top of one of the closed arms, vertically above the central square, sending images to a monitor and video recorder in an adjacent room. Photocell detectors (two infra-red transmitters and two receivers) were placed at the far ends of each of the four arms with output from the receivers 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 room was illuminated by a red light (40 W) and the experimenter remained outside the room during testing. A black curtain was extended around the apparatus during testing.

The dependent variables obtained for this test were the time spent on the open arms, time spent on the closed arms and the total number of arm entries.

2.4. Neurochemical analysis

At 10 weeks following MDMA or vehicle treatment, 16 of the 26 rats used in the study (8 MDMA and 8 vehicle treated) were randomly selected for neurochemical analysis. They were decapitated using a guillotine, and their brains rapidly removed. The brains were dissected according to the method of Heffner et al. (1980). Briefly, the brains were sectioned using a bank of razors glued together at 2-mm intervals that fitted into a 2-mm brain block (Zivic-Miller, Pittsburg, PA). This system divided the brain into a series of 2-mm coronal slices. Four regions of interest were manually dissected out over dry ice from individual slices: the olfactory bulb, the caudate-putamen, the amygdala (including surrounding temporal lobe) and the anterodorsal part of the hippocampus. Samples were individually placed in Eppendorf tubes and were stored in a freezer at −80 °C until assay.

Tissue samples were homogenized with a 250-μl ice-cold solution of 0.2 M perchloric acid containing 0.1% cysteine and 200 nmol/l of internal standard 5-hydroxy-*N*-methyltryptamine (5-HMeT). The homogenisate was centrifuged at 15,000 × *g* for 10 min at 4 °C. A 20-μl aliquot of the resulting supernatant fluid was then analysed for biogenic amines by high performance liquid chromatography (HPLC) with electro-chemical detection as described previously by Schworer et al. (1987) with slight modification.

Briefly, the GBC HPLC system (Melbourne, Australia) was composed of a LC1610 auto-injector, LC 1150 multi-solvent delivery pump, LC 1210 electrochemical detector (ECD) and WinChrom data management software. The mobile phase consisted of 0.1 mol/l phosphate buffer (pH 3.0), PIC B-8 octane sulphonic acid (0.74 mmol/l; Waters, Australia), sodium EDTA (0.3 mmol/l) and methanol (12% v/v). The flow rate was maintained at 1 ml/min. Dopamine, 5-hydroxyindoleacetic acid (5-HIAA), 5-HT and 5-HMeT were separated by a Merck LiChrospher 100 RP-18 reversed phase column. Quantification was achieved via ECD equipped with a glassy carbon working electrode set at +0.75 V. The calibration curve of each standard was obtained by the

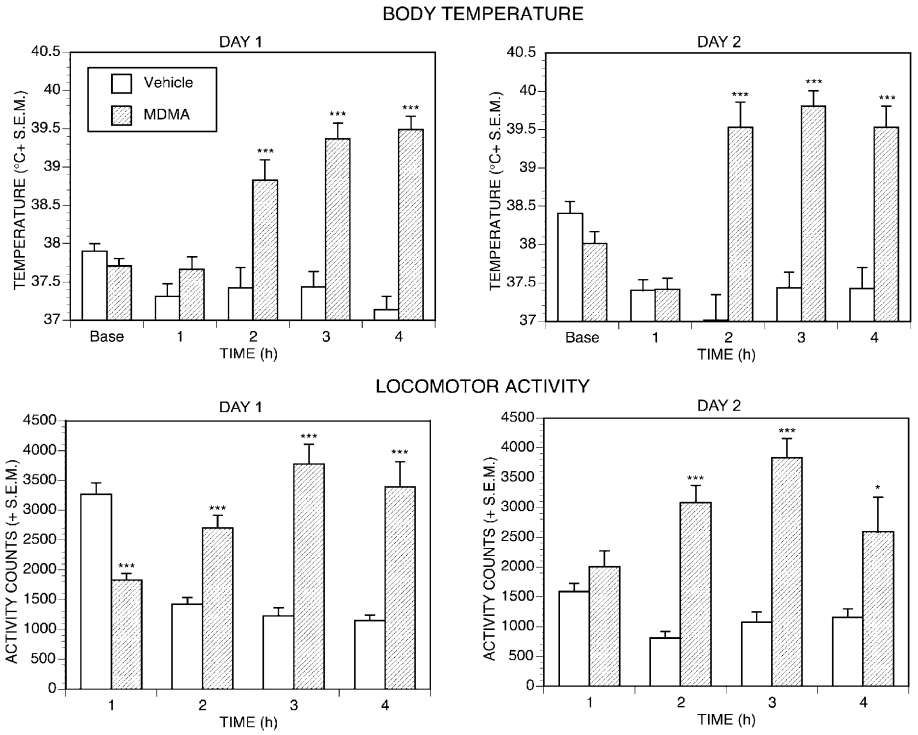

Fig. 1. Mean body temperature (upper) and locomotor activity (lower) in the vehicle and MDMA groups on day 1 (left) and day 2 (right) of drug treatment. *$P<0.05$, ***$P<0.001$, relative to vehicle group within each 1-h block. A 5 mg/kg dose of MDMA or vehicle was given at the beginning of each hour. Base = baseline temperature.

concentration versus the area ratio of the standard and internal standard.

2.5. Statistical analysis

Statistical analysis of the locomotor activity and temperature was accomplished via repeated measures analysis of variance (ANOVA), comparing the MDMA and vehicle-treated groups across each of the 4 h of drug administration. Group differences for each individual hour of testing were performed using simple *t*-tests.

Statistical analysis of data from the three anxiety tests was achieved by comparing MDMA and vehicle groups using one-way ANOVA.

Similarly, differences between MDMA- and vehicle-treated rats for 5-HT, 5-HIAA and dopamine content in individual brain regions were assessed by means of a one-way ANOVA.

The significance level for all statistical tests was set at $P<0.05$.

3. Results

3.1. Locomotor activity

The results for locomotor activity during acute drug administration are shown in Fig. 1. Rats given MDMA displayed significantly greater overall locomotor activity on day 1 ($F(1,24)=24.57$, $P<0.001$) and day 2 ($F(1,24)=37.66$, $P<0.001$) of drug administration. Comparison between groups for each individual hour of testing showed that the MDMA group had significantly greater activity in hours 2, 3 and 4 of testing on both day 1 and day 2. The vehicle group had significantly greater activity than the MDMA group during the first hour of testing on day 1.

3.2. Body temperature

The results for body temperature during acute drug administration are also shown in Fig. 1. MDMA-treated rats had a higher overall body temperature than controls on both day 1 ($F(1,24)=76.63$, $P<0.001$) and day 2 ($F(1,24)=54.18$, $P<0.001$) of drug administration. Comparison between groups for each individual hour of testing showed that the MDMA group had significantly greater

Table 1
Behaviour on the emergence test 4 weeks after vehicle or MDMA administration

Group	Vehicle ($n=13$)	MDMA ($n=13$)
Latency to emerge (s)	32.24 ± 8.69	64.73 ± 8.1[a]
Time in open field (s)	193.81 ± 11.94	143.89 ± 10.95[b]
Risk assessment (s)	28.53 ± 5.58	41.45 ± 6.13

[a] $P<0.05$ relative to vehicle group.
[b] $P<0.01$ relative to vehicle group.

Table 2
Behaviour in the social interaction test 6 weeks following vehicle or MDMA administration

Group	Vehicle ($n=16$ pairs)	MDMA ($n=14$ pairs)
Time spent interacting (s)	147.08 ± 9.53	70.38 ± 6.16[a]
No. of interactions	69 ± 2.78	49 ± 3.35[a]

[a] $P<0.001$ relative to control group.

temperature in hours 2, 3 and 4 of testing on both day 1 and day 2.

3.3. Emergence test

The results for the emergence test are shown in Table 1. Results revealed that rats receiving MDMA treatment 4 weeks previously had a longer latency to emerge from the hide box ($F(1,24)=6.58$, $P<0.05$) and spent less time in the open field than controls ($F(1,24)=9.48$, $P<0.01$). There were no significant differences between groups in risk-assessment ($F(1,24)=2.42$, $P=0.12$).

3.4. Social interaction test

Results for social interaction are shown in Table 2. Analysis indicated that rats previously treated with MDMA spent significantly less time in social interaction than controls ($F(1,28)=42.79$, $P<0.001$). The MDMA pre-treated rats also showed fewer total interactions than controls ($F(1,28)=22.54$, $P<0.001$).

3.5. Elevated plus-maze test

The results of the elevated plus-maze test are shown in Table 3. Analysis revealed that on average MDMA pre-treated rats spent less time on the open arms of the plus-maze ($F(1,24)=5.86$, $P<0.05$) and more time in the closed arms than controls ($F(1,24)=5.87$ $P<0.05$). There was no difference in the total number of arm entries between groups ($F<1$).

3.6. Neurochemical analysis

Results from the neurochemical analysis are presented in Table 4. Analysis revealed that 10 weeks after MDMA rats had significantly less 5-HT than controls in the amygdala ($F(1,14)=4.91$, $P<0.05$), hippocampus ($F(1,12)=8.52$, $P<0.05$) and caudate-putamen ($F(1,12)=6.75$, $P<0.05$).

Table 3
Behaviour on the elevated plus maze test 9 weeks after vehicle or MDMA administration

Group	Vehicle ($n=13$)	MDMA ($n=13$)
Open arm time (s)	120.22 ± 4.04	94.54 ± 10.12[a]
Closed arm time (s)	179.1 ± 4.11	205.15 ± 0.25[a]
Total arm entries	23.36 ± 0.95	22.85 ± 1.27

[a] $P<0.05$ relative to vehicle group.

Table 4
Results of HPLC analysis of brains 10 weeks after MDMA or vehicle

Region	Treatment	5-HT	5-HIAA	Dopamine
Caudate-putamen	Vehicle	193.3 ± 30.7	198.0 ± 18.9	5538.3 ± 860.0
	MDMA	101.9 ± 11.5[a]	105.6 ± 13.1[b]	5549.5 ± 753.4
Hippocampus	Vehicle	94.3 ± 2.5	123.2 ± 17.1	42.0 ± 7.8
	MDMA	64.4 ± 8.6[a]	64.1 ± 14.0[a]	35.1 ± 5.9
Amygdala	Vehicle	394.9 ± 41.6	232.6 ± 16.9	421.7 ± 88.7
	MDMA	269.4 ± 8.4[a]	148.0 ± 12.2[b]	338.5 ± 57.7
Olfactory bulb	Vehicle	91.8 ± 10.7	24.3 ± 8.6	47.8 ± 3.5
	MDMA	105.5 ± 10.4	27.5 ± 11.2	54.6 ± 3.1

[a] $P<0.05$ relative to vehicle group.
[b] $P<0.01$ relative to vehicle group.

There were no group differences in the olfactory bulb ($F<1$).

With 5-HIAA, analysis revealed that the MDMA pre-treated rats had significantly less 5-HIAA in the amygdala ($F(1,14)=16.47$, $P<0.01$), hippocampus ($F(1,12)=7.26$, $P<0.05$) and caudate-putamen ($F(1,12)=14.48$, $P<0.05$) but not in the olfactory bulb ($F<1$).

With respect to dopamine, analysis revealed no significant differences between groups in dopamine concentrations in any of the four areas examined (Fs <1.2).

4. Discussion

The results of the present study replicate and extend the recent findings of Morley et al. (2001) confirming that rats previously treated with MDMA show a magnification of anxiety-like behaviours on three separate tests: the emergence, social interaction and elevated plus-maze tests. The results on the social interaction test are particularly striking with MDMA pre-treated rats engaging in less than half the amount of social behaviour seen in controls. This is particularly interesting in that we have recently found that acute administration of MDMA causes a marked increase in social interaction between rats (Morley and McGregor, 2000). Thus, the long-term effects of MDMA in this case are precisely opposite to those seen in the short term. The long-term effects of MDMA on social interaction reported here with Wistar rats are also in agreement with those recently reported in Lister rats (Fone et al., 2002).

The acute effects of MDMA seen in the drug administration phase of the current study parallel those reported previously (Morley and McGregor, 2000). MDMA caused an initial locomotor hypoactivity, while a robust hyperactivity emerged in the later hours of drug administration as the cumulative dose of MDMA increased. The initial hypoactivity probably reflects the characteristic inhibition of exploratory behaviour that the drug produces in a novel environment (Callaway et al., 1990; Maldonado and Navarro, 2000; Morley and McGregor, 2000; Scearce-Levie et al., 1999). The subsequent hyperactivity is characteristic of higher doses of the drug and has been widely described in

the literature (Callaway et al., 1990; Morley et al., 2001; Morley and McGregor, 2000; Scearce-Levie et al., 1999; Stephenson et al., 1999).

Acutely, MDMA also produced a robust hyperthermia. Again this is an effect that has been widely described in the literature although it is an effect that depends upon the ambient temperature at which MDMA is administered (Dafters, 1995; Malberg and Seiden, 1998; O'Loinsigh et al., 2001; O'Shea et al., 1998). At cool ambient temperatures (e.g. <22 °C) MDMA can produce a hypothermia while at hotter temperatures such as the 28 °C used in the present study, hyperthermia is usually seen (Dafters, 1995; Fone et al., 2002; Malberg and Seiden, 1998; Morley et al., 2001). This hyperthermia may play an important role in the neurotoxic effects of MDMA (Malberg et al., 1996; Malberg and Seiden, 1998).

The present study shows that the magnification of anxiety-like behaviours seen in rats previously treated with MDMA is present at earlier time intervals than those reported by Morley et al. (2001). These researchers examined anxiety-like behaviours at 12 weeks following MDMA and found enhancement of anxiety in the emergence, elevated plus-maze and social interaction tests. The present findings that similar effects were obtained at 4, 6 and 9 weeks after MDMA are not altogether surprising, particularly if these effects reflect 5-HT neurotoxicity. It is well known that the 5-HT depleting effects of MDMA peak shortly after administration and that only very gradual recovery of tissue 5-HT concentrations and axonal density occurs over the weeks, months and years following MDMA (Battaglia et al., 1988; Hatzidimitriou et al., 1999; Lew et al., 1996; Sabol et al., 1996). Similarly, increased anxiety-like behaviours have been seen in rats in the open field at 4 weeks following MDA administration (Harkin et al., 2001) and in the social interaction test 12–29 days following MDMA (Fone et al., 2002).

One puzzling discrepancy is evident when comparing this pattern of results with those recently reported using Dark Agouti rats by Mechan et al. (2002b). These authors found no apparent differences in anxiety-like behaviour between MDMA-treated and control rats on the elevated plus-maze and open field at relatively early time intervals (8 and 29 days) yet an apparent long-term anxiolytic action of MDMA at 73 and 80 days following treatment (Mechan et al., 2002b). It is difficult to explain this opposite pattern of results. However, one salient feature is the very high baseline levels of anxiety-related behaviour in the Dark Aguoti strain (Mechan et al., 2002a). This might make it difficult to show a long-term anxiogenic action of MDMA due to ceiling effects. Further, it can be noted that these authors only injected a single dose of MDMA and did not verify the loss of 5-HT in their experimental subjects. Finally, these authors did not examine their rats in the social interaction test of anxiety, which in the present study and the study of Fone et al. (2002) was the most powerful index of long-term anxiogenic action of MDMA.

The present results do not prove that it is MDMA-induced 5-HT depletion that causes increases in anxiety-like behaviours. It might be argued that the effect reflects some broader non-neurotoxic action of stimulant drugs such as sensitisation. In this regard, it is worth noting that Morley et al. (2001) found no long-term increases in anxiety-like behaviour in rats treated with a dose regime of *d*-amphetamine administered 3 months earlier. Acutely, *d*-amphetamine produced acute hyperactivity and hyperthermia but, unlike MDMA, had no lasting effect on anxiety-like behaviour. However, Fone et al. (2002) report that decreased social interaction may be present in rats previously treated with MDMA in the absence of any detectable 5-HT neurotoxicity. This is a very important claim, suggesting that some mechanism other than 5-HT depletion underlies the long-term effects of MDMA on emotion. However, the HPLC results reported by Fone et al. showed a strong tendency towards 5-HT depletion in the hippocampus and the power of the study was limited due to a rather small sample size. Further, Fone et al. did not examine 5-HT or 5-HIAA in the amygdala, a region implicated in anxiety-related behaviour, and a region showing clear 5-HT depletion in the present study.

Nonetheless, supporting the conclusions that 5-HT depletion may not necessarily be causal in producing these emotional changes, Morley et al. (2001) found that a relatively mild regime of MDMA administration (2×5 mg/kg) that may not have neurotoxic consequences leads to long-term increases in anxiety-like behaviours in rats. It is therefore difficult to decide on the basis of the available evidence whether the changes in emotional behaviour seen in MDMA pre-treated rats are related to 5-HT depletion or not.

Traditional theory has posited that anxiety is due to increased rather than decreased 5-HT function or that 5-HT depletion produces behavioural disinhibition rather than any specific action on anxiety (Soubrie, 1986; Tye et al., 1977). Such theories sit uncomfortably with observations that most optimal treatments for human anxiety disorders involve interventions that increase synaptic 5-HT, warranting the inference that in some way lowered 5-HT function is implicated in anxiety disorders. Further, there is some evidence from animal models that manipulations that decrease brain 5-HT may increase anxiety-like behavior. Recently, Hall et al. (1999) showed that rats given a relatively low dose of the serotonergic neurotoxin 5,7-*d*-hydroxytryptamine (5,7-DHT) displayed a significant increase in anxiety-like behavior on the elevated plus-maze 2 weeks later in parallel with a modest decrease in brain 5-HT levels (Hall et al., 1999).

To further understand the mechanisms underlying the magnification of anxiety reported here and elsewhere with MDMA, it would clearly be advantageous to know the effects of 5-HT depletion on anxiety-like behaviours when the depletion is limited to specific brain regions such as the amygdala, hippocampus or prefrontal cortex. It is to be hoped that future studies will address this important issue.

Future studies comparing the long-term behavioural effects of different dosing regimes of MDMA (with different neurotoxic consequences) are also clearly warranted.

Finally, it is important to reiterate that elevated anxiety is increasingly evident in recent human studies of heavy human MDMA users (Gamma et al., 2000; Parrott et al., 2000; Verkes et al., 2001; Wareing et al., 2000). Whether such effects are a direct result of MDMA use and whether they reflect MDMA-induced 5-HT depletion is, at present, uncertain.

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