

Annis O. Mechan · Paula M. Moran · J. Martin Elliott  
Andrew M. J. Young · Michael H. Joseph  
A. Richard Green

## A study of the effect of a single neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”) on the subsequent long-term behaviour of rats in the plus maze and open field

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**Abstract Rationale:** Decreased 5-HT function has been shown to induce behaviour consistent with an “anxiolytic” effect. Administration of a single dose of 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”) 12.5 mg/kg IP to rats results in prolonged damage to central serotonergic nerve terminals. Thus we wished to assess whether an MDMA-induced lesion may have longer-term behavioural consequences. **Objective:** The study was designed to examine the behaviour of MDMA-pretreated and control animals in the elevated plus-maze and open field at a number of time-points, up to 80 days, after the administration of a single neurotoxic dose of MDMA (12.5 mg/kg IP). **Results:** MDMA-pretreated Dark Agouti rats demonstrated a statistically significant reduction in anxiety-related behaviour, compared to saline-pretreated control rats, in both the elevated plus-maze and open field when the rats were tested on day 73 (open field) and day 80 (plus maze) after MDMA administration. **Conclusions:** The behavioural consequences of a single neurotoxic dose of MDMA can be demonstrated over 2 months after administration of the compound, thereby indicating that long-term adaptive changes occur within the brain following the neurodegeneration of 5-HT neurones produced by this recreationally used drug.

**Keywords** 3,4-Methylenedioxymethamphetamine · MDMA · Ecstasy · Anxiety-related behaviour · Elevated plus-maze · Open field · Long-term neurotoxicity

A.O. Mechan · J.M. Elliott · A.R. Green (✉)  
Department of Pharmacology, School of Pharmacy,  
De Montfort University, The Gateway, Leicester LE1 9BH, UK  
e-mail: richard.green@astrazeneca.com

P.M. Moran · A.M.J. Young · M.H. Joseph  
School of Psychology, University of Leicester,  
University Road, Leicester LE1 7RH, UK

A.R. Green  
AstraZeneca R&D Charnwood, Loughborough LE11 5RH, UK

### Introduction

Administration of 3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”) to several species, including primates, induces an acute release of 5-hydroxytryptamine (5-HT) and dopamine from nerve endings in the brain and produces a behavioural syndrome and hyperthermia (Nash et al. 1988; Gordon et al. 1991; Colado et al. 1993; Dafters 1994). Either a single high dose or several lower repeated doses of MDMA also produce long lasting damage to 5-HT nerve terminals, which is reflected in a decrease in cerebral 5-HT content and loss of [<sup>3</sup>H]-paroxetine binding sites (Stone et al. 1987b; O’Hearn et al. 1988; Molliver et al. 1990; Colado et al. 1997; O’Shea et al. 1998). While evidence that similar long-term changes also occur in the brain of human recreational MDMA users has been controversial (see Green and Goodwin 1996; Saunders 1996), recent studies have provided sufficient data to indicate that MDMA may well produce neurodegenerative changes (McCann et al. 1998, 1999).

5-HT is a major neurotransmitter in the central nervous system and is implicated in the control of many functions including sleep, mood, cognition, temperature control and sexual activity (Myers 1980; Sandler et al. 1991; Oliver et al. 1998; Anderson and Mortimore 1999; Fink et al. 1999; Meneses 1999; Leonard 2000; Portas et al. 2000). It is therefore reasonable to propose that persons taking high or regular doses of MDMA might display altered physiological or psychological symptoms that are indicative of functional damage to central serotonergic systems. However, investigation of such matters in MDMA users is problematic. Firstly, only retrospective studies can be undertaken, and these are complicated by problems of ascertaining accurate information concerning the frequency of drug use and the doses ingested and this information is notoriously inaccurate when the drug is manufactured, obtained and

used illicitly (see Green et al. 1995). Secondly, it is difficult to separate the acute adverse effects of the drug, which are often anecdotal in the form of case reports (for example, Winstock 1991), from any underlying long-term psychopathology (McGuire et al. 1994; Green et al. 1995).

Very few studies have examined the long-term behavioural consequences of MDMA administration in laboratory animals. Decreased 5-HT function has long been reported to result in behaviour consistent with an "anxiolytic" effect (Tye et al. 1977, 1979; Briley et al. 1990). It is reasonable, therefore, to propose that an MDMA-induced lesion might result in altered responses in animal models of anxiety-related behaviour. Two models that are used to examine anxiety-related behaviour in experimental animals, the elevated plus maze and the open field (Rodgers and Dalvi 1997; Schmitt and Hiemke 1998), have been used in the current study to assess the long-term consequences of MDMA administration.

Both the elevated plus-maze and open-field behavioural test paradigms were employed at three time-points over a period of 80 days: (1) days 8–11, or 1 week after the administration of MDMA, at which time 5-HT content in rat brain is already markedly decreased (see Colado et al. 1995; O'Shea et al. 1998) and we would therefore expect any behavioural consequences to begin to appear; (2) days 29–32, or approximately 1 month after MDMA administration, which we believed allowed a suitable time period to elapse to prevent any habituation effects; (3) days 71–73 (open field) and day 80 (plus-maze) or approximately 10–11 weeks after MDMA, at which time neurodegenerative effects should still be present, prior to the onset of serotonergic recovery, which has been demonstrated 16 weeks after drug administration (Scanzello et al. 1993).

The study undertaken examined effects in the Dark Agouti strain of rat since a single dose of MDMA produced significant neurodegeneration of 5-HT pathways in this strain (Colado et al. 1995, 1998; O'Shea et al. 1998) in contrast to Sprague-Dawley, Lister Hooded and Wistar rats where several doses are required (Colado et al. 1993; Aguirre et al. 1998; Shankaran and Gudelsky 1999). Furthermore, we have accumulated substantial information on the behavioural and biochemical changes which occur in this strain following MDMA (e.g. Colado et al. 1995, 1997, 1998).

In addition, we also recently demonstrated that a single neurotoxic dose of MDMA in this strain produces a long-term defect in thermoregulation (Mechan et al. 2001a), illustrating that the size of the serotonergic lesion produced by this dose is sufficient to produce a functional abnormality. Therefore, we wished to see whether the relatively modest 5-HT loss induced by this dose of MDMA could produce other long-term functional changes. Such data are likely to be more relevant to any possible long-term clinical health problems than effects only seen after a major MDMA-induced neurotoxic loss of 5-HT has been produced.

## Materials and methods

### Animals, drug administration and experimental design

Adult male Dark Agouti rats (Harlan Olac, Bicester, UK) weighing 160–200 g at the start of the experiment were used. They were housed in groups of two or three, in conditions of constant temperature ( $21 \pm 2^\circ\text{C}$ ) and a 12-h light/dark cycle (lights on 0700 hours), with free access to food and water.

MDMA hydrochloride (Sigma, Dorset, UK), was dissolved in saline (0.9% NaCl w/v) and administered as a single dose of 12.5 mg/kg IP, in a volume of 1 ml/kg. The dose quoted refers to the base weight. This dose has previously been shown to produce a significant long-term neurotoxic loss of 5-HT in several brain regions in this strain of rat (O'Shea et al. 1998).

A group of rats was administered a single dose of MDMA (12.5 mg/kg IP) or saline ( $n=8$ , in each case). Rectal temperature was measured over the following 3 h by use of an MC 8700 thermometer, with digital readout, and a lubricated H-RB3 rectal probe (EXACON A/S, Roskilde, Denmark). Each rat was lightly restrained by hand for about 20 s while the probe was inserted approximately 2.5 cm into its rectum and a steady reading was obtained.

Both groups were tested on the elevated plus-maze (8, 29 and 80 days post-injection) and in the open field (9–11, 30–32 and 71–73 days post-injection) and observed for locomotor activity and anxiety-related behaviours.

### Plus maze apparatus and behavioural assessment

The plus-maze, consisting of two open arms (each 16×46 cm), two closed arms (each 16×46 cm, with a 10 cm surrounding wall) and a central square (16×16 cm) constructed from black plastic, was positioned 50 cm above the floor. The apparatus was situated in a darkened room, illuminated by a single 60 W white light bulb located approximately 50 cm above the centre of the maze. Experiments were recorded by a video camera, suspended approximately 100 cm above the centre of the plus-maze. A weak cider vinegar solution (10%) was used to clean the apparatus prior to the introduction of each animal.

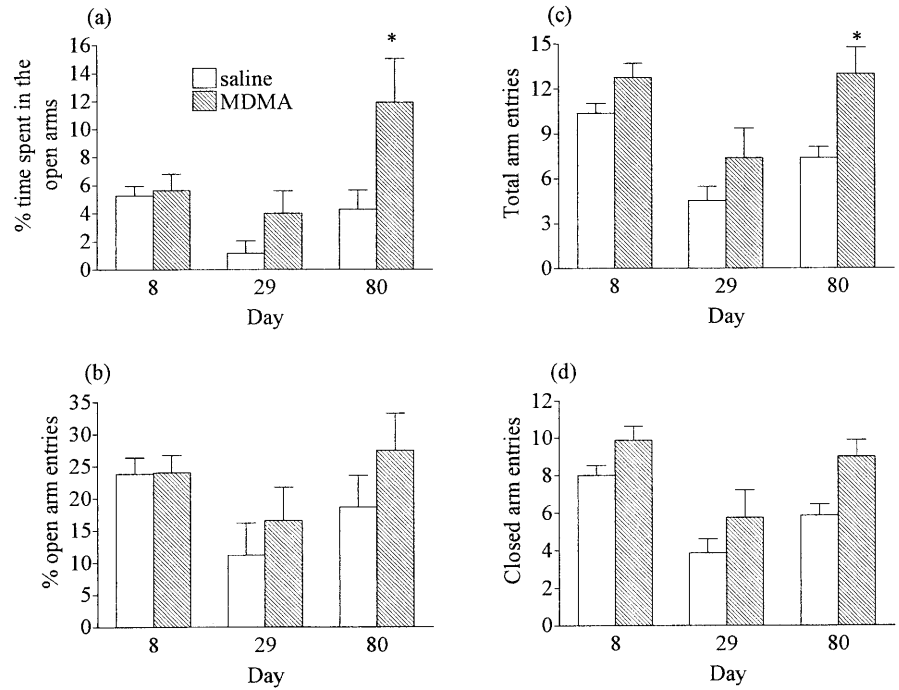
Since few studies have been performed with Dark Agouti (DA) rats in this behavioural paradigm initial studies were performed comparing the behaviour of DA rats with Sprague-Dawley (SD) rats on the elevated plus maze. While the overall activity of DA rats as indicated by the total number of arm entries was similar to that of SD rats, the amount of time spent in the open arms as a percentage of total time on all arms was lower in DA rats (Mechan et al. 2001b). In order to adjust for this lowered response rate, we therefore used a 10-min observation period in the current study rather than the frequently used 5-min period.

Rats (control and MDMA-treated groups,  $n=8$  in each case) were tested on the maze in randomised order. Each rat was placed in the centre of the plus-maze, facing one of the open arms, observed for 10 min by an experimenter seated approximately 1 m from the apparatus, and video-recorded for future analysis.

Arm entries were only counted when all four paws had entered either a closed or an open arm. Total arm entry data enabled calculation of the percentage of open and closed arm entries.

A number of simple anxiety-altered behaviours were also noted. These behaviours have been examined in a variety of investigations and have been proposed to reflect more ethologically based measures of anxiety (Espejo 1997; Rodgers and Dalvi 1997; Holmes and Rodgers 1999). Behaviours noted included: rearing (sitting on the hindpaws only, apart from while grooming), head dips (lowering the head, either over the edge of an open arm or over the surrounding wall of a closed arm), stretched attend posture (SAP; full body stretch, usually with hindpaws in the central square and forepaws in an open arm), directed sniffing (sniffing of the base or surrounding wall of the maze), non-directed sniffing (sniffing of the air, often while rearing with forepaws positioned on the top edge of the surrounding wall), grooming and defecation. Behaviour in the maze was observed on days 8, 29 and 80 following the injection of saline or MDMA (12.5 mg/kg) on day 1.

**Fig. 1a–d** Long-term effect of MDMA on arm entries in the elevated plus-maze. **a** Time spent in the open arms, as a percentage of total time spent in open and closed arms. Overall, MDMA-pretreated group different from control group [ $F(1,21)=7.23$ ,  $P<0.05$ ] with post-hoc analysis demonstrating a statistical difference on day 80 ( $*P<0.05$ ). **b** Number of open arm entries, as a percentage of total arm entries. MDMA-pretreated group not different from control group on any of the days tested. **c** Total number of arm entries. Overall, MDMA-pretreated group different from control group [ $F(1,21)=9.23$ ,  $P<0.01$ ] with post-hoc analysis demonstrating a statistical difference on day 80 ( $*P<0.05$ ). **d** Number of closed arm entries. Overall, MDMA-pretreated group different from control group [ $F(1,21)=8.42$ ,  $P<0.01$ ]



#### Open field apparatus and behavioural assessment

The open field was constructed from a white painted board, with three concentric circles (15.5 cm, 45.5 cm and 81.5 cm in diameter) delineated in black with a sheet metal surrounding wall of height 50 cm. Zone 2, surrounding the innermost circle, was further divided into six areas while zone 1, the outermost circle, was subdivided into 12 marked areas. The apparatus was situated in a darkened room and was illuminated by a single 60 W white or red light bulb located approximately 65 cm above the centre of the arena. Experiments were recorded by a video camera suspended approximately 150 cm above the open field arena. A weak cider vinegar solution was used to clean the apparatus prior to the introduction of each animal.

Groups of rats (control and MDMA-treated,  $n=8$  in each case) were tested over 3 consecutive days. The first 2 days were performed under white-light conditions, in order to assess any effects of habituation, while the third day was performed under red-light conditions. On each day, each rat was placed in zone 1 of the open field arena, observed and videotaped for 5 min. Locomotor behaviour was recorded by noting the number of crossings within each zone, with all four paws. The behaviours observed were: freezing (no movement within any particular section), rearing, grooming and defecation.

All experiments were performed according to the University of Leicester guidelines on the use of experimental animals and under appropriate project and personal licence authority under the Animals (scientific procedures) Act, 1986.

#### Statistics

Statistical analysis was performed using GraphPad Prism (San Diego, Calif., USA). All data is presented as mean $\pm$ SEM. Two-way analysis of variance (ANOVA) with repeated measures, with Day (day following drug treatment) $\times$ Treatment (MDMA or saline) as factors, followed by a Bonferroni post-hoc test was performed on all plus-maze and open field data, where appropriate. Mann-Whitney  $U$ -tests were performed on individual time-point data, except percent entries and percent time data in the plus-maze, where unpaired  $t$ -tests were performed. Two-way ANOVA was also performed on the acute temperature response to MDMA, where Treatment was the between-subjects factor and Time the repeated measure.

## Results

### Acute rectal temperature response to MDMA administration

Following administration of MDMA (12.5 mg/kg IP) the temperature of the rats increased above that of the saline injected animals, reaching a peak 80 min post-injection when their mean temperature was  $1.5\pm 0.1^\circ\text{C}$  greater than the control group. This difference was still apparent 3 h post-injection and is an acute measurable response to the drug. Significant main effects of both Treatment [ $F(1,56)=307.4$ ,  $P<0.0001$ ] and Time [ $F(7,56)=2.81$ ,  $P<0.05$ ] and a significant interaction [ $F(7,56)=4.47$ ,  $P<0.001$ ] were observed. Post-hoc analysis confirmed a statistically significant difference between MDMA- and saline-treated animals from 20 min post-injection, which was sustained for over 3 h ( $t=20$ ,  $P<0.05$ ;  $t_{40-180}$ ,  $P<0.001$ ).

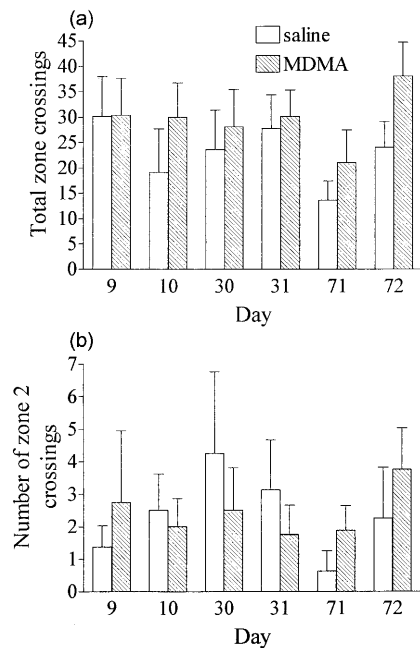
### Long term effects of MDMA on behaviour of rats on the plus maze

MDMA-pretreated animals spent significantly longer periods of time in the open arms, calculated as a percentage of total time spent in arms (Fig. 1a), resulting in significant main effects of both Day [ $F(2,21)=5.28$ ,  $P<0.05$ ] and Treatment [ $F(1,21)=7.23$ ,  $P<0.05$ ], but no significant interaction. Post-hoc analysis demonstrated a significant difference between pretreated and untreated animals on day 80 ( $P<0.05$ ). MDMA-pretreated animals also demonstrated a difference in overall locomotor activity, indicated by increased numbers of both total and closed

**Table 1** Long-term effect of MDMA on ethological variables in the elevated plus-maze

	Day 8		Day 29		Day 80		Two-way ANOVA		
	Control	MDMA	Control	MDMA	Control	MDMA	Day	Treatment	Interaction
Rearing	26.3±1.6	29.1±3.5	22.9±3.2	29.6±5.7	27.9±2.7	35.1±1.5*	$F(2,21)=1.42$ , ns	$F(1,21)=4.02$ , ns	$F(2,21)=0.28$ , ns
Defecation	3.0±0.96	3.5±0.8	4.4±0.8	5.6±0.9	2.6±1.2	2.4±1.1	$F(2, 21)=3.84^*$	$F(1,21)=0.37$ , ns	$F(2,21)=0.33$ , ns
Head dips	3.1±0.7	4.5±1.4	2.9±1.1	4.5±1.5	6.6±0.9	10.0±1.7	$F(2,21)=13.08^{***}$	$F(1,21)=3.32$ , ns	$F(2,21)=0.56$ , ns
Grooming	4.4±0.8	2.6±0.2	3.3±0.5	4.3±0.4	4.6±0.6	4.8±0.5	$F(2,21)=2.35$ , ns	$F(1,21)=0.26$ , ns	$F(2,21)=3.93$ , ns
SAP	8.5±0.9	10.0±0.9	3.1±0.9	3.4±1.0	3.4±0.6	5.0±0.5	$F(2,21)=33.32^{***}$	$F(1,21)=2.72$ , ns	$F(2,21)=0.46$ , ns

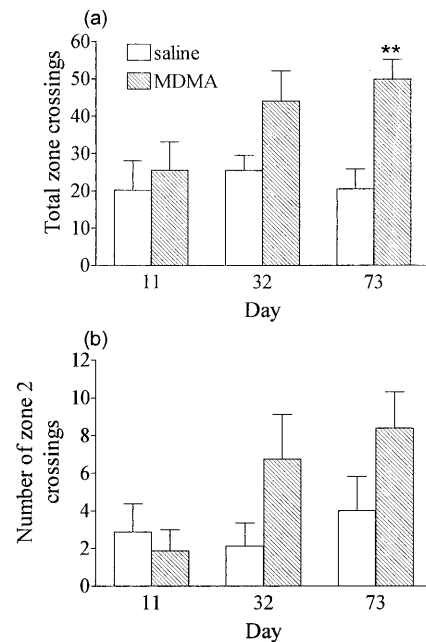
MDMA-treated group different from control group where \* $P<0.05$ ; \*\* $P<0.001$



**Fig. 2a, b** Long-term effect of MDMA on zone crossings in the open field, under white-light conditions. **a** Total zone crossings. MDMA-pretreated group not different from control group. **b** Zone 2 crossings. MDMA-pretreated group not different from control group

arm entries (Fig. 1c and d). This resulted in significant main effects of both day [total arm entries:  $F(2,21)=15.18$ ,  $P<0.0001$ ; closed arm entries:  $F(2,21)=13.20$ ,  $P<0.001$ ] and treatment [total arm entries:  $F(1,21)=9.23$ ,  $P<0.01$ ; closed arm entries:  $F(1,21)=8.42$ ,  $P<0.01$ ], but no significant interactions. In the case of total arm entries, post-hoc analysis again demonstrated a significant difference between the two treatment groups on day 80 ( $P<0.05$ ). The percent number of open arm entries, however, did not differ between MDMA-pretreated and control animals (Fig. 1b) but a decrease in both treatment groups on day 29, compared to day 8, resulted in a significant effect of Day [ $F(2,21)=3.45$ ,  $P=0.05$ ].

The results obtained on each individual test day are now examined in greater detail. Eight days after drug administration, there was no difference between MDMA-pretreated and saline groups in number of total or closed arm entries (Mann-Whitney  $U$ -tests,  $P>0.05$  in each case), the percent number of open arm entries compared to total



**Fig. 3a, b** Long-term effect of MDMA on zone crossings in the open field, under red-light conditions. **a** Total zone crossings. Overall, MDMA-pretreated group different from control group [ $F(1,21)=11.10$ ,  $P<0.01$ ] with post-hoc analysis demonstrating a statistical difference on day 73 (\* $P<0.05$ ). **b** Zone 2 crossings. MDMA-pretreated group not different from control group

arm entries, or the percent time spent on the open arms (unpaired  $t$ -tests,  $P>0.05$ , in each case), when compared with the behaviour of saline injected animals (Fig. 1).

At day 29, the responses of both saline- and MDMA-treated rats were modestly decreased with regard to these four parameters, compared with the day 8 values (Fig. 1). However, the responses of both groups were similar ( $P>0.05$ , in each case). On day 80, the MDMA-pretreated rats displayed a significant increase in the numbers of total and closed arm entries (total arm entries: Mann-Whitney  $U$ -value=11.50,  $P<0.05$ , Fig. 1c; closed arm entries: Mann-Whitney  $U$ -value=10,  $P<0.05$ , Fig. 1d), and the percent time spent on the open arms ( $t$ -test,  $t=2.24$ ,  $P<0.05$ , Fig. 1a). There was a trend towards increased percent open arm entries in the MDMA treated group (Fig. 1b); however, this did not reach significance ( $t$ -test,  $P>0.05$ ).

**Table 2** Long-term effect of MDMA on ethological variables in the open field, under red-light conditions

	Day 11		Day 32		Day 73		Two-way ANOVA		
	Control	MDMA	Control	MDMA	Control	MDMA	Day	Treatment	Interaction
Rearing	9.6±3.3	13.4±3.5	14.0±2.0	18.6±3.7	12.9±2.4	21.0±1.9*	$F(2,21)=2.04$ , ns	$F(1,21)=5.73^*$	$F(2, 21)=0.31$
Defecation	3.8±0.7	5.3±0.6	4.4±1.0	5.1±1.7	4.0±0.7	1.9±1.0	$F(2,21)=1.71$ , ns	$F(1,21)=0.003$ , ns	$F(2, 21)=1.62$
Grooming	4.4±0.8	2.6±0.2	3.3±0.5	4.3±0.4	4.6±0.6	4.8±0.5	$F(2,21)=0.13$ , ns	$F(1,21)=0.20$ , ns	$F(2, 21)=0.51$

MDMA-treated group different from control group where \* $P<0.05$

With regards to the ethological variables tested, there were no differences in grooming behaviour, or defecation, on any of the days tested, while rearing behaviour was increased significantly in the MDMA-pretreated group at day 80 (Mann-Whitney  $U$ -value=11.50,  $P<0.05$ , Table 1). On day 80, the mean number of head dips performed by the MDMA-pretreated group was 50% greater than those of the control group, (Table 1) but this was not statistically significant. Stretch attend postures were also performed in greater numbers by the MDMA-pretreated group, on day 80, but did not reach significance. Directed and non-directed sniffing were not altered at any time point by MDMA-pretreatment (data not shown).

#### Long term effects of MDMA on behaviour of rats in the open field

Overall, under white-light illumination, there were no significant differences between MDMA-pretreated and control rats with respect to total and zone 2 crossings during the first two trials (days 9–10 and days 30–31). By day 72, however, MDMA-pretreated rats performed approximately 59% greater numbers of total crossings and approximately 67% greater numbers of zone 2 crossings, than control rats. However, these effects were not statistically significant, thus there were no significant effects of Day or Treatment and no significant interaction ( $P>0.05$ , in each case, Fig. 2). The ethological variables measured, freezing, grooming, defecation and rearing, were unchanged (data not shown).

Under red light conditions, the MDMA-pretreated rats showed an increase in total zone crossings over the testing period, which reached significance by day 73 (Mann-Whitney  $U$ -value=4.00,  $P<0.01$ , Fig. 3a) and a non-significant increase in the number of zone 2 crossings over the testing period (two-way ANOVA: Day, Treatment and Day×Treatment,  $P>0.05$ , Fig. 3b). Rearing behaviour was greater in MDMA-treated animals on day 73 (Mann-Whitney  $U$ -value=13.00,  $P<0.05$ ), while the defecation behaviour was decreased compared with control animals this difference was not statistically significant (Mann-Whitney  $U$ -value=15.50,  $P=0.08$ , Table 2). Both grooming (Table 2) and freezing behaviour (data not shown) were unchanged.

## Discussion

There have been a substantial number of studies on the acute effects of MDMA on behaviour. MDMA is an amphetamine derivative and therefore, not unexpectedly, its administration to rats induces a series of behavioural changes including hyperthermia, locomotion and other more subtle behavioural changes, the pharmacology of which has been examined in some detail (Nash et al. 1988; Callaway et al. 1990, 1992; Callaway and Geyer 1992a, 1992b; Colado et al. 1993; McCreary et al. 1999). Recently Lin et al. (1999) examined the acute effect of MDMA administration on the behaviour of mice in the elevated plus maze and demonstrated that the drug had an apparent anxiogenic effect at low dose (4 mg/kg IP) and an anxiolytic effect at high dose (20 mg/kg IP). MDMA has also been reported to be anxiogenic in mice using the light/dark box test (Maldonado and Navarro 2000). However, one qualification about interpretation of these studies is that MDMA has a very different interaction with monoaminergic systems in the brain of the mouse compared to most other species including rats, primates and probably humans (Stone et al. 1987a; O'Callaghan and Miller 1994; O'Shea et al. 2001).

While it is clearly vital to understand the pharmacological and behavioural consequences of acute MDMA administration, there continues to be a problem of clarifying the long-term effects of this commonly used recreational drug. There is now substantial evidence that the compound can cause damage to serotonergic nerve endings in the brain of several species (O'Hearn et al. 1988; Molliver et al. 1990; Colado et al. 1997), including humans (McCann et al. 1998, 1999) and there has been discussion as to whether this effect will produce functional changes (Green and Goodwin 1996). There are reports that heavy recreational use of MDMA can result in users displaying significant psychiatric changes, but these reports are often anecdotal (see Green et al. 1995) and there remains the problem of assessing whether the change seen relates to the acute effect of the drug, long-term damage occurring in the brain, or is unrelated to MDMA ingestion (see Green et al. 1995). The only study, of which we are aware, investigating the long-term consequences of MDMA administration on the functional responses of rats, is that of Marston et al. (1999). This study demonstrated that a deficit in delay non-match to place performance developed over 20 days, and is therefore suggestive of cognitive impairment.

We have now attempted to answer whether other changes in behaviour develop in rats following the administration of a neurotoxic dose of MDMA. The behaviour of the rats in both the plus maze and open field was investigated at 3 time points over 80 days following a single neurotoxic dose of MDMA. Results obtained therefore reflect the long-term neurotoxic consequences of MDMA administration and are not related to any acute action of the drug. Both behavioural tests employed here allowed ethological measures of risk assessment behaviour and have previously been used to examine the effects of drugs altering 5-HT function (Köhler and Lorens 1978; Griebel et al. 1997). In general terms the data obtained was consistent across the models and indicated that MDMA-treated animals showed decreased anxiety and increased risk-associated behaviour.

A single dose of MDMA of 10–15 mg/kg has been shown previously to induce dose dependent acute hyperthermia and long-term neurotoxic degeneration of 5-HT nerve terminals in Dark Agouti rats (O'Shea et al. 1998; Mehan et al. 2001a). A dose of 12.5 mg/kg was also recently shown to result in Dark Agouti rats displaying an acute temperature rise similar to that observed in the current investigation and a 5-HT loss in several brain regions of more than 30% (Mehan et al. 2001a). Therefore it can be assumed that the rats in the current study also had a decrease in cerebral 5-HT content of greater than 30%. We feel such a relatively modest change makes our data more relevant to the possible consequences of the recreational use of MDMA by young persons, since it is probable that any damage produced by repeated or high dose ingestion of MDMA (see O'Shea et al. 1998) is likely to be modest.

There is evidence that repeat testing of rodents on the plus maze can alter the response seen in the animals on the second and subsequent exposure (for example Espejo 1997; Schmitt and Hiemke 1998; Holmes and Rodgers 1999), although some groups have observed stable test-retest profiles (Pellow et al. 1985; Lister 1987).

Repeated exposure of rats to the elevated plus-maze has been reported to alter the type of "anxiety" which is being measured and, in particular, alters the response to benzodiazepines in later trials. It has been suggested that trial 2 anxiety, which is unaffected by treatment with benzodiazepines or barbiturates, is dependent upon learning which occurs during experience of open arms during trial 1 (File 1993), leading to increased avoidance of the open arms in trial 2 (Bertoglio and Carobrez 2000). More specifically, as demonstrated in Swiss-Webster mice, open arm avoidance is apparent by the third minute of the first 5-min trial and open arm entries are further reduced in trials 2 and 3, each trial being separated by a 24-h interval (Holmes and Rodgers 1998). In addition, the duration of exposure to the elevated plus-maze has been shown to affect the level of tolerance to the anxiolytic effects of benzodiazepines (File et al. 1990). However, the "one-trial tolerance" phenomenon has generally been demonstrated with far shorter intervals between trials than those employed in our study. File et

al. (1990) demonstrated a tolerance effect where there was an inter-trial interval of 24 h and further demonstrated that this effect was just as strong with a 2-week interval between trials. Thus, a 3-week interval between trials 1 and 2 and a 7-week interval between trials 2 and 3 were expected to be sufficient time periods to prevent any habituation effects from occurring. In fact, in our earlier study (Mehan et al. 2001b) where DA and SD rats were tested on the elevated plus-maze on two occasions, 25 days apart, there was little difference in locomotor or anxiety-related behaviours between the two trials. However, bearing in mind the reduction in overall locomotor activity and percent time spent in the open arms on day 29 compared to day 8 in the current study, some habituation to the elevated plus-maze may have been present in trial 2.

File et al. (1993) investigated the response of male hooded Lister rats to diazepam (2 mg/kg IP) in a two-trial study, the trials being of 5 min duration and separated by 24 h. The anxiolytic effect of diazepam (indicated by the percent number of open arm entries) was abolished in the second trial. However, by increasing the duration of the test to 10 min, this effect was reversed. Diazepam-treated rats then demonstrated a similar increase in percent open arm entries, compared to the control rats, as in trial 1. Therefore, since the DA strain has previously been demonstrated to exhibit reduced locomotor activity and spends less time in the open arms than SD rats (Mehan et al. 2001b), an increased duration of exposure to the plus-maze (10 min) in the current investigation appeared appropriate.

No statistically significant changes were seen in the MDMA-pretreated rats, compared to control animals, on day 8 or day 29 in terms of the percent open arm entries or percent time spent on the open arms, although the mean values of both these measures were decreased at day 29. However, on day 80 there was an unequivocal increase in the percent time spent on the open arms. The total number of arm entries of the MDMA-pretreated rats, on day 80, was also greater than that of control animals, but was no greater than the value seen on day 8. Nevertheless, it appears that the MDMA-pretreated rats do display increased motor activity compared to control animals, which is consistent with, but not mutually exclusive from, decreased anxiety-related behaviour. There was also an increase in rearing behavior in these animals, a behaviour that has also been claimed to indicate increased motility (Cruz et al. 1994).

On day 80, in comparison to control animals, the mean number of head dips increased by 50% in MDMA-treated rats, albeit not statistically significantly, while stretch attend postures increased by 47%, but again this change failed to reach statistical significance ( $P=0.08$ ). Increases in both of these behaviours have been reported in rats pretreated with anxiolytic drugs and examined on the elevated plus maze (Cruz et al. 1994; Griebel et al. 1997). The other behaviours measured (grooming, defecation, directed and non-directed sniffing) did not differ, compared to control animals, on any of the days

tested. These data therefore indicated that MDMA-pretreated rats explored more, were less “anxious” and were more prepared to take risks 80 days after a neurotoxic dose of the drug.

This conclusion was supported by the studies in the open field, at least under red light conditions. MDMA-pretreated rats did perform increased numbers of both total crossings and zone 2 crossings, under white light conditions, on day 72 but these effects did not reach statistical significance. Under red light conditions, MDMA-pretreated rats clearly demonstrated different behaviour from that of control animals, where both the total zone crossings and the number of zone 2 crossings increased with time (11–73 days) in the MDMA-lesioned rats.

Under white light conditions the ethological measures were also not seen to be different. However, under red light conditions on day 73, the MDMA-pretreated rats displayed a 63% increase in rearing behaviour and defecation was decreased by 53%, compared to control animals. These results indicate that white light illumination provides a particularly aversive environment and only under red light conditions do the differences between MDMA-pretreated and control animals become apparent.

The involvement of 5-HT in anxiety responses in rodents is clear, but the data obtained with drugs that alter 5-HT function have often been complex and sometimes conflicting (Wright et al. 1992; Handley and McBlane 1993; Handley et al. 1993; Griebel et al. 1997; Setem et al. 1999). Nevertheless, there is good evidence that lesioning of 5-HT pathways or decreasing cerebral 5-HT levels and function produces an apparent anxiolytic effect in rodents (Tye et al. 1977, 1979; Briley et al. 1990). One might, therefore, predict that an MDMA-induced lesion of forebrain 5-HT would result in anxiolytic-type behaviour. This paper reports such an effect, although it is surprising that it is not apparent until at least 2 months after the lesion. Given that 5-HT content in rat brain is markedly decreased 7 days after MDMA administration (e.g. Colado et al. 1995; O’Shea et al. 1998) and remains lowered for several months (Scanzello et al. 1993), it is clear that the observed effect on behaviour does not relate simply to the decreased 5-HT concentration. Other mechanisms must be involved and it seems likely that the change in the behaviour is related to adaptive changes occurring in the brain.

The increase in locomotor response and rearing behaviour in both the open field and elevated plus maze, in addition to the decrease in defecation and the increase in time spent on the open arms in the plus maze, can be interpreted in terms of the animals having less emotional reactivity (Escorihuela et al. 1999; Liebsch et al. 1998) and, perhaps, increased impulsivity.

The increase in locomotor activity can be suggested to be a confounding factor in interpreting data from both the plus maze and the open field. However while overall activity increased in the MDMA-treated rats on the plus maze (as measured by either total arm entries or number of closed arm entries) by around 50% at day 80, the percent time spent on the open arms increased by over

180% at this time. This suggests that there is no close correlation between these measures. Similarly, the increase in crossings in the red light conditions of the open field by MDMA-pretreated rats was considerably greater than the increase under white light conditions, again suggesting that no close relationship exists between locomotor activity and anxiolytic-like responses in the test. Finally several ethological measures also suggested a lower state of anxiety-like behaviour in the MDMA-pretreated rats and these measures would not be expected to be markedly influenced by increased locomotor behaviour. It therefore seems reasonable to conclude that the increased locomotion reflects decreased “anxiety” and increased risk-taking behaviour in the rats.

The important question here is whether these data can, in any way, be associated with clinical data concerning recreational use of MDMA. Clinical data are not particularly consistent, due to the problems of usage, dose variability and separation of the subacute actions of the drug from any long-term psychopathology (see Introduction). Both Gamma et al. (2000) and Parrott et al. (2000) have suggested, on the basis of their studies on recreational users of MDMA, that users demonstrated higher levels of anxiety. However, both groups acknowledge the possibility that the results may reflect either the simultaneous use of other drugs or may be due to a pre-existing condition. In contrast, Morgan (1998) observed little change in anxiety but increased impulsivity, a behavior that has repeatedly been associated with lowered 5-HT functional activity (see Evenden and Ryan 1999; Söderpalm and Svensson 1999). Using a psychopharmacological approach, McCann et al. (1999) administered *m*-chlorophenylpiperazine (*m*-CPP) to a group of control subjects and a group of MDMA users. The MDMA users were less sensitive to the anxiogenic effects of this 5-HT<sub>2C</sub> agonist than the control subjects and less likely to report an *m*-CPP induced panic attack. It is tempting to propose that this reflects a long-term MDMA-induced change in their basal level of anxiety/risk taking behaviour, as was observed in rats in the current study. If this were the case, it indicates that recreational MDMA use may produce a change in the psychological status of the user, which may be irreversible.

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