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The effects of MDMA pretreatment on the behavioural effects of other drugs of abuse in the rat elevated plus-maze test

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Abstract

Few preclinical studies have found long-term behavioural consequences of the serotonergic neurotoxicity produced by 3,4-methylenedioxymethamphetamine (MDMA). This study investigated whether pretreatment with MDMA altered the behavioural effects of other drugs of abuse. Adult male Lister hooded rats ($n=10$/group) were pretreated with 10 mg/kg MDMA or 1 ml/kg saline vehicle intraperitoneally every 2 h for 6 h. Fourteen days later, the behavioural effects of D-amphetamine (2 mg/kg), cocaine (10 mg/kg), ethanol (2.0 g/kg), heroin (0.5 mg/kg), or MDMA (10 mg/kg) were assessed in the elevated plus-maze test. MDMA pretreatment produced approximately 20–25% decrease in hippocampal 5-HT and 5-HIAA concentrations, and [^{3}H]paroxetine binding when analysed 2 weeks later. Despite inducing neurotoxicity, this regimen had no effect upon the plus-maze behaviour induced by ethanol, heroin, and MDMA. Acutely, and independent of neurotoxic pretreatment, MDMA produced a clear anxiogenic-like behavioural profile with a reduction of open arm entries and suppression of explorative behaviours. Despite being acutely anxiogenic, pretreatment with a neurotoxic regimen of MDMA has little effect on the anxiety-related effects of other drugs of abuse. It is possible that extended time points would produce significant changes, although the available evidence suggests that the plus-maze may not be a suitable model for detection of behavioural dysfunction after neurotoxic MDMA.
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1. Introduction

In animals, acute (±) 3,4-methylenedioxymethamphetamine (MDMA) produces dose-dependent effects upon anxiety-related behaviours. Low-dose MDMA facilitates social interaction but increasing the dose reduces this response (Bhattacharya et al., 1998; Capurro et al., 1997; Miczek and Haney, 1994; Morley and McGregor, 2000). The unique effects of low doses of MDMA upon animal behaviour distinguish it from other commonly abused drugs that generally have no effect upon social behaviour, or facilitate social withdrawal in animals (Cole and Sumnall, 2003b). All low to medium doses (< 10 mg/kg) tested to date have produced increased fear-like behaviour in rats on the elevated plus-maze but very high doses (≥ 15 mg/kg) have facilitated fear reduction in rats and mice (Ho et al., 2004). In the rat elevated plus-maze, 5–10 mg/kg ip produced a dose-dependent increase in anxiety-like behaviour (Bhattacharya et al., 1998). Over a range of lower doses (< 5 mg/kg), and in contrast to the effects of the same doses on social interaction, MDMA also produced an increase in anxiety-like behaviour on the plus-maze (Morley and McGregor, 2000). Similar increases in anxiety were obtained from the emergence and cat odour avoidance tests. In the emergence test, < 5 mg/kg decreased the frequency of emergence and caused a concomitant increase in emergence latency, whilst 5 mg/kg produced a significant decrease in approach time towards a worn cat collar. There was also a dose-dependent reduction in the number of vocalisations produced by footshock (Morley and McGregor, 2000).

Recent work has produced conflicting evidence of the effects of neurotoxic MDMA pretreatment on the anxiety-like behaviour of rats, with one study showing anxiolysis and others showing anxiogenesis (Fone et al., 2002; Gurt-

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man et al., 2002; McGregor et al., 2003b; Mechan et al., 2002; Morley et al., 2001). Administration of the behaviourally active neurotoxic metabolite 3,4-methylenedioxyamphetamine (MDA) was without effect (Cho et al., 1990; Harkin et al., 2001). Studies in young Lister hooded rats (postnatal day 28) have shown model-specific effects, with reports of no-baseline behavioural change (and after mCPP challenge) in the plus-maze, but decreased social interaction (Bull et al., 2003). These findings appeared unrelated to gross measures of serotonergic function. In the rat, behavioural dysfunction on the plus-maze has only been observed at extended survival times (>80 days), and in the absence of neurotoxicity, but the direction of change is strain dependent, and findings are not consistent across laboratories (Green and McGregor, 2002; Sumnall et al., submitted for publication).

Within the dance music (rave) culture, concomitant polydrug use is the norm; alcohol, cannabis, cocaine, and amphetamine sulphate are the most popular drugs ingested alongside ecstasy (Cole and Sumnall, 2003a; Winstock et al., 2001). Preclinical studies have shown that repeated exposure to a high-dose regimen of MDMA alters the rewarding properties of MDMA, cocaine, and ethanol, and pharmacological perturbation reveals underlying neurochemical change (Cole et al., 2003; Horan et al., 2000; Kalivas et al., 1998; Morgan et al., 1997; Ratzenboeck et al., 2001). These have largely been described as behavioural consequences of neurotoxicity and/or sensitisation.

The present study aimed to further characterise the effects of MDMA pretreatment upon anxiety in rats using the elevated plus-maze, a well validated animal model of anxiety (Rodgers and Cole, 1994). Furthermore, in order to model patterns of drug use in humans, we investigated the effects of pretreatment with MDMA upon the behavioural effects of five popular drugs of abuse. Drugs chosen were D-amphetamine, cocaine, ethanol, heroin, and MDMA, based upon reports of their use in human users of ecstasy (e.g., (Winstock et al., 2001).

2. Materials and methods

2.1. Animals

Male Lister hooded rats (Charles River UK, Kent, UK), weighing 180–210 g on arrival, were used for all experiments. Rats were individually housed in standard acrylic cages under a 12-h reversed lighting schedule (lights off at 1400 h) at a constant room temperature of 21 ± 1 °C and relative humidity of 65%. Food (Special Diet Services, Witham, UK) and water were available ad lib. All animals were experimentally naïve and all testing took place during the dark cycle. All procedures were carried out under conditions prescribed by Home Office Project licence 40/1905 according to guidelines described in the UK Animals (Scientific Procedures) Act (1986).

2.2. Drugs

MDMA hydrochloride was custom manufactured as the racemic form by Ultrafine Chemicals (Manchester, UK). Ethanol was received from the Department of Chemistry, University of Liverpool. D-Amphetamine sulphate was obtained from Sigma-Aldrich (Dorset, UK). Heroin hydrochloride and cocaine hydrochloride were purchased from MacFarlen Smith (Edinburgh, UK). Drug solutions were freshly prepared on each experimental day, dissolved in 0.9% w/v physiological saline and administered intraperitoneally in a volume of 1 ml/kg and tested at 20 min postinjection. Administered doses were calculated as the free-base weight. Ethanol was prepared as a 20% w/v stock solution with 0.9% physiological saline, and was administered in a volume appropriate for the weight of the animal.

2.3. Procedure

2.3.1. Neurotoxic MDMA administration

Rats were randomly assigned to groups ($n = 10$ per group; vehicle/vehicle, MDMA/vehicle, vehicle/drug of interest, and MDMA/drug of interest). MDMA-pretreated animals received 10 mg/kg MDMA intraperitoneally every 2 h for 6 h, for a total of 40 mg/kg. Control animals received the equivalent volume of saline vehicle. This regimen was chosen because of its reported ability to produce extensive serotonergic neurotoxicity at a relatively low total dose (e.g., (Fischer et al., 1995a; Scanzello et al., 1993)). Furthermore, pilot experiments found that this treatment did not have lethal consequences in Lister hooded rats, unlike other popular protocols (e.g., 20 mg/kg every 12 h for 96 h; Battaglia et al., 1987).

Rats were closely monitored during the neurotoxic regimen, and for 2 h afterwards; any adverse effects were noted. All MDMA-treated animals displayed overt signs of the 'serotonin syndrome' but no unforeseen reactions occurred and the drug was generally well tolerated. Behavioural testing commenced 14 days after MDMA administration. During the interim, animals were weighed daily and their health was monitored in accordance with the Home Office licence conditions. No unusual homecage behaviour was observed during this period. It was therefore unnecessary to withdraw any rats from testing.

2.3.2. Acute behavioural testing

Behaviourally active doses of investigated drugs were identified in a series of pilot dose–response experiments. Doses (2 mg/kg D-amphetamine; 10 mg/kg cocaine; 2 g/kg ethanol; 0.5 mg/kg heroin; and 10 mg/kg MDMA) were chosen on the basis of statistically significant behavioural effects in the plus-maze compared to vehicle and/or other doses and the ranges were chosen from the literature (Bhattacharya et al., 1998; Crawford et al., 1994; Hall et al., 1998; Hoffmann and Wise, 1993; Morato et al., 2001; Morley and

McGregor, 2000; Pellow et al., 1985; Pulvirenti et al., 1991; Rogerio and Takahashi, 1992; Vale and Green, 1996).

Two weeks after neurotoxic pretreatment, the plus-maze testing began. The plus-maze apparatus was a black, plus-shaped wooden maze with a black Plexiglas floor consisting of two open (10 × 50 cm) and two enclosed (10 × 50 × 50 cm) arms extending from a central platform (10 × 10 cm). The sides of the enclosed arms were made of transparent Plexiglas and the whole maze was mounted on a black metal base elevated to a height of 50 cm. Retention on the open arms was facilitated by the incorporation of raised edges around the perimeter of the maze (at a height of 0.5 cm). Maze sessions were videotaped under red light using a Sony video camera (model SLV-SE39) mounted on a tripod at approximately 45° to the maze and linked to a video recorder (Sony SLV-SE30) and monitor (Saville XPM20) in the laboratory control room. An additional infrared light source increased illumination of the maze, removing shadows and allowing for equal illumination of all arms.

On testing days, rats were assigned to treatment groups in a counterbalanced manner, administered the drug under investigation, and were then returned to their home cages. Twenty minutes later, rats were placed on the central platform of the maze, facing an enclosed arm, and were allowed to freely explore the apparatus for a single 5-min test. To avoid auditory and visual distractions, the experimenter withdrew to an adjacent laboratory during testing where the experimental session could be monitored via video link. Between each animal, the maze was thoroughly cleaned with water and dried with paper towels. Animals that fell off the maze were not replaced, the stress caused by the incident and subsequent recapture was considered to have had confounding effects upon behaviour. This resulted in unequal group sizes for many of the statistical analyses.

2.4. Behavioural analysis

Videotapes were retrospectively scored by a trained observer blind to the treatment condition using the Hindsight ethological analysis software package (version 1.4, developed by Dr. S. Weiss, Vernalis) installed on a desktop PC. This package enables the measurement and collation of a range of specific user-defined behaviours and spatiotemporal parameters (Rodgers and Johnson, 1995). Behavioural parameters consisted of both traditional and ethologically derived measures (Rodgers and Cole, 1994). Traditional measures recorded were total, open (OA) and closed arm (CA) entries, % OA entries ([OA entries/total arm entries] × 100), % OA and CA time ([arm time/300] × 100). Ethological measures were total number of stretched attend postures (SAPs, a forward extension and retraction of the head and shoulders), head dips (a directed exploratory behaviour consists of a downwards head movement over the side of the open arms directed from either closed or open arms), rears (vertical movement against the maze walls), CA returns (two forepaws and head exiting arm, investigation and return/doubling back into the same arm), central platform latency (time taken from initial maze placement to first arm entry, expressed as % total maze time), and grooming (cleaning of body parts with teeth, mouth, forepaws or tongue). Rearing, head dipping and SAPs were additionally differentiated (% of total) depending upon location in the maze (i.e., protected or unprotected; Rodgers and Johnson, 1995; Treit et al., 1993).

2.4.1. Measurement of hippocampal 5-HT/5-HIAA concentrations and density of cortical [^{3}H]paroxetine binding

MDMA-pretreated rats and their controls were selected from the ethanol study for neurochemical analysis. These animals were selected at random. Rats were killed by cervical dislocation and decapitation and brains were rapidly removed and the hippocampus and cerebral cortex dissected out on ice. Tissue samples were homogenised and hippocampal 5-HT and 5-HIAA were analysed by high-performance liquid chromatography. The mobile phase (flow rate approximately 0.7 ml/min) comprised potassium phosphate (0.05 M), sodium-1-octane sulphonic acid (0.16 mM), EDTA (0.1 mM), and methanol (16%), and was adjusted to pH 3 with phosphoric acid. The working electrode potential was set at 0.75 V. The approximate retention times for 5-HT and 5-HIAA were 9.5 and 11.9 min, respectively. Working external standards (10^{-7} M 5-HT, 10^{-7} M 5-HIAA in perchloric acid) were prepared from external standard stock solutions (10^{-2} M; 5-HT prepared from creatinine sulphate complex, 5-HIAA prepared as the free acid). The [^{3}H]paroxetine binding protocol followed the methods outlined by Hewitt and Green (1994). To determine total binding, the assay solution (1 ml) contained 800 μl tissue preparation, 100 μl [^{3}H]paroxetine (final concentration 1 nM; specific activity 25 Ci/mmol; Dupont NEN, Boston, USA) Tris buffer. For the determination of nonspecific binding, Tris buffer was substituted with 100 μl 5-HT (final concentration 100μM; creatinine sulphate complex; Sigma, UK). Each reaction was performed in triplicate. Incubation proceeded for 60 min at 22 °C. The binding reaction was terminated by rapid filtration with ice-cold buffer solution through filters (Brandel GF/B Fired Filters; Brandel, USA) presoaked in 0.05% polyethylenimine using a Brandel cell harvester (Brandel). Filter disks and 100 μl ligand standards were placed in 4-ml liquid scintillant and were left overnight (protected from light) before radioactivity was counted (3-min count) by liquid scintillation spectroscopy (1214 RackBeta Liquid Scintillation Counter, LKB Wallac; counting efficiency approximately 36%). Protein content was determined using the method of Lowry et al. (1951).

3. Statistics

All data were statistically analysed using the software package SPSS for Windows (version 11.0; SPSS, USA).

Body weight data in the 2 weeks following neurotoxic treatment was analysed by repeated-measures ANOVA. Acute plus-maze behaviour after neurotoxic pretreatment was analysed by two-way ANOVA with pretreatment (MDMA or saline) and maze treatment (drug of interest or vehicle) as between-subject fixed-factors and behavioural measure as the dependent variable. Significant treatment-related *interactions* were further analysed using simple-effect ANOVA. Significant treatment *main effects* or *simple effects* were analysed further by step-down comparison procedures. Significance was set at $P < .05$.

4. Results

MDMA pretreated animals gained significantly less weight than groups pretreated with saline over the 14 days between neurotoxic administration and behavioural testing [$F(1,38) = 5.25$, $P < .01$; data not shown]. For example, compared to saline-treated rats, the loss of body weight in MDMA animals was 4% on Day 1, 8% on Day 7, and 7% on Day 14. Again, these differences were statistically significant ($P < .05$; $P < .01$; and $P < .05$, respectively).

(i) *Amphetamine*: 2 mg/kg D-amphetamine failed to reproduce the anxiogenic profile (see Table 1) observed in the dose–response experiment. No changes in arm entries were observed and head dips and rears, previously suppressed by drug treatment, were comparable to saline controls. Similarly, there was no effect of any treatment regimen upon SAP. In the absence of change in the absolute number, rears were more predominant in the unprotected areas of the maze [$F(1,35) = 14.19$, $P < .01$], suggesting locomotor stimulation. MDMA pretreatment failed to affect any baseline measures of behaviour and as generally there were no acute effects of D-amphetamine, no interactions between levels of pretreatment and the D-amphetamine maze challenge were observed.

(ii) *Cocaine*: The behaviour of both cocaine-treated groups was similar to that of their saline controls (see Table 2). MDMA pretreatment failed to significantly affect baseline behaviours although cocaine-induced head dips were (nonsignificantly) reduced in the MDMA pretreated animals [$F(1,35) = 3.91$, $P = .056$].

(iii) *Ethanol*: Ethanol maze treatment produced a behavioural profile that suggests a reduction in fear (see Table 3). OA entries were significantly increased by acute drug treatment [$F(1,32) = 4.19$, $P < .05$] and there was a nonsignificant trend for increased % OA time and % OA entries in these animals. Total SAP [$F(1,32) = 5.48$, $P < .05$] and rears [$F(1,32) = 34.16$, $P < .01$] were decreased, but these were also accompanied by a reduction in the number of CA [$F(1, 32) = 5.35$, $P < .05$] and total arm entries [$F(1,32) = 3.43$, NS], and an increase in nonexploratory behaviours [$F(1,32) = 19.50$, $P < .01$]. This profile suggested a nonspecific sedative-like effect of ethanol. There was

Table 1
Effects of MDMA pretreatment upon the behavioural effects of 2.0 mg/kg i.p. D-amphetamine in rats on the elevated plus-maze

Behaviour	Treatment condition				Statistical interactions and effects		
	Vehicle × Vehicle ($n = 9$)	MDMA × Vehicle ($n = 10$)	Vehicle × D-Amphetamine ($n = 10$)	MDMA × D-Amphetamine ($n = 10$)	MDMA pretreatment, $F(1,35)$	D-Amphetamine maze treatment, $F(1,35)$	Pretreatment × Maze Treatment interaction, $F(1,35)$
OA entries	4.0 ± 0.4	4.6 ± 0.4	4.5 ± 0.3	5.1 ± 0.4	2.57	1.78	0.00
CA entries	8.4 ± 0.5	8.2 ± 0.5	8.7 ± 0.3	9.6 ± 0.4	0.57	3.63	1.73
Total entries	12.4 ± 0.9	12.8 ± 0.8	13.2 ± 0.6	14.7 ± 0.7	1.50	3.07	0.57
% OA entries	32.2 ± 2.3	35.9 ± 1.5	34.1 ± 1.6	34.7 ± 0.1	0.04	0.03	0.04
% OA time	22.1 ± 3.6	25.6 ± 2.5	24.0 ± 5.2	24.3 ± 2.9	0.01	2.37	1.97
% CA time	55.8 ± 3.5	51.0 ± 2.3	56.3 ± 5.4	61.9 ± 3.1	0.09	3.22	1.46
% Central platform latency	2.1 ± 0.4	3.4 ± 0.6	3.7 ± 0.5	3.7 ± 0.7	1.42	3.02	1.47
Total SAP	2.8 ± 0.9	4.3 ± 1.0	3.4 ± 0.6	2.4 ± 1.0	0.32	0.19	1.27
% up SAP	19.3 ± 11.4	41.1 ± 11.9	35.0 ± 11.0	33.8 ± 12.3	0.78	0.13	0.96
Total HD	18.6 ± 1.5	18.9 ± 1.3	16.4 ± 2.0	18.4 ± 1.7	0.50	0.64	0.25
% up HD	86.0 ± 4.3	86.6 ± 3.4	87.1 ± 3.9	90.0 ± 2.4	0.24	0.39	0.11
CA returns[a]	–	–	–	–	–	–	–
Total rears	11.9 ± 1.6	11.6 ± 0.9	11.9 ± 2.0	11.7 ± 1.3	0.03	0.00	0.00
% up rears	19.7 ± 4.1	13.5 ± 3.0	46.4 ± 11.1	40.3 ± 6.7	0.75	14.19 *	0.00
NEB[a]	–	–	–	–	–	–	–

Acute plus maze behaviour was examined 14 days after a neurotoxic regimen of MDMA (4 × 10 mg/kg ip every 2 h for 6 h), or an equivalent volume of vehicle. Data expressed as mean values (± S.E.M.). OA = open arms, CA = closed arms, SAP = stretched attend postures, HD = head dips, up = unprotected, and NEB = Nonexploratory behaviours.

[a] Behaviour expressed too infrequently for analysis.

* $P < .01$, significant effect of D-amphetamine maze treatment.

Table 2
Effects of MDMA pretreatment upon the behavioural effects of 10 mg/kg ip cocaine in rats on the elevated plus-maze

Behaviour	Treatment condition				Statistical interactions and effects		
	Vehicle × Vehicle ($n=10$)	MDMA × Vehicle ($n=10$)	Vehicle × Cocaine ($n=10$)	MDMA × Cocaine ($n=9$)	MDMA pretreatment $F(1,35)$	Cocaine maze treatment $F(1,35)$	Pretreatment × Maze Treatment interaction $F(1,35)$
OA entries	4.0 ± 0.4	3.5 ± 0.3	4.4 ± 0.2	4.3 ± 0.5	0.00	3.74	0.64
CA entries	8.0 ± 0.4	7.7 ± 0.3	8.4 ± 0.4	8.7 ± 0.4	0.54	2.54	0.31
Total entries	12.0 ± 0.8	11.2 ± 0.6	12.8 ± 0.5	13.0 ± 0.9	0.19	3.53	0.52
% OA entries	33.3 ± 1.3	31.3 ± 1.7	34.4 ± 1.5	33.3 ± 1.9	0.70	0.06	0.00
% OA time	18.9 ± 5.3	19.7 ± 2.9	16.8 ± 3.5	20.7 ± 4.1	1.45	2.96	0.05
% CA time	48.6 ± 5.2	41.7 ± 3.0	41.1 ± 3.3	35.8 ± 3.9	2.40	2.88	0.04
% Central platform latency	3.0 ± 0.4	3.7 ± 0.8	2.14 ± 0.7	3.5 ± 0.6	3.14	0.85	0.36
Total SAP	5.9 ± 1.2	4.1 ± 0.9	4.9 ± 0.9	3.8 ± 1.1	1.99	0.41	0.11
% up SAP	18.8 ± 5.3	14.4 ± 7.6	26.7 ± 7.4	16.9 ± 8.1	1.00	0.54	0.14
Total HD	20.6 ± 1.8	25.9 ± 2.0	24.3 ± 1.5	21.8 ± 2.1	0.49	0.01	3.91 *
% up HD	81.4 ± 4.6	82.8 ± 2.1	86.2 ± 5.1	89.7 ± 3.8	0.37	2.03	0.07
CA returns[a]	–	–	–	–	–	–	–
Total rears	7.9 ± 1.3	9.5 ± 0.9	7.2 ± 0.9	7.1 ± 1.4	0.44	1.85	0.55
% up rears	22.6 ± 7.1	20.2 ± 4.3	26.1 ± 5.3	41.3 ± 8.2	1.02	3.80	1.94
NEB[a]	–	–	–	–	–	–	–

Acute plus-maze behaviour was examined 14 days after a neurotoxic regimen of MDMA (4 × 10 mg/kg ip every 2 h for 6 h), or an equivalent volume of vehicle. Data expressed as mean values (± S.E.M.). OA = open arms, CA = closed arms, SAP = stretched attend postures, HD = head dips, up = unprotected, and NEB = nonexploratory behaviours.

[a] Behaviour expressed too infrequently for analysis.

* P=.056.

no effect of MDMA pretreatment upon any of the undrugged behaviours recorded. In contrast, there was a single significant interaction between pre- and maze treatments. Inspection of means found that head dips were significantly increased in those ethanol-treated animals that had previously received MDMA compared to those that had received vehicle [$F(1,32)=5.18$, $P<.05$].

Table 3
Effects of MDMA pretreatment upon the behavioural effects of 2.0 g/kg ip ethanol in rats on the elevated plus-maze

Behaviour	Treatment condition				Statistical interactions and effects		
	Vehicle × Vehicle ($n=10$)	MDMA × Vehicle ($n=10$)	Vehicle × Ethanol ($n=8$)	MDMA × Ethanol ($n=8$)	MDMA pretreatment $F(1,32)$	Ethanol maze treatment $F(1,32)$	Pretreatment × Maze Treatment interaction $F(1,32)$
OA entries	4.2 ± 0.3	4.1 ± 0.3	4.4 ± 0.5	5.0 ± 0.3	0.28	4.19 *	3.04
CA entries	7.5 ± 0.3	7.2 ± 0.3	6.2 ± 0.4	7.0 ± 0.3	0.71	5.35 *	2.98
Total entries	11.7 ± 0.5	11.3 ± 0.6	10.7 ± 0.9	12.0 ± 0.5	0.54	3.43	3.53
% OA entries	35.7 ± 1.5	36.3 ± 0.6	41.6 ± 2.8	41.7 ± 1.7	0.09	3.06	0.01
% OA time	26.5 ± 3.2	26.4 ± 3.8	27.3 ± 6.9	31.2 ± 3.8	0.22	2.22	3.28
% CA time	44.1 ± 3.1	41.6 ± 3.9	39.0 ± 6.2	34.9 ± 3.8	0.23	2.86	0.31
% Central platform latency	2.4 ± 0.5	2.1 ± 0.3	3.8 ± 0.8	4.0 ± 0.5	0.01	12.66	0.00
Total SAP	3.9 ± 1.1	2.4 ± 0.5	1.2 ± 0.6	1.8 ± 0.5	0.43	5.48 *	1.93
% up SAP	13.0 ± 7.3	14.8 ± 6.4	13.3 ± 11.1	15.6 ± 10.5	0.04	0.05	0.02
Total HD	24.4 ± 1.6	25.3 ± 1.6	20.2 ± 2.7	28.3 ± 2.9	4.56	0.79	5.18 *
% up HD	83.9 ± 2.3	86.1 ± 3.9	86.1 ± 3.4	88.0 ± 3.1	0.28	1.27	0.31
CA returns[a]	–	–	–	–	–	–	–
Total rears	10.5 ± 1.7	8.4 ± 0.6	3.4 ± 0.9	3.6 ± 0.9	0.07	34.13 * *	1.26
% up rears	23.0 ± 5.6	27.3 ± 7.0	24.7 ± 12.1	26.2 ± 14.3	0.04	0.00	0.01
NEB	1.5 ± 1.4	4.1 ± 1.6	10.6 ± 2.4	11.8 ± 3.1	0.40	19.50 * *	0.50

Experimental details as before. Data expressed as mean values (±S.E.M.). OA = open arms, CA = closed arms, SAP = stretched attend postures, HD = head dips, up = unprotected, and NEB = nonexploratory behaviours.

[a] Behaviour expressed too infrequently for analysis.

* $P<.05$, significant effect.

** $P<.01$, significant effect.

Table 4
Effects of MDMA pretreatment upon the behavioural effects of 0.5 mg/kg ip heroin in rats on the elevated plus-maze

Behaviour	Treatment condition				Statistical interactions and effects		
	Vehicle × Vehicle ($n=10$)	MDMA × Vehicle ($n=8$)	Vehicle × Heroin ($n=10$)	MDMA × Heroin ($n=9$)	MDMA pretreatment $F(1,33)$	Heroin maze treatment $F(1,33)$	Pretreatment × Maze Treatment interaction $F(1,33)$
OA entries	6.4 ± 0.6	6.9 ± 0.3	8.8 ± 0.5	9.0 ± 0.3	0.97	23.32 * *	0.09
CA entries	5.5 ± 0.3	6.0 ± 0.4	7.3 ± 0.7	7.1 ± 0.3	0.11	9.50	0.53
Total entries	11.9 ± 0.5	12.9 ± 0.6	16.1 ± 1.2	16.2 ± 0.6	0.44	16.95 * *	0.33
% OA entries	53.8 ± 2.1	53.4 ± 1.8	54.7 ± 2.9	56.0 ± 1.2	0.01	1.47	0.02
% OA time	36.9 ± 4.4	36.3 ± 5.3	40.6 ± 5.2	38.7 ± 3.2	0.65	0.17	1.48
% CA time	48.7 ± 4.0	50.2 ± 4.5	47.7 ± 5.5	48.6 ± 3.5	0.73	0.66	1.01
% Central platform latency	4.4 ± 1.2	2.6 ± 0.4	1.7 ± 0.8	2.7 ± 0.4	0.21	2.33	2.79
Total SAP	3.7 ± 0.4	3.6 ± 0.9	3.5 ± 0.7	4.4 ± 0.7	0.41	0.21	0.57
% up SAP	8.3 ± 5.7	9.5 ± 6.3	13.2 ± 5.5	19.6 ± 11.1	0.26	1.00	0.12
Total HD	19.3 ± 1.5	25.5 ± 1.2	17.8 ± 1.9	21.4 ± 2.0	8.08 * *	2.57	0.54
% up HD	87.5 ± 3.1	92.6 ± 3.5	87.6 ± 3.2	90.0 ± 2.2	1.49	0.16	0.20
CA returns	–	–	–	–	–	–	–
Total rears	6.2 ± 1.5	8.9 ± 1.1	3.9 ± 0.7	8.7 ± 1.7	7.66 * *	0.87	0.61
% up rears	24.4 ± 9.5	33.1 ± 6.1	12.5 ± 6.7	36.0 ± 6.7	4.49 *	0.35	0.96
NEB[a]	–	–	–	–	–	–	–

Experimental details as before. Data expressed as mean values (±S.E.M.). OA = open arms, CA = closed arms, SAP = stretched attend postures, HD = head dips, up = unprotected, and NEB = nonexploratory behaviours.
[a] Behaviour expressed too infrequently for analysis.
* $P<.05$, significant effect.
* * $P<.01$, significant effect.

(iv) *Heroin*: Table 4 summarises the behaviours recorded after the different treatment regimes. MDMA pretreatment significantly affected measures of directed exploration and locomotion. Rats who had previously received MDMA performed more head dips than saline controls [$F(1,33)=8.08$, $P<.01$] and rears [$F(1,33)=$

Table 5
Effects of pretreatment with MDMA upon the behavioural effects of 10.0 mg/kg MDMA in rats on the elevated plus-maze

Behaviour	Treatment condition				Statistical interactions and effects		
	Vehicle × Vehicle ($n=10$)	MDMA × Vehicle ($n=10$)	Vehicle × MDMA ($n=6$)	MDMA × MDMA ($n=10$)	MDMA pretreatment $F(1,32)$	MDMA maze treatment $F(1,32)$	Pretreatment × Maze Treatment interaction $F(1,32)$
OA entries	4.6 ± 0.5	4.5 ± 0.5	3.7 ± 0.6	3.2 ± 0.5	0.33	5.04 *	0.14
CA entries	4.7 ± 0.5	4.6 ± 0.5	3.8 ± 0.6	3.4 ± 0.5	0.27	3.97	0.10
Total entries	9.3 ± 0.9	9.1 ± 0.9	7.5 ± 1.2	6.6 ± 0.9	0.32	4.86 *	0.13
% OA entries	49.5 ± 3.3	49.5 ± 3.3	48.9 ± 4.3	48.5 ± 3.3	0.20	0.55	0.32
% OA time	43.8 ± 4.4	46.7 ± 4.4	27.0 ± 5.6	28.0 ± 4.4	0.17	14.12 * *	0.04
% CA time	43.9 ± 4.5	40.6 ± 4.5	60.8 ± 5.8	58.0 ± 4.5	0.39	12.62 * *	0.00
% Central platform latency	2.3 ± 0.5	2.7 ± 0.5	2.2 ± 0.7	4.0 ± 0.5	2.07	1.03	1.38
Total SAP	3.5 ± 1.5	3.0 ± 1.5	9.5 ± 1.9	8.8 ± 1.5	0.14	13.77 * *	0.00
% up SAP	7.8 ± 5.5	5.0 ± 5.5	14.9 ± 7.1	19.5 ± 5.5	0.02	3.30	0.38
Total HD	21.8 ± 2.4	21.8 ± 2.4	16.5 ± 3.0	12.9 ± 2.4	0.50	7.75 * *	0.50
% up HD	84.6 ± 6.3	91.0 ± 6.3	79.3 ± 8.1	68.4 ± 6.3	0.11	4.20 *	1.61
CA returns[a]	–	–	–	–	–	–	–
Total rears	10.8 ± 1.5	11.9 ± 1.5	4.3 ± 1.9	4.5 ± 1.5	0.16	19.21 * *	0.09
% up rears	23.1 ± 5.7	30.2 ± 4.0	4.3 ± 3.0	2.3 ± 1.5	0.84	30.28 * * *	1.98
NEB[a]	–	–	–	–	–	–	–

Experimental details as before. Data expressed are mean values ± S.E.M. OA = open arms, CA = closed arms, SAP = stretched attend postures, HD = head dips, up = unprotected, and NEB = nonexploratory behaviours.
[a] Behaviour expressed too infrequently for analysis.
* $P<.05$, significant effect.
* * $P<.01$, significant effect.
* * * $P<.001$, significant effect.

7.66, $P<.01$], the latter being predominant in the OA [$F(1,33)=4.49$, $P<.05$]. Independent of the level of pretreatment present, acute heroin increased both OA and total arm entries [$F(1,33)=23.32$, $P<.01$ and $F(1,33)=16.95$, $P<.01$, respectively], and there was a nonsignificant increase in the number of CA entries [$F(1,33)=9.50$]. The remaining behaviours were not affected by acute drug treatment and there were no interactions between any of the measures recorded and levels of pre- and maze treatments.

(v) *MDMA*: Acute MDMA maze treatment produced a clear increase in anxiety-related behaviour (Table 5). Total [$F(1,32)=4.86$, $P<.05$] and OA [$F(1,32)=5.04$, $P<.05$] entries were significantly decreased, as was the % time spent in the OA [$F(1,32)=14.12$, $P<.01$]. However, CA entries were also nonsignificantly reduced, suggesting that some of the behavioural responses may have been a result of locomotor suppression. The total number of head dips [$F(1,32)=7.75$, $P<.01$] and rears [$F(1,32)=19.21$, $P<0.01$] were suppressed in MDMA-treated animals and in accordance with the pattern of spatial localisation, there was a redistribution of these two behaviours from the OA to the CA [$F(1,32)=4.20$, $P<.05$ and $F(1,32)=30.28$, $P<.001$, respectively]. However, total SAP were increased after MDMA [$F(1,32)=13.77$, $P<.01$] and there was a corresponding nonsignificant increase in the number of these taking place in the OA. There were no closed arm returns or nonexploratory behaviours. In contrast to its acute effects, MDMA pretreatment had no effect on any of the behaviours observed, although there was a nonsignificant increase in % central platform latency. There were no interactions between pre- and maze treatments.

4.1. Neurochemistry

In the animals selected for analysis, MDMA pretreatment significantly reduced hippocampal concentrations of 5-HT and 5-HIAA. Specific cortical [^{3}H]paroxetine binding was also significantly decreased by MDMA (see Table 6). Post hoc analysis showed that ethanol maze treatment had no effect upon these markers (data not shown).

Table 6
Effects of pretreatment with MDMA (10 mg/kg ip every 2 h for 6 h) or saline (1 ml/kg ip) upon cortical [^{3}H]paroxetine binding (fmol/mg tissue) and hippocampal 5-HT and 5-HIAA concentrations (pmol/mg tissue)

Region	Hippocampus		Cortex	
Treatment	Saline	MDMA	Saline	MDMA
5-HT	1.65 ± 0.08	1.26 ± 0.06**	ND	ND
5-HIAA	2.00 ± 0.07	1.63 ± 0.11*	ND	ND
[^{3}H]Paroxetine	ND	ND	197.96 ± 9.16	148.29 ± 9.10**

Values expressed as mean ± S.E.M., $n=9-10$ rats/treatment condition., significant effect of MDMA pretreatment upon marker. ND, not determined.
* $P<.01$, significant effect of MDMA pretreatment upon marker.
** $P<.001$, significant effect of MDMA pretreatment upon marker.

5. Discussion

The regimen of MDMA employed throughout this series of experiments produced a significant, although a mild (approximately 20–25%; Farfel and Seiden, 1995; Fischer et al., 1995b; Russell and Laverty, 2000; Scanzello et al., 1993) decrease in markers of serotonergic function. The reduction in specific cortical [^{3}H]paroxetine binding reflects a loss of the 5-HT uptake sites located on 5-HT nerve terminals indicating neurodegeneration. As these measures were only taken from a single experiment, it is unknown whether the other animals experienced a similar change, although there are no apparent reasons why this should not be the case. However, the relationship between this degree of change in the serotonergic system and the observed behaviour should be interpreted cautiously.

Acutely, 10 mg/kg MDMA was clearly anxiogenic. Administration resulted in a decrease in the total number and OA entries, the percentage of time spent in unprotected arms, and a reduction in the number of locomotor behaviours taking place in the OA. These findings were in accordance with consensual literature conclusions that low to middling systemic doses of MDMA (i.e., <10 mg/kg) are anxiogenic in a variety of behavioural paradigms.

MDMA pretreatment produced very few changes in the behavioural response to MDMA, ethanol, and heroin challenge on the elevated plus-maze. MDMA pretreatment was associated with a decrease in the total number of head dips after cocaine challenge and an increase after ethanol. In the rat, the incidence of head dips has been validated as an ethological index of anxiety (Rodgers and Cole, 1994). Whilst alterations in risk assessment measures have been reported in the absence of changes in arm activity (Rodgers and Cole, 1994), as in the present study, the significance of a change in a single measure should not be overstated. Unfortunately, there has been no previous work investigating the effects of selective serotonergic neurotoxicity upon the effects of these drugs in animal models of anxiety, and thus no parallels can be drawn between these results and the literature. Further examination of these particular treatment combinations in the hole-board test, from which plus-maze head dipping was derived, may help to validate these findings (File and Wardill, 1975).

The data also suggest that the effects of ethanol after MDMA pretreatment may have been subject to the influences of motor suppression. Whilst open arm entries were increased compared to saline-treated rats, there was a large decrease in the total number of rears. However, closer inspection of the type of nonexploratory behaviours exhibited showed that they mainly consisted of OA grooming episodes, which was compatible with fear reduction. Fur-

thermore, there was no evidence for a decrease in supported rears, which may have indicated sedative effects. SAP may have been reduced as a consequence of the spatial redistribution of behaviour towards the OA, where this behaviour is usually less common.

The lack of effect after acute amphetamine and cocaine administration is problematic. Doses of the two drugs used in the challenge experiment were behaviourally active in the dose-finding studies, and there is literature support to suggest we targeted an active dose range (see Materials and methods). Superficially, at least our observations were surprising. Whilst it is beyond the scope of the current discussion to explore underlying reasons in detail, we would suggest that disparate periods of single housing and handling (7 days in dose-finding experiments, 21 days in neurotoxicity work) and unreported variations in previvarium history may have played some part in the inconsistent profiles (Boix et al., 1990; Brett and Pratt, 1990; Rodgers and Cole, 1994). Subsequently, whilst it is possible that MDMA pretreatment may theoretically produce changes in behaviourally inactive drug doses, perhaps through cross-sensitisation, the current data does not allow us to discount or confirm the original experimental hypotheses. Pilot work from this laboratory suggested (Sumnall et al., 2000), in common with the modulating effects of MDMA upon cocaine-induced reward (Horan et al., 2000), an interaction between neurotoxic MDMA and psychostimulatory behaviours produced by 20 mg/kg cocaine ip. However, as this was also accompanied by stereotypy, it is important that this work is confirmed using more moderate doses.

There was considerable variation in the spontaneous behaviour exhibited on the plus-maze in these experiments; therefore, comparisons between the behaviour exhibited in different experiments is unwise. Given that these experiments were run sequentially during a 3-month period using animals from the same supplier held under identical environmental conditions, this variation is most likely an inherent feature of models measuring spontaneous behaviour. However, as there were appropriate vehicle-treated controls used, the effects of MDMA pretreatment can be established within experiments. It is possible that this variation in baseline behaviour may have masked effects produced both by MDMA pretreatment and the drug under investigation on the plus-maze. Therefore, these results and those in other studies where no behavioural change was detected should be interpreted cautiously.

Previous investigations of the effects of MDMA pretreatment on anxious behaviours in adult rats have produced inconsistent results. Wistar rats displayed *increased* anxiety-related behaviour in the elevated plus-maze, emergence, and social interaction tests 3 months after multiple-dose MDMA pretreatment (administered at 28 °C to maximise neurotoxic potential, but no measures of 5-HT function were made; Morley et al., 2001). In a subsequent study where 5-HT neurotoxicity was assessed, it was found that increased anxiety-related behaviour was seen in Wistar rats with reduced 5-HT in the amygdala, hippocampus, and caudate putamen (Gurtman et al., 2002). These findings have been supported by more recent observations using social models of anxiety (McGregor et al., 2003a,b). In contrast, Dark Agouti rats displayed evidence of *reduced* anxiety-related behaviour in the plus-maze 80 days after a single 12.5-mg/kg ip dose of MDMA (Mechan et al., 2002). Earlier time points (8 and 29 days after pretreatment) were not associated with behavioural change. Furthermore, a recent strain comparison study performed in this laboratory (manuscript under review) reported no behavioural dysfunction at this extended survival time. Ho et al. (2004) grouped rats on the basis of previous plus-maze exposure (rats were designated as having high or low anxiety) but found no individual differences in plus-maze response after MDMA. This is noteworthy considering suggestions that MDMA-induced clinical complaints may manifest in susceptible individuals with premorbid disposition towards psychiatric dysfunction (Green et al., 1995). Also in keeping with the general findings of the studies reported here, work examining the effects of MDA-induced 5-HT neurotoxicity (MDA is a neurotoxic metabolite of MDMA; Ricaurte et al., 1985) found no effect on the plus-maze behaviour of Wistar rats, 2 weeks after the last dose (Harkin et al., 2001). This inconsistency across studies is a challenge to accurate determination of the long-term effects of MDMA, but it is interesting to note that these cited studies, including the present one, have shown no behavioural change at time points where gross 5-HT depletion and transporter loss is thought to be greatest ($t=+2$ weeks; Battaglia et al., 1988). Classic theories propose that excess 5-HT increases anxiety whilst manipulations that reduce transmission are anxiolytic (Griebel, 1995; Handley et al., 1993; Handley, 1995). Behavioural dysfunction on the plus-maze has been observed after generalised (all brain regions and raphé projections) or localised (specific to DRN fibres, thus mimicking the effects of MDMA) depletion of at least 50% of central 5-HT (Briley et al., 1990; Critchley and Handley, 1992). If gross concentrations of 5-HT were the only determinant of this type of behaviour, then it would be expected that across studies, all MDMA-pretreated rats would exhibit a decrease in anxious behaviour on the plus-maze. However, microdialysis work using the plus-maze has shown that regionally specific patterns of 5-HT release are more indicative of behavioural responses to stressful stimuli than the magnitude of 5-HT release per se (Voigt et al., 1999). McGregor et al. (2003a) have suggested that subtle, regionally specific alterations in 5-HT_{1B} and 5-$HT_{2A/2C}$ receptor binding density or function underlie anxiogenesis at extended survival times, as serotonergic neurons recover from initial MDMA-induced damage. Unfortunately, no studies have yet examined *recovery* of the 5-HT system after MDMA in detail, having so far being restricted to gross transmitter concentration and transporter density (e.g., (Fischer et al., 1995b; Scanzello et al., 1993). Measuring

transporter density, for example, may have limited use when describing the neurotoxic effects of MDMA as it is not a structural element of the nerve terminal and thus, is susceptible to pharmacological regulation, maturation, ageing, and food restriction in common with other dynamic cellular components (Kekuda et al., 1997; Lopez et al., 1994; Vicentic et al., 1999; Zhou et al., 1996). It was therefore interesting to note that in the present experiment, the rate of weight gain in MDMA-treated animals was less than controls. More extreme loss of body weight than recorded in the present experiment (26%) produced 30% reduction in [^{3}H]paroxetine binding to frontocortical 5-HT transporters in male Wistar rats (Zhou et al., 1996). What consequences the differences recorded in the present study had upon the range of behavioural data obtained (albeit limited) is unclear. Reduced weight gain, perhaps related to decreased food intake (Frith et al., 1987), is unlikely to have resulted in 5-HT terminal destruction but may have at least caused down-regulation in the number of expressed transporters.

6. Conclusion

As human users of MDMA are polydrug abusers, it is possible that they are at risk of developing problems with other drugs due to altered neurochemical and behavioural responses. These data suggest that mild 5-HT neurotoxicity will not produce such changes. It is uncertain whether humans who use MDMA will experience similar serotonergic changes to those reported in animals in this paper, but is a critical point for discussion. Integration of results from several studies which have used the elevated plus-maze indicate that it may not be a reliable model for the detection of behavioural neurotoxicity after MDMA. Caution should be applied in extrapolating derived results.

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