Psychopharmacology (2002) 159:437–444
DOI 10.1007/s00213-001-0931-z

ORIGINAL INVESTIGATION

Kevin C.F. Fone · Simon R.G. Beckett
Ian A. Topham · Jennifer Swettenham · Melanie Ball
Laura Maddocks

Long-term changes in social interaction and reward following repeated MDMA administration to adolescent rats without accompanying serotonergic neurotoxicity

Received: 12 July 2001 / Accepted: 20 September 2001 / Published online: 20 November 2001

Abstract *Rationale:* MDMA is a popular drug of abuse in adolescents which causes serotonergic neurotoxicity in adult but not young rodents. However, few studies have examined the long-term behavioural consequence of MDMA and it is unclear whether such changes occur in the absence of neurotoxicity. *Objectives:* The present study examined whether treatment of young rats with MDMA produced long-term behavioural alterations without accompanying serotonergic neurotoxicity. *Method:* Male Lister hooded rats (n=36, postnatal day (PND) 39) received MDMA (7.5 mg/kg IP, twice daily for 3 days) or saline (l ml/kg IP) and the acute effect on open field behaviour and body temperature was monitored. Following drug withdrawal, social interaction in pre-treatment- and weight-matched rat pairs, cortical [^{3}H]paroxetine binding and hippocampal and frontal cortical serotonin and dopamine levels (PND 53, n=12) and conditioned place preference (PND 70, n=24) to cocaine (5 mg/kg IP) were analysed. *Results:* MDMA elicited the expected immediate serotonin syndrome with significant hyperlocomotion, decreased rearing and hypothermia. Twelve to 29 days after the last MDMA injection social interaction was significantly attenuated (by 41%) and the sub-threshold conditioned place preference to cocaine was significantly enhanced compared with that in saline controls, although no significant side preference to cocaine occurred in the latter. MDMA pre-treatment did not alter 5-HT levels or cortical [^{3}H]paroxetine binding. *Conclusion:* MDMA administration to adolescent rats reduced social interaction and enhanced the sub-threshold rewarding effect of cocaine at adulthood, despite an absence of accompanying serotonergic and dopaminergic neurotoxicity.

K.C.F. Fone (✉) · S.R.G. Beckett · I.A. Topham · J. Swettenham
M. Ball · L. Maddocks
School of Biomedical Sciences, Queen's Medical Centre,
Nottingham University, Nottingham NG7 2UH, UK
e-mail: kevin.fone@nottingham.ac.uk
Tel.: +44-115-9709469, Fax: +44-115-9709259

Keywords MDMA · Social interaction · Conditioned place preference · Serotonin · Cocaine

Introduction

The popular recreational drug, 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy" or "E") elicits a characteristic psychoactive syndrome comprising euphoria, enhanced well-being, derealisation and cognitive alterations in man (Peroutka et al. 1988). Although the precise underlying pharmacological mechanisms have not been established in humans, carrier-mediated release and/or inhibition of reuptake of 5-hydroxytryptamine (5-HT) and dopamine appear to account for the acute behavioural correlate seen with similar doses in rodents (Green et al. 1995; Iravani et al. 2000; Morgan 2000). In addition, MDMA reduces the activity of the biosynthetic enzyme tryptophan hydroxylase (Schmidt and Taylor 1987) and causes a longer-term depletion of the product, 5-HT, preferentially from fine-diameter neurones arising from the dorsal raphé nucleus (Mamounas et al. 1991). This is accompanied by depletion of serotonin transporter sites (Battaglia et al. 1998; O'Shea et al. 1998) and immunohistochemical evidence of 5-HT nerve terminal damage (Commins et al. 1987; O'Hearn et al. 1988; Fischer et al. 1995). Both effects are indicative of serotonergic neurotoxicity, which lasts for months in rats and years in non-human primates (Hatzidimitriou et al. 1999; Ricaurte et al. 1992). Increasing evidence suggests that MDMA also causes serotonergic neurotoxicity, reducing 5-HT transporter (5-HTT) levels, in the human cortex (McCann et al. 1998; Semple et al. 1999) associated with cognitive impairments (Morgan 1999) and heightened impulsivity (Parrott et al. 1998) in regular users. Furthermore, two groups (McCann et al. 1999; Gerra et al. 2000) have reported a blunted prolactin response to 5-HT agonist challenge following 3 weeks and 12 months of abstinence in regular MDMA users, consistent with a long-lasting loss of serotonergic neuronal function following drug with-

drawal. Given the increasing use of MDMA by young adults, who generally perceive the drug to be safe (Green et al. 1995; Curran 2000), it is pertinent to examine the long-term behavioural impact of treating young rats with low doses of MDMA.

Although the neurotoxic effect has received much attention, few studies have assessed the long-term behavioural consequence of MDMA in rats and it is unclear whether the neurotoxic and behavioural changes are interrelated or independent. However, in the rhesus monkey behavioural tolerance to MDMA has been reported without accompanying depletion of 5-HT in any brain region examined, although hippocampal 5-HT uptake was reduced (Frederick et al. 1995), suggesting dissociation of the two effects. The extent of MDMA-induced serotonergic neurotoxicity in rodents depends on the dosage regimen, strain, body temperature and age (Broening et al. 1995; Green et al. 1995; O'Shea et al. 1998). Of particular interest, rats up to postnatal day (PND) 35–40 are resistant to the neurotoxic effects of MDMA (following single injections, 10–40 mg/kg SC), which causes no significant decrease in paroxetine labelled 5-HTT density or 5-HT levels (Aguirre et al. 1998; Broening et al. 1994). However, only one very recent study has examined the long-term behavioural effect of a non-neurotoxic dosage regimen in young rats (Broening et al. 2001). In a preliminary study (Bellerby et al. 1999), we showed that MDMA (20 mg/kg IP, twice daily for 2 days) produced a long-term reduction in social interaction without accompanying 5-HT neurotoxicity when given to young rats (PND 28–29). The present study extends these observations, concomitantly examining the long-term effect on neurochemical markers of both serotonergic and dopaminergic neuronal function and social and reward-related behaviour.

Materials and methods

Animals

Male Lister hooded rats (Charles River, UK, *n*=36), weighing 165±l g at the start (PND 39), were housed in groups of four in a temperature controlled (21±2ºC) environment on a 12-h light/dark cycle (lights on 0700–1900 hours) in the Biomedical Sciences Unit (The University of Nottingham). Rats had access to food (Beekay-Standard rat and mouse diet) and water ad libitum and were weighed twice a week throughout the study.

On PND 39–41, each rat was injected with either MDMA (7.5 mg/kg IP, racemic; Sigma, UK) or, vehicle, saline (0.154 M NaCl, 1 ml/kg IP) twice daily (between 1000–1100 and 1630 and 1700 hours) for 3 consecutive days. On PND 39 rectal temperature was recorded before and 45 min after the first injection, using a Portec Instrumentation Ltd, P9005, thermocouple probe and open field behaviour measured as detailed below. In addition, following 12 and 26–29 days withdrawal, respectively, social interaction and conditioned place preference with cocaine were recorded and neurochemical evidence of serotonergic and dopaminergic neurotoxicity determined in post-mortem brain tissue, as described below.

The dose and duration of MDMA treatment was chosen following preliminary experiments that showed this to elicit a profound acute serotonin syndrome without subsequent animal loss. The treatment age was selected on the basis of previous studies showing this to be the critical point at which susceptibility to serotonergic neurotoxicity begins (Aguirre et al. 1998; Broening et al. 1994).

All experiments were performed in accordance with the UK animals (Scientific Procedures Act 1986) using a blind drug treatment protocol. Between each behavioural test, all apparatus were cleaned with 20% (v/v) ethanol to remove odour cues.

Acute open field behaviour

Fifteen minutes after the first MDMA injection on PND 39, rats (*n*=36) were placed in separate activity monitor chambers (Medical Physics, University of Nottingham) to record locomotion and rears separately. Each chamber consisted of a clear acrylic open field box, 40×20×25 cm high, with a wire mesh lid. Five parallel infrared beams, 7.5 cm apart, crossed the chamber at two different heights. The lower beams (3.5 cm above the floor) measured locomotion and the upper beams (7 cm above the floor) rears. To prevent false recording of locomotion by small movements such as grooming, a count was only generated from the lower detectors when two adjacent beams were broken simultaneously in a consecutive sequence. For each rat activity was recorded for 30 min (15–45 min post-injection) in 5-min epochs using "Activity Monitor" software on an Apple Macintosh IIcx computer (Clemett et al. 1998).

In addition, components of the serotonin behavioural syndrome, (reciprocal forepaw treading, lateral head weaving and flat body posture), were scored separately by an individual who sat 1 m away and screened by a curtain from the chamber. Each behaviour was rated (0 if absent, 1 present <10 s, 2 if present >10 s and 3 if continuous) for 20 s on four occasions at one minute intervals from 20 to 24 min post-injection. Thus for each behaviour the maximum possible summated score was 12. As an index of sympathetic activity and anxiety, the number of faecal pellets produced in the chamber was also recorded.

Social interaction

Given the considerable evidence that MDMA affects social behaviour in both man and animals the impact of pre-treatment on social interaction was studied. Twelve days after the last injection, without further drug administration, social interaction between pairs of rats was examined in a circular (75 cm diameter 45 cm high) arena with a matt black floor divided by white lines into eight outer and eight inner radial zones, under dim light (50 Lux) familiar conditions. Each rat was familiarised to the arena for 15 min on 2 consecutive days in the week preceding the test. Each pair of rats had received the same pre-treatment (either saline or MDMA), were weight matched (to within 20 g) and were from a different litter. At the start of the test, two rats were placed at opposite sides of the arena facing each other and activity monitored for 10 min. Aggressive (biting or boxing each other), avoidance (going over or under the other rat), passive (lying motionless in contact with each other) or exploratory (sniffing the other rat or following with its head over the base of the other rat's tail) interaction were timed separately using a computerised video-tracking system (Ethovision ColourPro videotrack system; Noldus Information Technology, The Netherlands). Summation of all four forms of interaction for each rat was used to calculate the total social interaction. Locomotion was analysed from a video-recording by counting the number of lines crossed by both front paws. For analysis, the mean score of the two paired rats was calculated for each individual behaviour, the total interaction and the locomotion and the mean value used for statistical analysis, since the behaviour of each rat within the pair is not independent.

Conditioned place preference

To ascertain whether reward mechanisms were altered after MDMA, the impact on condition place preference to cocaine was

determined using a protocol similar to that utilised in previous studies (Cheer et al. 2000). Commencing on PND 68, (26 days after the last injection) conditioned place preference was carried out in a square open field arena (30×60×40 cm high, at 80 Lux) that could be divided into two equal size compartments by a removable guillotine door. In one compartment, the walls had 2.5 cm thick, vertical black and white stripes, while the other side had thin (1 cm) black and white stripes. The floor was the same colour (black) and texture (smooth) in both compartments. Each rat was handled 5 days a week throughout the whole study and acclimatised to the box prior to testing in a preconditioning phase, by being placed in the centre of the whole arena for 10 min, on 3 consecutive days (PND 62–64) prior to the test day. During these trials movement was tracked, and any rat spending >350 s on one side would have been excluded, thus ensuring that no natural side preference was expressed.

During the conditioning phases, on PND 68 and 69, rats were assigned at random to receive either cocaine (5 mg/kg, IP) or saline (0.154 M; 1 ml/kg IP) and immediately post-injection confined (for 15 min) in one compartment of the box. On the next day, in the test phase, each rat was placed facing the side wall at the intersection of the two compartments and allowed to explore (10 min) to assess preference for the drug-paired compartment using a computerised video-tracking system and Ethovision software.

Neurochemical analyses

Since drug treatment during the conditioned place preference test would affect subsequent neurochemical measures, six rats from each treatment group (MDMA or saline), selected at random were stunned and decapitated immediately after the social interaction test. The frontal cortex, hippocampus and brainstem were rapidly removed, frozen in liquid nitrogen and stored at –80°C for subsequent analysis by HPLC as detailed below. In addition, the left cortical hemisphere was placed in TRIS buffer for [^{3}H]paroxetine binding (see below).

HPLC of 5-HT, dopamine and their metabolites

5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine, DOPAC and homovanillic acid (HVA) were separated from frontal cortex, hippocampus and brainstem extracts and measured by HPLC-ED using a Hypersil 3 µm C18 column (10 cm×4.6 mm ID; Phenomenex) and values compared with those of 2 pmol standards. Samples were extracted in perchloric acid (1 ml, 0.1 M containing 1.6 mM sodium metabisulphite) by sonication on ice (30 s, Soniprep 150, MSE) followed by centrifugation (13,000 *g*, for 10 min at 4°C; Beckman, USA, Microfuge 12). The resultant supernatant was filtered (0.45 µm syringe filters, Millipore) and quantified by electrochemical detection (Antec Leyden, The Netherlands) at a potential of +0.7 V with a mobile phase consisting of 0.15 M NaH_2PO_4, 0.1 mM EDTA, 0.5 mM 1-octanesulphonic acid and 13% (v/v) methanol (pH 3.8).

Cortical [^{3}H]paroxetine binding

[^{3}H]Paroxetine binding to cortical homogenates (800 µl) was performed in triplicate using 50 mM TRIS (pH 7.4 containing 120 mM NaCl and 5 mM KCl), 10 nM paroxetine and 1 mM 5-HT to define specific binding according to the method of Hewitt and Green (1994). In brief, hemi-cortex was homogenised in 25 vol ice cold TRIS, centrifuged (30,000 *g*, for 10 min at 4°C, Sigma) and the pellet washed and re-centrifuged twice prior to suspension in TRIS buffer at 10 mg tissue/ml. Assay tubes were incubated for 60 min at room temperature and terminated by rapid filtration through filters pre-soaked in 0.05% v/v polyethylenimine using a Brandel cell harvester (model M-24, Maryland, USA).

Statistics

Behaviours recorded objectively or timed and neurochemical data were analysed using Student's unpaired *t*-test, while components of the serotonin behavioural syndrome that were rated were analysed with the Mann-Whitney *U*-test. Two- and one-way ANOVA followed by Duncan's New Multiple Range post-hoc test was used to assess conditioned place preference and time course affects. $P<0.05$ was considered significant.

Results

Effect of acute MDMA administration on rectal temperature and behaviour

Immediately following the first injection saline treated rats showed a low level of locomotion in the open field that declined, as they became familiar with the activity box (Fig. 1A). In contrast, MDMA (7.5 mg/kg IP) significantly increased locomotion which was sustained throughout the 30 min observation period, such that there was a significant main effect of treatment [ANOVA, $F(1,204)=419.0$, $P=0.0001$; Fig. 1A] and a marked increase in total locomotion (Student's *t*-test, $P<0.0001$). By comparison, the total number of rears (Fig. 1B) was significantly less in MDMA than in saline controls ($P<0.0001$) and there was a significant treat-

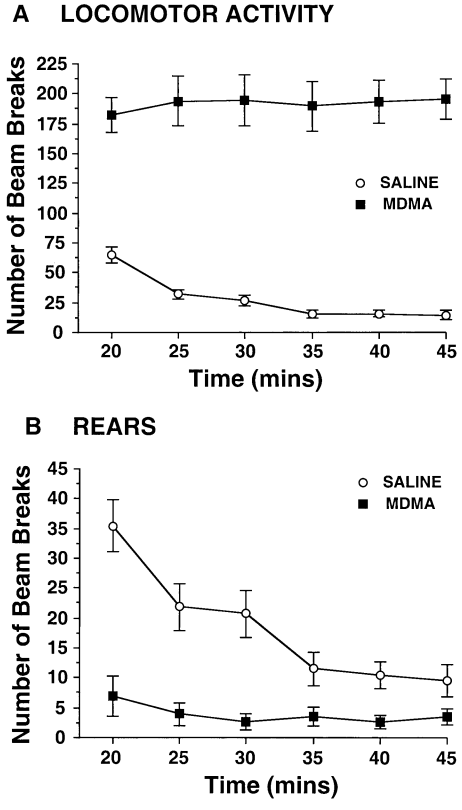

Fig. 1 Comparison of the effect of the first injection of MDMA (7.5 mg/kg IP, $n=18$) or saline (1 ml/kg, $n=18$) on; **A** locomotor activity and **B** rearing (mean±SEM counts over 5 min, plotted against time from the injection at the end of the recording epoch)

Table 1 Comparison of the effect of the first injection of MDMA (7.5 mg/kg IP) or saline (1 ml/kg) on stereotype behaviours [median (interquartile (range)] and the number of faecal pellets and body temperature (mean±SEM)

Measurement	Saline	MDMA
Reciprocal forepaw treading	0 (0)	6 (5) ***
Lateral head weaving	0 (0)	3.5 (3) ***
Flat body posture	0 (0)	8.5 (4) ***
Faecal boluses	3±1	3±0
Pre-injection temperature °C	38.2±0.1	38.3±0.1
Post-injection temperature °C	38.3±0.3	37.4±0.1††

For all the stereotype behaviours, comparison was by Mann-Whitney *U*-test from saline injected controls (***P<0.001) while for body temperature the comparison was from the temperature prior to MDMA in the same rat according to Student's *t*-test (††P<0.001)

ment×time interaction [F(5,204)=4.87, P=0.001] and a main effect of both time [F(5,204)=8.17, P=0.0001] and treatment [F(5,204)=80.62, P=0.0001].

MDMA also concomitantly elicited extensive reciprocal forepaw treading (P<0.0001), lateral head weaving (P<0.0001) and flat body posture (P<0.0001) that was not exhibited in saline-treated rats (Table 1). There were a similar number of faecal pellets in the activity boxes after open field behaviour, irrespective of the pre-treatment given (Table 1). MDMA also decreased body temperature by 0.9°C (P<0.001) when measured immediately following activity assessment, 45 min post-injection (Table 1).

Effect of previous repeated MDMA on social interaction

The total social interaction was significantly reduced by 41% in rats pre-treated with MDMA compared with that in saline controls (P<0.01; Fig. 2). Of the component behaviours, only exploratory behaviour (sniffing and following the partner), was significantly reduced by MDMA pre-treatment (P<0.05; Fig. 2). However, both aggressive (10±4 and 2±1 s in saline and MDMA, respectively) and passive behaviour (Fig. 2) also tended to be reduced in the MDMA pre-treated group, but these effects just failed to reach significance. During the test, MDMA and saline pre-treated rats entered an almost identical number of zones (84±5 with saline and 82±4 with MDMA), showing that the reduction in social interaction was not accompanied by a change in total locomotion.

Effect of previous repeated MDMA on conditioned place preference

Conditioning with cocaine just failed to produce a significant place preference in saline pre-treated controls (Fig. 3), main effect of cocaine [F(1,20)=3.505, P=0.0759]. However, ANOVA showed a significant main effect of MDMA pre-treatment [F(1,20)=6.302, P=0.0208], such that MDMA pre-treated rats spent significantly longer in the drug-paired compartment following cocaine treatment than rats receiving the saline+saline combination (P<0.05). In addition, the saline place aversion seen in saline pre-treated rats was converted to a preference for the injection side following MDMA (P<0.05, Fig. 3).

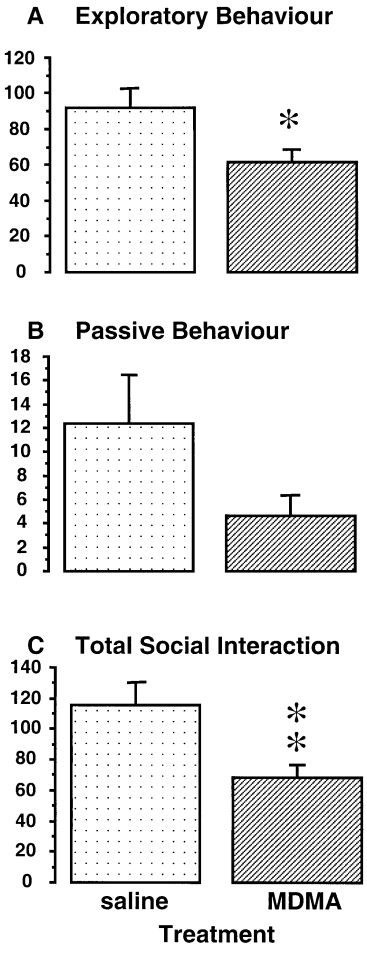

Fig. 2 Change in **A** exploratory, **B** passive and **C** total social interaction (mean±SEM) 12 days after the last injection of MDMA (7.5 mg/kg IP ×2 for 3 days) or saline (1 ml/kg IP) in pre-treatment and weight-matched pairs of rats (n=18 per treatment group). *P<0.05 and **P<0.01 from saline pre-treated control rats calculated by Student's *t*-test

[^{3}H] paroxetine binding and 5-HT and dopamine levels

The level of 5-HT and its major metabolite, 5-HIAA, and of dopamine was unaltered in the brain stem, frontal cortex and hippocampus, irrespective of whether rats had been pre-treated with saline or MDMA (Table 2). MDMA pre-treatment also failed to alter the 5HIAA:5-HT or (DOPAC+HVA):dopamine ratios in any brain region (data not shown).

In addition, the B_{max} value for [^{3}H]paroxetine binding to cortical homogenates was unaltered by previous MDMA administration (Table 2).

Table 2 Comparison of the effect of pre-treatment with either MDMA (7.5 mg/kg×6, IP) or saline (1 ml/kg) on cortical [^{3}H]paroxetine binding (fmol/mg wet weight), the 5-HT, 5-HIAA (pmol/mg wet weight) and dopamine levels (fmol/mg wet weight, mean±SEM, *n*=6 except dopamine in cortex and hippocampus *n*=3–5) in the hippocampus, frontal cortex and raphé nuclei region of the brainstem. ND=not determined

Region Treatment	Cortex		Hippocampus		Brain stem	
	Saline	MDMA	Saline	MDMA	Saline	MDMA
5-HT	1.68±0.21	1.83±0.71	1.92±0.42	1.37±0.28	4.45±0.61	3.81±0.24
5-HIAA	1.95±0.15	1.75±0.25	2.12±0.28	1.86±0.33	4.18±0.27	3.85±0.21
Dopamine	222±1	254±38	1.79±0.19	2.57±0.77	843±45	867±24
[^{3}H]Paroxetine	8.10±0.63	8.20±0.73	ND	ND	ND	ND

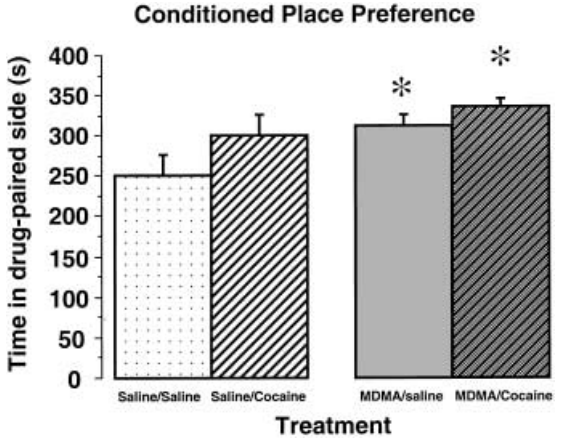

Fig. 3 Rats received saline (1 ml/kg IP) or MDMA (7.5 mg/kg IP) twice a day for 3 days. Following habituation to the conditioned place preference box, rats were conditioned by pairing either saline (1 ml/kg IP) or cocaine (5 mg/kg IP) injection, as indicated, with confinement to one of the two distinctive compartments. The effect of MDMA on the time spent (s, mean±SEM, *n*=6 each) in the drug-paired compartment on the test day (29 days after the last MDMA or saline injection) in the conditioned place preference test is shown. **P*<0.05 from saline-pre-treated saline conditioned control rats according to Duncan's New Multiple Range test following a significant ANOVA

Discussion

In the current study, the first injection of MDMA produced the expected hyperlocomotion and components of the serotonin syndrome including flat body posture (Slikker et al. 1989; Spanos and Yamamoto 1989; Marston et al. 1999), consistent with an acute increase in synaptic levels of serotonin and dopamine (Shankaran and Gudelsky 1998; Iravani et al. 2000). This was followed, at 45 min post-injection, by a significant, approximately 1°C, drop in core body temperature. The body temperature change elicited by MDMA critically depends on ambient room temperature. The hypothermia observed was anticipated at a room temperature of 22°C (Malberg and Seiden 1998), as these rats were housed singly and unable to aggregate, which contributes to the hyperthermia often caused by amphetamine-like compounds (Green et al. 1995).

In humans the acute effects of MDMA include euphoria and enhanced feelings of closeness (Peroutka et al. 1988; Green et al. 1995), while in contrast, one of the more common adverse psychiatric symptoms seen in regular users is pathological anxiety and panic attacks (McCann and Ricaurte 1991; Morgan 2000). MDMA injection elicits anxiogenic-like behaviour on social encounters (Maldonado and Navarro 2001) and in the light dark box in mice (Maldonado and Navarro 2000), and in the elevated plus maze in both rats (Bhattacharya et al. 1998; Morley and McGregor 2000) and, at low doses, in mice (Lin et al. 1999). Several groups have suggested a greater impact of MDMA on tests involving social behaviour than on other aversive behavioural paradigms. Yet there are conflicting reports of the acute effect of MDMA in the rat social interaction test, the same dose being reported to produce anxiolysis (Morley and McGregor 2000) or an anxiogenic response (Bhattacharya et al. 1998). In contrast, few animal studies have examined the longer-term behavioural impact of exposure of adolescent rats to MDMA and only one recent study assessed whether such changes occur independent of serotonergic degeneration (Broening et al. 2001), which was an aim of the current study. Marston et al. (1999) showed a delay-dependent deficit in performance in the delayed non-match to place test 16 days post-MDMA treatment in rats, consistent with impairment of short-term memory. As this was accompanied by a 40–60% reduction in cortical and hippocampal 5-HT and 5-HTT sites, cognitive impairment may have been due to serotonergic degeneration. Similarly, MDMA injection in PND 11–20 rats dose-dependently impaired subsequent sequential and spatial learning and memory with only small (<15%) but significant reductions in both hippocampal and frontal cortex 5-HT, which was therefore not thought to account for the cognitive change (Broening et al. 2001).

In the current study adolescent MDMA treatment did not change 5-HT levels or [^{3}H]paroxetine binding in cortical homogenates, implying that there was no overt serotonergic neurotoxicity. The absence of acute hyperthermia during MDMA treatment in this study would tend to protect against long-term serotonergic neurotoxicity (Broening et al. 1995; Malberg and Seiden 1998; Colado et al. 1999). However, adult rats given MDMA show extensive 5-HT depletion, even when injected at an ambient temperature of 10°C, which is accompanied by acute hypothermia. Thus although rats aged PND 35–40

show an attenuated thermoregulatory response and appear to be protected against the neurotoxic effect of MDMA (Broening et al. 1994, 1995), it is unlikely that the lack of toxicity is entirely due to the absence of hyperthermia (Aguirre et al. 1998). Interestingly, the increase in free radical formation, thought to contribute to neurotoxicity in the adult rat, does not appear to occur in neonatal rat brain (Colado et al. 1997). Furthermore, as both serotonergic and dopaminergic neurones continue to develop during the early postnatal period this alteration and/or a deficiency in an enzyme involved in conversion of MDMA to the neurotoxic metabolite/s (Miller et al. 1997) could also contribute to the aged-dependent neurotoxic susceptibility to MDMA.

Despite the absence of serotonergic neurotoxicity, MDMA pre-treatment markedly reduced social interaction following 12 days of drug withdrawal. The reason for this reduction is unclear. It could result from a change in post-synaptic serotonin receptor function. In particular, enhanced 5-HT_{1A} receptor function in the dorsal hippocampus, basolateral amygdala (Gonzalez et al. 1996) or lateral septum (Cheeta et al. 2000) could produce such a change. Although enhancement in the latter region would also be expected to reduce open arm entries in the elevated plus maze, which was not found in our previous study (Bellerby et al. 1999). However, in two studies at 12 and 30 days after MDMA, 8-OHDPAT-induced reciprocal forepaw treading (but not other behaviours) was attenuated (Granoff and Ashby 2001), yet ACTH release was enhanced and prolactin release decreased (Poland 1990), suggesting that MDMA may cause brain region dependent effects on 5-HT_{1A} receptor function. Alternatively, it could involve a reorganisation of developing serotonergic innervation, in particular of the dorsal raphé neurones, which are known to be critical for social interaction behaviour (File et al. 1979). In mice most reports indicate that MDMA is neurotoxic to dopaminergic neurones (O'Callaghan and Miller 1994) especially those in the striatum, but this is thought to be a species specific effect not produced in rats (Stone et al. 1987; Shankaran and Gudelsky 1998) and there was no change in dopamine levels in this study. Further studies are thus required to delineate the neurochemical basis of the change in social interaction.

MDMA is rewarding when given to rats, as shown by the induction of a conditioned place preference (Bilsky et al. 1991; Schechter 1991) associated with increased dopamine release in the nucleus accumbens (Marona Lewicka et al. 1996) and lower intracranial self-stimulation thresholds (Hubner et al. 1988; Lin et al. 1997). The acute positive reinforcing effect of MDMA has also been established in a drug self-administration paradigm in the monkey (Lamb and Griffiths 1987) and is retained following a neurotoxic dosage regimen in the rat (Schechter 1991). The cocaine treatment protocol used herein failed to induce a robust conditioned place preference, making interpretation difficult. Nonetheless, MDMA pre-treatment significantly enhanced the time that cocaine-treated rats spent in the drug paired compartment post-conditioning. This is consistent with a recent demonstration that MDMA enhanced the rewarding effect of cocaine in a similar conditioned place preference test (Horan et al. 2000), but it is unlikely to be due to serotonergic depletion, as suggested by these workers.

Overall, the current study shows that following withdrawal from repeated MDMA administration in adolescent rats there was a marked reduction in social interaction and enhancement of the sub-threshold reward associated with cocaine administration, in the absence of serotonergic neurotoxicity. Clearly only tentative extrapolations can be made from animal studies to potential human implications. Nonetheless, evidence should be sought to determine whether chronic exposure of adolescents to MDMA causes long-term changes in social anxiety and/or increases the vulnerability to cocaine abuse.

References

Aguirre N, Barrionuevo M, Lasheros B, Del Rio J (1998) The role of dopaminergic systems in the perinatal sensitivity to 3,4-dimethoxymethamphetamine induced neurotoxicity in rats. J Pharmacol Exp Ther 242:1159–1165

Battaglia G, Yeh SY, De Souza B (1998) MDMA-induced neurotoxicity: parameters of degeneration and recovery of brain serotonin neurons. Pharmacol Biochem Behav 29:269–274

Bellerby N, Bodley J, Copas D, Dowling S, Furby J, Husband M, Townsend A, Wood TJB, Bennett GW, Fone KCF, Kendall DA, Marsden CA, Parker TL (1999) Long-term change in social interaction without accompanying serotonergic neurotoxicity following MDMA treatment of young adult rats. Br J Pharmacol 128:8P

Bhattacharya SK, Bhattacharya A, Ghosal S (1998) Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy): an experimental study. Biogenic Amines 14:217–237

Bilsky EJ, Hubbell CL, Delconte JD, Reid LD (1991) MDMA produces a conditioned place preference and elicits ejaculation in male-rats – a modulatory role for the endogenous opioids. Pharmacol Biochem Behav 40:443–447

Broening HW, Bacon L, Slikker W (1994) Age modulates the long-term but not the acute effects of the serotonergic neurotoxicant 3,4-methylenedioxymethamphetamine. J Pharmacol Exp Ther 271:285–293

Broening HW, Bowyer JF, Slikker W (1995) Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (±)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. J Pharmacol Exp Ther 275:325–333

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

Cheer JF, Kendall DA, Marsden CA (2000) Cannabinoid receptors and reward in the rat: a conditioned place preference study. Psychopharmacology 151:25–30

Cheeta S, Kenny PJ, File SE (2000) The role of 5-HT_{1A} receptors in mediating the anxiogenic effects of nicotine following lateral septal administration. Eur J Neurosci 12:3797–3802

Clemett DA, Cockett MI, Marsden CA, Fone KCF (1998) Antisense oligonucleotide-induced reduction in 5-hydroxytryptamine(7) receptors in the rat hypothalamus without alteration in exploratory behaviour or neuroendocrine function. J Neurochem 71:1271–1279

Colado MI, Esteban B, O'Shea E, Granados R, Green AR (1999) Studies on the neuroprotective effect at pentobarbitone on MDMA-induced neurodegeneration. Psychopharmacology 142: 421–425

Colado MI, O'Shea E, Granados R, Misra A, Murray TK, Green AR (1997) A study of the neurotoxic effect of MDMA ("ecstasy") on 5-HT neurones in the brains of mothers and neonates following administration of the drug during pregnancy. Br J Pharmacol 121:827–833

Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987) Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 241:338–345

Curran HV (2000) Is MDMA ("ecstasy") neurotoxic in humans? An overview of evidence and of methodological problems in research. Neuropsychobiology 42:34–41

File SE, Hyde JRG, Macleod NK (1979) 5,7-dihydroxytryptamine lesions of dorsal and median raphé nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. J Affect Disord 1:115–122

Fischer C, Hatzidimitriou G, Wlos J, Katz J, Ricaurte G (1995) Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (±)3,4-methylenedioxymethamphetamine (MDMA, Ecstasy). J Neurosci 15:5476–5485

Frederick DL, Ali SF, Slikker W Jr, Gillam MP, Allen RR, Paule MP (1995) Behavioral and neurochemical effects of chronic methylenedioxymethamphetamine (MDMA) treatment in rhesus monkeys. Neurotox Teratol 17:531–543

Gerra G, Zaimovic A, Ferri M, Zambelli U, Timpano M, Neri E, Marzocchi GF, Delsignore R, Brambilla F (2000) Long-lasting effects of (±)3,4-methylene-dioxymethamphetamine (ecstasy) on serotonin system function in humans. Biol Psychiatry 47:127–136

Gonzalez LE, Andrews N, File SE (1996) 5-HT_{1A} and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. Brain Res 732:145–153

Granoff MI, Ashby CR (2001) Effect of the repeated administration of (±)-3,4-methylenedioxymethamphetamine on the behavioral response of rats to the 5-HT1A receptor agonist (±)-8-hydroxy-(di-n-propylamino)tetralin. Neuropsychobiology 43:42–48

Green AR, Cross AJ, Goodwin GM (1995) Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). Psychopharmacology 119:247–260

Hatzidimitriou G, McCann UD, Ricaurte GA (1999) Altered serotonin innervation patterns in the forebrain of monkeys treated with (±)-3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. J Neurosci 19:5096–5107

Hewitt KE, Green AR (1994) Chlormethiazole, dizocilpine and haloperidol prevent the degeneration of serotonergic nerve-terminals induced by administration of MDMA (Ecstasy) to rats. Neuropharmacology 33:1589–1595

Horan B, Gardner EL, Ashby CR (2000) Enhancement of conditioned place preference response to cocaine in rats following subchronic administration of 3,4-methylenedioxymethamphetamine (MDMA). Synapse 35:160–162

Hubner CB, Bird M, Rasnick S, Kornetsky C (1988) The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. Psychopharmacology 95:49–51

Iravani MM, Asari D, Patel J, Wieczorek WJ, Kruk ZL (2000) Direct effects of 3,4-methylenedioxymethamphetamine (MDMA) on serotonin or dopamine release and uptake in the caudate putamen, nucleus accumbens, substantia nigra pars reticulate, and the dorsal raphe nucleus slices. Synapse 36:275–285

Lamb RJ, Griffiths RR (1987) Self-injection of D,1–3,4-Methylenedioxymethamphetamine (MDMA) in the baboon. Psychopharmacology 91:268–272

Lin HQ, Jackson DM, Atrens DM, Christie MJ, McGregor IS (1997) Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. Brain Res 744:351–357

Lin HQ, Burden PM, Christie MJ, Johnston GAR (1999) The anxiogenic-like and anxiolytic-like effects of MDMA on mice in the elevated plus-maze: a comparison with amphetamine. Pharmacol Biochem Behav 62:403–408

Malberg JE, Seiden LS (1998) Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. J Neurosci 18:5086–5094

Maldonado E, Navarro JF (2000) Effects of 3,4-methylenedioxymethamphetamine (MDMA) on anxiety in mice tested in the light-dark box. Prog Neuropsychopharmacol Biol Psychiatry 24:463–472

Maldonado E, Navarro JF (2001) MDMA ("ecstasy") exhibits an anxiogenic-like activity in social encounters between male mice. Pharmacol Res 44:27–31

Mamounas LA, Mullen CA, O'Hearn E, Molliver ME (1991) Dual serotoninergic projections to forebrain in the rat – morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J Comp Neurol 314:558–586

MaronaLewicka D, Rhee GS, Sprague JE, Nichols DE (1996) Reinforcing effects of certain serotonin-releasing amphetamine derivatives. Pharmacol Biochem Behav 53:99–105

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

McCann UD, Ricaurte GA (1991) Lasting neuropsychiatric sequelae of (±)methylenedioxymethamphetamine (Ecstasy) in recreational users. J Clin Psychopharmacol 11:302–305

McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA (1998) Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet 352:1433–1437

McCann UD, Eligulashvili V, Mertl M, Murphy DL, Ricaurte GA (1999) Altered neuroendocrine and behavioral responses to m-chlorophenylpiperazine in 3,4-methylenedioxymethamphetamine (MDMA) users. Psychopharmacology 147:56–65

Miller TR, Lau SS, Monks TJ (1997) 2,5-bis-(glutathion-S-yl)-alpha-methyldopamine, a putative metabolite of (±)-3,4-methlenedioxyamphetamine, decreases brain serotonin concentrations. Eur J Pharmacol 323:173–180

Morgan MJ (1999) Memory deficits associated with recreational use of "ecstasy" (MDMA). Psychopharmacology 141:30–36

Morgan MJ (2000) Ecstasy (MDMA): a review of its possible persistent psychological effects. Psychopharmacology 152:230–248

Morley KC, McGregor IS (2000) (±)-3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") increases social interaction in rats. Eur J Pharmacol 408:41–49

O'Callaghan JP, Miller DB (1994) Neurotoxicity profiles of substituted amphetamines in the C57bl/6j mouse. J Pharmacol Exp Ther 270:741–751

O'Hearn E, Battaglia G, De SAouza EB, Kuhar MJ, Molliver ME (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 8:2788–2803

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

Parrott AC, Lees A, Garnham NJ, Jones M, Wesnes K (1998) Cognitive performance in recreational users of MDMA or "ecstasy": evidence for memory deficits. J Psychopharmacol 12:79–83

Peroutka SJ, Newman H, Harris H (1988) Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. Neuropsychopharmacology 1:273–277

Poland RE (1990) Diminished corticotropin and enhanced prolactin responses to 8-hydroxy-2(di-N-propylamino)tetralin in methylenedioxymethamphetamine pretreated rats. Neuropharmacology 29:1099–1101

Ricaurte GA, Martello AL, Katz JL, Martello MB (1992) Lasting effects of (±)-3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in nonhuman-primates – neurochemical observations. J Pharmacol Exp Ther 261:616–622

Schechter MD (1991) Effect of MDMA neurotoxicity upon its conditioned place preference and discrimination. Pharmacol Biochem Behav 38:539–544

Schmidt CJ, Taylor VL (1987) Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem Pharmacol 36: 4095–4102

Semple DM, Ebmeier KP, Glabus MF, O'Carroll RE, Johnstone EC (1999) Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ("ecstasy") users. Br J Psychiatry 175:63–69

Shankaran M, Gudelsky GA (1998) Effect of 3,4-methylenedioxymethamphetamine (MDMA) on hippocampal dopamine and serotonin. Pharmacol Biochem Behav 61:361–366

Slikker W, Holson RR, Ali SF, Kolta MG, Paule MG, Scallet AC, McMillan DE, Bailey JR, Hong JS, Scalzo FM (1989) Behavioral and neurochemical effects of orally-administered MDMA in the rodent and non human primate. Neurotoxicology 10:529–542

Spanos LJ, Yamamoto BK (1989) Acute and subchronic effects of methylenedioxymethamphetamine [(±) MDMA] on locomotion and serotonin syndrome behavior in the rat. Pharmacol Biochem Behav 32:835–840

Stone DM, Hanson GR, Gibb JW (1986) Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. Neuropharmacology 26:1657–1661