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ORIGINAL INVESTIGATION

High-dose MDMA does not result in long-term changes in impulsivity in the rat

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Abstract
Rationale Evidence suggests that recreational users of (±) 3,4-methylenedioxymethamphetamine HCl (MDMA, "ecstasy") have cognitive and behavioral deficits and show increased impulsivity consistent with 5-hydroxytryptamine (5-HT) neurotoxicity. MDMA effects on impulsivity in users are difficult to establish being confounded by polydrug use and individual predisposition to impulsivity or behavioral inhibition.
Objective We previously observed a long-term anxiolytic effect of a neurotoxic dose of MDMA on elevated plus maze behavior in Dark Agouti (DA) rats while other strains were reported to show anxiogenesis. We have now examined whether MDMA influences impulsivity producing disinhibited behavior interpretable as anxiolysis.
Methods Impulsivity was measured using an operant visuospatial discrimination procedure. Male DA rats (n=24) were trained to lever press for food reward in response to a light-stimulus and subsequently required to withhold responding. Correct responses, premature responses, and response latencies were used as measures of accuracy and impulsivity. Trained rats were administered MDMA (5 mg/kg, i.p. at 3-h intervals to a total of three injections). Performance was measured at 3 h and 7, 27, 49, and 80 days posttreatment.
Results There was a short-term effect of MDMA on the percentage of correct responses at 3 h and day 1 with recovery to control levels by days 7–8 and no significant long-term changes up to day 80. There was no effect of MDMA on premature responses on any of the days measured. MDMA reduced cortical 5-HT content (MDMA 363±14 ng/g and control 440±10 ng/g tissue).
Conclusion These results suggest that impulsivity may not be directly altered by MDMA despite serotonergic neurotoxicity.

Keywords Serotonin · Impulsivity · MDMA ("ecstasy") · Vigilance · Dark Agouti

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Introduction

(±)3,4-Methylenedioxymethamphetamine HCl (MDMA, "ecstasy") is a recreational drug widely used by young people in Europe and the USA. There is considerable evidence from both animal and human studies that MDMA is a selective neurotoxin at 5-hydroxytryptamine (5-HT) nerve terminals in most species, including the rat (see Green et al. 2003 for review). In contrast, it is the dopaminergic system that is damaged in the mouse brain (see Colado et al. 2004).

MDMA causes an acute rapid release of 5-HT and dopamine from nerve terminals and while this release produces little change in the tissue content of dopamine (see Colado et al. 2004), there is a major depletion in the tissue concentration of 5-HT in the forebrain, which lasts

for several hours (Schmidt and Taylor 1987). The cerebral 5-HT concentration returns toward normal by 24 h (Schmidt and Taylor 1987), but there then follows a sustained depletion of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), which reflects neurotoxic damage that lasts for several months in rats (Battaglia et al. 1988).

As the serotonergic system underpins a wide variety of psychological and cognitive functions, such as effects on behavioral inhibition, learning, memory, and attention, it is of particular importance to assess whether MDMA has long-term effects on these functions.

There is increasing evidence that users of MDMA have long-term changes in impulsivity (Rodgers et al. 2001; Morgan 1998; Thomasius et al. 2003; Butler and Montgomery 2004; Hanson and Luciana 2004; Halpern et al. 2004), which is consistent with long-term damage to 5-HT neurons because 5-HT is suggested to play a role in impulsivity. In animal studies, a 5,7-dihydroxytryptamine-induced lesion of the dorsal-raphé reduces tolerance to delay of reward (Bizot et al. 1999) and a similar effect is seen after administration of the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA) (Denk et al. 2005). A precise role of MDMA on higher order functions such as impulsivity or cognition is, however, difficult to establish unequivocally from human studies as they are confounded by polydrug use and variable psychologically and genetically mediated predispositions to impulsivity and behavioral inhibition. Consequently, animal studies are a necessary approach to reduce these experimental confounds.

Impulsivity has been characterized as a multidimensional construct (Gerbing et al. 1987; Malle and Neubauer 1991; Moeller et al. 2001). Impulsivity has been suggested to include orientation, diminished ability to delay gratification, behavioral disinhibition, risk taking, sensation seeking, boredom proneness, reward sensitivity, lack of perseverance, and poor planning (Petry 2001; Semple et al. 2005). Impulsivity is therefore a complex psychological term and the ways in which it is measured in human studies reflect the heterogeneity of the construct, including self-report measures such as retrospective questionnaires, behavioral measures such as reaction times or event-related potential (ERP) correlates of inhibition.

In general, substance abusers have higher levels of impulsivity compared to nonabusers (Sher et al. 2000; Sher and Trull 1994). Morgan (1998) showed increased impulsivity in MDMA users measured both as self-report and behavioral measures. Schifano et al. (1998) suggested that 14% of MDMA users meet the *Diagnostic and Statistical Manual of Mental Disorders* III criteria for impulse and control disorder, however, Gamma et al. (2005) did not find any differences in the P3 ERP correlate of inhibition, implying no changes in impulsivity.

We have previously shown an anxiolytic effect of MDMA treatment on elevated plus maze behavior in Dark Agouti (DA) rats (Mechan et al. 2002a), which contrasts with studies showing an anxiogenic effect of MDMA using social interaction measures in other strains of rats such as Wistar (McGregor et al. 2003). One possible explanation of this discrepancy is that MDMA may be differentially influencing impulsivity to produce disinhibited behavior interpretable as anxiolysis in the elevated plus maze task. Disinhibited behavior consequent to MDMA would be consistent with the number of reports in human studies (e.g., Halpern et al. 2004), indicating changes in impulsivity after MDMA.

In this study, we addressed two questions. First, whether a known neurotoxic dose regimen of MDMA induces long-term changes in behavioral measures of relevance to impulsivity. Second, whether such potential changes mirror the pattern of long-term but not short-term effects we have previously established in the elevated plus maze test of anxiety. We measured behavioral inhibition aspects of impulsivity using an operant visuospatial discrimination procedure in which rats were trained to withhold responding to a light stimulus for food reward.

Materials and methods

Animals and drug administration

Male DA rats (Harlan UK, Bicester, Oxon, UK) were used weighing 175–200 g at the start of the experiment. They were housed in groups of three at an ambient temperature of 21±2°C and a 12-h light/dark cycle (lights on at 07 h 00 min). Food (Dietex International, Witham, Essex, UK) was freely available for the first 10 days and then the animals were placed on a food restriction regimen. After day 10 and until the end of the experiment the animals were fed for 2 h a day at 14 h 00 min. Water was freely available.

MDMA was obtained from Ultrafine Chemicals, Manchester, UK, dissolved in 0.9% w/v saline, and injected intraperitoneally at a volume of 1 ml/kg. To induce neurotoxicity of 5-HT in the brain, MDMA (5 mg/kg, i.p.) was given at 3-h intervals to a total of three injections during 1 day. This dose regime was chosen as it produces approximately 30% depletion of 5-HT in the cortex and hippocampus (Green et al. 2005) and which may have relevance to the damage produced in the brain of heavy recreational users of MDMA (see "Discussion"). A similar change follows this dose given in a single administration (O'Shea et al. 1998), but the divided dose regime was chosen to mimic binge-dosing, which has recently become more popular (Weir 2000; Parrott 2002). In addition, a recent study demonstrated that the loss of 5-HT in both cortex and

hippocampus is detectable for at least 32 weeks (226 days) after a similar dose of MDMA (O'Shea et al. 2006). To produce a greater neurotoxic loss of 5-HT, sustained multiple dosing of MDMA is required, which does not reflect the human recreational ingestion regimes. Higher acute dosing to this strain of rats also tends to produce mortality.

All procedures were carried out after approval by the University Experimental Ethics Committee and in accordance with the United Kingdom Home Office Regulations, which ensure humane and proper care of research animals.

Measurement of 5-HT in cerebral tissue after a neurotoxic dose of MDMA

The rats in the main study were killed on day 82, 24 h after the final behavioral evaluation. Further groups of animals not subjected to behavioral examination were killed 30 min, 24 h, and 7 days after the final three doses of MDMA to examine the profile of 5-HT loss in the frontal cortex of animals administered the same dose schedule of MDMA. Rats were killed by cervical dislocation and decapitation, the brains rapidly removed, and cortex dissected out on ice. The dissected tissue was snap frozen using supercooled isopentane (a beaker surrounded by dry ice) and stored at −80°C until analysis when it was homogenized and 5-HT was measured by high-performance liquid chromatography (HPLC) with electrochemical detection. The method used was based on that published in detail by Colado et al. (2004). Briefly, the mobile phase, which consisted of KH_2PO_4 (1.0 M), octanesulfonic acid (0.25 mM), EDTA (0.1 mM), and methanol (10%), was adjusted to pH 3 with phosphoric acid, filtered, and degassed. The flow rate was 1.2 ml/min and the working electrode potential was set at +0.8 V. The HPLC system consisted of a pump (Pharmacia LKB 2150) linked to a stainless steel reversed-phase column (C18 Phenomenex Lichrosher Select B, 5 μm, 150×4.6 mm) with a precolumn and an LC-4C amperometric detector (Bioanalytical Systems, Congleton, Cheshire, UK). The current produced was monitored by using integration software (SP4400 Chromjet with Nelson chromatography software).

Behavioral methods

Behavioral experiments were conducted in six operant conditioning chambers (Med Associates, VT, USA, ENV-007) with internal dimensions of 20×20×20 cm. The chambers were connected to a MED-PC interface system that controlled the experimental events and recorded the data. Ventilation was provided by a fan on the wall of the box and light was provided by a main overhead house light and each chamber contained a grid floor. Light stimuli were two stimulus lights (48 V) above two retractable levers on either sides of a food hopper. All programs were written in MedState notation language. Food reinforcement was provided by Noyes Precision Pellets (45 mg) (Sandown Scientific, Middlesex, UK).

Animals were trained 5 days/week. "Days" in the following protocol refers to experimental day. The animals were trained as follows:

Day 1: acclimatized to the conditioning chamber for one 30-min session (levers withdrawn, house light on).

Days 2–5: habituated to delivery of food pellets on a fixed-interval, 1-min schedule (levers withdrawn, house light on) for one daily 30-min session.

Days 6–11: trained to lever press with both levers extended, using combined fixed ratio 1 schedule and successive approximation controlled by the experimenter. Rats received one daily 30-min session or until at least 100 lever presses.

Days 12–25: trained to press individual lever signaled by a light stimulus. A single lever extended simultaneously with onset of light stimulus located over the lever, when the lever was pressed the light stimulus turned off, the lever retracted, and food was delivered. The order of left or right lever presentation was randomized between but not within days. Rats received one daily 30-min session or until at least 100 lever presses.

Days 25–51: trained to press individual left or right lever signaled by light stimulus. A single lever was presented simultaneously with onset of light above the lever. The order of left–right lever presentations was pseudorandomized such that no more than three successive presentations of the lever were on any one side. The lever remained extended until a lever press occurred then the light turned off, the lever retracted, and food pellet was delivered. If no lever was pressed after 30 s, the lever would retract and light would turn off and the house light would extinguish for a 5-s timeout period. The intertrial interval (ITI) was 10 s and the session duration was 30 min or until 70 higher lever presses.

Days 51–116: trained to press one of the two levers presented, signaled by simultaneous light stimulus above lever. Both levers were presented, one of which had a light stimulus on over the lever. Pressing the lever corresponding to the light stimulus (CORRECT RESPONSE) resulted in re-

traction of both levers, stimulus light turning off, and food reward. Pressing the lever without a corresponding stimulus light (INCORRECT RESPONSE) resulted in retraction of both levers, no food delivery, and turning off of the house light until the next trial. Rats continued training until they performed 70% correct responses on three consecutive days.

Days 116–226: trained to press one of two levers signaled by delayed presentation of a light stimulus above lever. Both levers were presented. After a delay of 2 s a light stimulus was presented above one of the levers. Pressing the lever corresponding to the light stimulus (CORRECT RESPONSE) resulted in retraction of both levers, stimulus light turning off, and food reward followed by a 10-s ITI. Pressing the lever without a corresponding stimulus light (INCORRECT RESPONSE) resulted in retraction of both levers, no food delivery, and turning off of the house light and a 20-s timeout followed by the 10 s ITI until the next trial. Pressing a lever before the onset of a light stimulus (PREMATURE RESPONSE) resulted in retraction of both levers, turning off of house light, and 20-s timeout followed by 10 s ITI; no food was delivered. If no lever was pressed (OMISSION) after 30 s the lever would retract and light would turn off and the house light would extinguish for a 5-s timeout period and a 10 s ITI.

When rats were performing consistently (over three consecutive days) at 70% correct responses, they were trained on the same procedure but with a 5-s delay between lever presentation and onset of light stimulus. Rats continued training until they performed 70% correct responses on three consecutive days. If no lever was pressed after 30 s the lever would retract, the light would turn off, and the house light would extinguish for a 5-s timeout period.

Behavioral measures were thus: correct response, incorrect response, omission, and premature response calculated as percentages of total numbers of trials completed. In addition, mean response latencies (RL) in seconds was also measured for each premature and correct response.

Once trained according to criterion, rats were allocated into two experimental groups matched for their percentage of correct performance. Rats were injected with either saline or MDMA and tested using the 5-s delay paradigm at 3 h after injection, and then on days 1, 7, and 8; 27 and 28; 49 and 50; and 80 and 81. Between the test sessions, free access to food in the home cage was restored and rats resumed food restriction 7 days before testing. With the exception of the 3-h and day 1 tests, for the purposes of analysis, time points noted above were collapsed into blocks of two sessions, the mean of the 2 days sessions representing one time point. This was to account for any practice effect that may have occurred because no behavioral testing was carried out between test days. This design was implemented as an a priori precaution against any overtraining that may be associated with such a long training procedure. Thus, time points represented are predrug (3 days testing before injection), day 0 (3 h after injection), day 1 (the day after injection), day 7 (mean days 7 and 8), day 27 (mean days 27 and 28), day 49 (mean days 49 and 50), and day 80 (mean days 80 and 81).

The mean (SD) number of trials completed by groups at each time point were:

Day	MDMA group Mean (SD)	Saline group Mean (SD)
Predrug	97.6 (4.8)	99.9 (0.2)
0	51.0 (10.2)	77.3 (20.4)
1	77.3 (20.4)	97 (9.2)
7	93.4 (9.2)	98.7 (3.0)
27	94.9 (10.1)	97.6 (4.3)
49	93.1 (9.2)	95.6 (6.7)
80	95.6 (6.7)	99.0 (3.9)

Statistics

Statistics were carried out using SPSS (version 12.0). Within or between subjects analyses of variance (ANOVA) or analyses of covariance were carried out as appropriate with days (predrug, 0, 1, 7, 28, 50, and 81), drug (saline and MDMA), or lever (left and right) as factors. Post hoc *t* tests after significant ANOVA were carried out as appropriate with Bonferroni alpha correction for multiple comparisons. Correlations were carried out using the nonparametric Spearman's rank.

Results

Percentage of correct measure

Within subjects ANOVA indicated a significant effect of day $F(6, 21)=65.6$, $p<0.0001$ on percentage of correct responses and a significant drug × day interaction $F(6, 21)=$

15.5, $p<0.001$. There was a significant difference between subjects on the effect of drug $F(1, 21=14.128, p<0.001)$. Graphical results (Fig. 1a) indicated that this difference was localized to day 0 and day 1, which was confirmed by post hoc t tests between saline and MDMA groups with Bonferroni correction: predrug $T(21)=-0.07$, N.S.; day 0 $T(21)=-3.66$, $p<0.05$; and day 1 $T(21)=-5.238$, $p<0.001$. None of the other days showed significant differences: day 7 $T(21)=-2.44$, day 27 $T(21)=-1.4$, day 49 $T(21)=0.02$, and day 80 $T(21)=0.24$.

Percentage of premature responses

Within subjects ANOVA indicated a significant effect of day [$F(6, 21)=18.19$, $p<0.0001$] on percentage of premature responses and a significant drug × day interaction [$F(6, 21)=2.52$, $p<0.05$]. The between subjects effect of drug [$F(1, 21=3.96$, $p=0.06)$] just failed to reach significance. Figure 1b indicates differences between means on this measure on day 0 and day 1. Post hoc t tests did not show a significant difference between groups at any time point when Bonferroni correction for alpha slippage was applied: predrug $T(21)=-2.1$, day 0 $T(21)=-2.7$, day 1 $T(21)=-2.2$, day 7 $T(21)=-0.34$, day 27 $T(21)=-1.4$, day 49 $T(21)=-1.5$, and day 80 $T(21)=-0.71$.

Percentage of omissions

Within subjects ANOVA indicated a significant effect of day $F(6, 21)=48.67$ $p<0.0001$ on percentage of omissions and a significant drug × day interaction $F(6, 21)=13.73$, $p<0.001$. There was a significant difference between subjects on the effect of drug $F(1,21=17.46$, $p<0.001)$. Figure 1c indicates that this difference is localized to days 0 and 1, which is confirmed by post hoc t tests between saline and MDMA groups with Bonferroni correction: predrug $T(21)=2.2$, N.S.; day 0 $T(21)=3.86$, $p<0.01$; and day 1 $T(21)=5.09$, $p<0.001$. None of the other days showed significant differences: day 7 $T(21)=-1.54$, day 27 $T(21)=0.54$, day 49 $T(21)=0.57$, and day 80 $T(21)=0.01$.

Bias

To assess for potential bias effects either in training or as a consequence of drug treatment, we examined whether there was significant difference in left or right lever responses for percentage of correct data on any day between saline and drug groups. There were no significant differences on any day between any group, indicating that bias toward responding on any one lever was not a factor in the pattern of results reported.

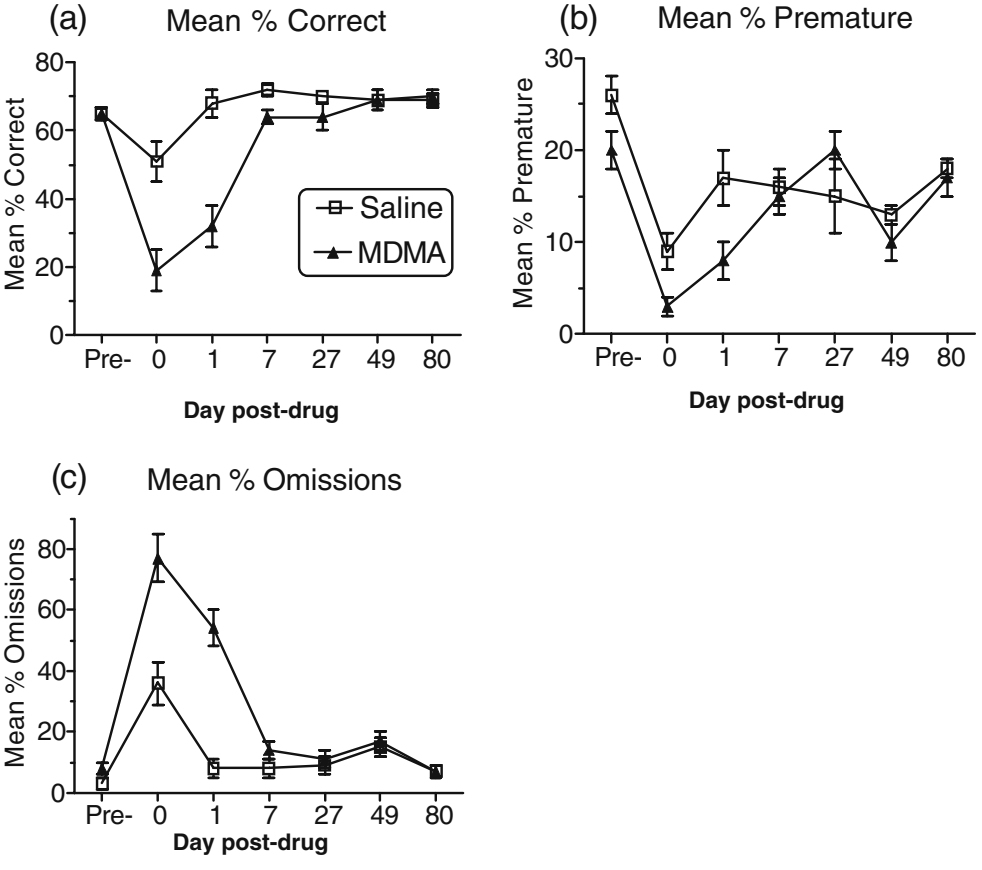

Fig. 1 **a** Mean percentage of correct trials (±SEM) at each of the indicated time points after drug administration for MDMA- and saline-treated groups. **b** Mean percentage of premature trials (±SEM) at each of the indicated time points after drug administration for MDMA- and saline-treated groups. **c** Mean percentage of correct trials (±SEM) at each of the indicated time points after drug administration for MDMA- and saline-treated groups

Correlations between percentage of correct responses and percentage of omissions

There were significant correlations between percentage of correct responses and percentage of omissions at all of the time points measure with the exception of predrug (r=−0.321, N.S.): day 0 r=−0.978, p<0.0001; day 1 r=−0.875, p<0.001; day 7 r=−0.69, p<0.001; day 27 r=−0.541, p<0.01; day 49 r=−0.773, p<0.0001; and day 80 r=−0.4, p<0.05.

Response latencies of premature and correct responses

RL were calculated for correct responses and premature responses. The RL for premature responses were faster in the MDMA groups at early time points and slower for correct responses (Fig. 2). There was a significant effect of drug on RL for premature responses on days 0 [F(1, 112)=10.49, p<0.002] and 1 [F(1, 260)=16.16, p<0.0001] only (see Fig. 2). There was also a significant effect of drug on RL for correct responses on predrug [F(1, 4,404)=38.6, p<0.0001], day 0 [F(1, 642)=49.55, p<0.0001], day 1 [F(1, 1,054)=146.82, p<0.0001], and day 49 [F(1, 3,032)=16.71, p<0.0001].

To assess potential subtle effects on vigilance, we also analyzed within session increases in RL and decreases in percentage of correct responses, comparing RL and percentage of correct in first ten trials of a day's session with the last ten trials of a day's session. Within session, RL increases for the predrug period mean (SD) RL T1–T10 predrug [7.6 (2.6)] and RL of the last ten trials [11.44 (7.7)], which are significantly different: T(22)=18.55 and p<0.0001. Similarly, mean percentage of correct responses decrease within the session. Mean (SD) percentage of correct responses for the predrug period T1–T10 is 73.4 (17.9) and mean (SD) percentage of correct responses for the last ten trials is 45 (12.67), which are significantly different [T(22)=5.43, p<0.0001]. Using ANOVA, there were no significant drug × trial block (two levels: first 10 trials/last 10 trials) interactions seen on any day for within session increase in RL on correct responses or percentage of correct responses on day 7 or later (data not shown).

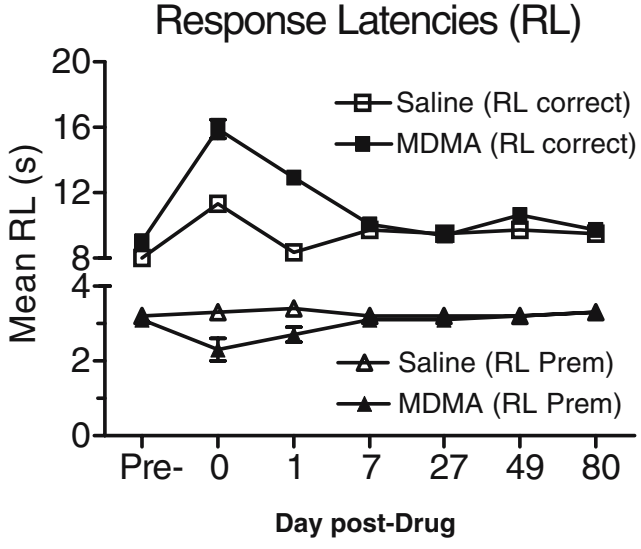

Fig. 2 Mean *RL* in seconds±SEM for correct responses only and for premature responses only at each of the indicated time points after drug administration for MDMA- and saline-treated groups

Biochemistry

In the cohort of animals used for the behavioral experiments, there was a significant decrease in cortical 5-HT of 18% (Fig. 3) and 5-HIAA (43%) at day 82. In cohorts of rats killed at specific time points after MDMA administration, but without behavioral testing, there were also significant decreases in 5-HT with an 80% decrease 30 min after the final MDMA injection and an approximate 30% decrease at 24 h and 7 days later (Fig. 3).

Discussion

These data demonstrate that there are no long-term behavioral effects of MDMA treatment either on measures of impulsivity (premature responses or RL) or vigilance (correct responses or RL) in this lever-pressing task in the DA rat.

MDMA treatment significantly decreased the percentage of correct responses but these are largely confined to 3 h (day 0) and day 1 after drug treatment, suggesting that these effects are associated with acute effects of the drug rather than any longer term effect (Fig. 1a,b). A similar though

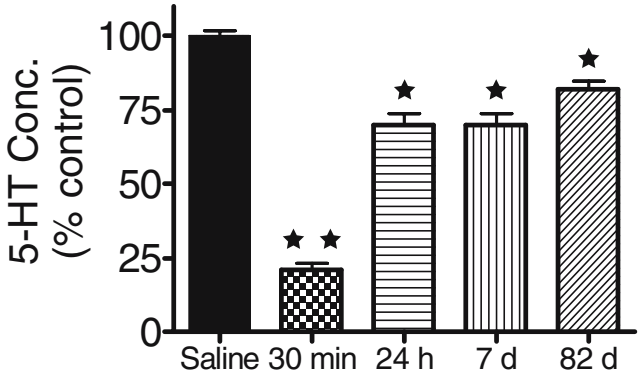

Fig. 3 The percentage of 5-HT in the frontal cortex at various times after MDMA (5 mg/kg given at 3-h intervals to a total of three doses) compared to an appropriate saline-injected control group. Different from control (saline-injected) group: **p<0.01 and *p<0.05, calculated using the absolute concentration data. Control group for the 82 days study was 440±10 ng/g tissue and for the cohort short-term groups 487±12 ng/g tissue (30-min and 24-h data) and 416±16 ng/g tissue (7 days data)

nonsignificant trend was seen for the percentage of premature responses with control performance also decreasing; this is likely to reflect the lower number of response opportunities as reflected in the data on percentage of omissions. This is clearly seen to recover by day 7. The mean percentage of omissions also follows this temporal pattern with increases on day 0 and day 1 and recovery to control levels by day 7 (Fig. 1c). It is difficult to disentangle these acute/early effects of the drug from a general behavioral disruption, manifested as a large number of trials where animals did not respond on day 0 and day 1 after drug treatment, and confirmed by significant negative correlations between percentage of correct and omission scores. However, it is clear these parameters have recovered to control levels by day 7.

For premature responses, we demonstrated a significant decrease in RL to make a premature response on days 0 and 1 in MDMA-treated rats, which may be consistent with an early/acute increase in inappropriate responsivity; however, this decrease recovers completely by day 7 and is not accompanied by an increase in the *number* of premature responses. There were significant increases in RL on correct responses in MDMA-treated animals; these increases are similarly confined to day 0 and day 1, clearly recovering to control values by day 7. These increased RL for correct responses also map directly onto to the pattern of increased percentage of correct responses on days 0 and 7 after MDMA treatment. Increased reaction times to simple visual stimuli were reported in former MDMA users challenged acutely with dexfenfluramine (Verkes et al. 2001), but it is unknown whether long-term recovery occurs.

We observed a significant (approximately 20%) depletion of 5-HT in the frontal cortex in rats treated 82 days earlier with a high dose of MDMA, consistent with the known neurotoxic action of the drug as was extensively reported previously (see "Materials and methods"). The 5-HT concentration measured in cohort groups killed after 30 min, 1 day, and 7 days postdrug treatment confirmed that a rapid and major (about 80%) depletion of cortical 5-HT occurs rapidly after MDMA administration, which is primarily due to 5-HT release from the terminals (Green et al. 2003). There is then some recovery of stores by 24 h followed by a long-term neurotoxic loss of 5-HT as seen at 7 and 80 days. These data confirm another recent study on the time course of 5-HT loss in the cortex after MDMA (O'Shea et al. 2006). The frontal cortex was chosen as an indicator of the 5-HT loss after MDMA because previous studies suggest that similar changes can be observed in most regions of the forebrain, with the exception of the striatum where the neurotoxic loss may only occur for about a month (Green et al. 2003; O'Shea et al. 2006).

A 5-HT loss of this magnitude may have clinical relevance as a comparable loss in 5-HT markers was reported to occur in the human brain after heavy recreational use of MDMA (McCann et al. 1998). However, these data are controversial and opinion remains divided as to whether a long-term loss of 5-HT does occur in the brain of human recreational users of MDMA (Green et al. 2003). If such loss does occur, our results suggest (assuming the validity of the rat model) that this loss is unlikely to lead to a long-term change in impulsivity.

While our impulsivity results in the rats concur with some human studies (Travers and Lyvers 2005; McCann et al. 1994), there are other investigations that do find a change in MDMA users. Butler and Montgomery (2004), Morgan (1998), and Parrott et al. (2000) have all reported higher impulsiveness and/or venturesomeness in drug users than nondrug using controls, an effect also seen in polydrug users without history of MDMA use. Schifano et al. (1998), using a different approach, also suggested evidence of impulsivity among ecstasy users attending an addiction treatment unit with 14% being diagnosed with impulse control disorders. Human studies are necessarily confounded by their retrospective nature, making preexisting impulsive personality traits difficult to isolate from drug-induced effects. There is also an inability to control dose or timing of dose or the purity of compounds bought as "ecstasy." In the present study using a known dose of pure MDMA, and having confirmed significant depletion of cortical 5-HT, we found no evidence for an effect of MDMA on impulsivity. Some authors have suggested that findings of elevated impulsivity may predate drug use (Butler and Montgomery 2004) and the present study might indirectly support such an idea.

Impulsivity is a multidimensional construct, and even in animal paradigms different dimensions are addressed by different behavioral paradigms such as tolerance to delayed gratification or reward (Bizot et al. 1999), cost–benefit decision about delay or effort measured in T-maze paradigms (Denk et al. 2005), or the inability to inhibit inappropriate responses measured in the five-choice task (Chudasama et al. 2003). The present paradigm most closely addressed the behavioral disinhibition aspect where animals were trained to withhold inappropriate responding, i.e., a lever press until a light presentation signaling the reinforced side. While we clearly do not see a long-term effect of MDMA on this behavior, it is possible that MDMA might have selective effects on other aspects of impulsivity such as the delayed gratification aspects of impulsivity. Denk et al. (2005) suggested that depletion of 5-HT, effected by the synthesis inhibitor PCPA, has selective effects on the ability to tolerate delay rather than increased effort to obtain a larger reward. It may be possible that MDMA effects on impulsivity are seen only in tasks that include this specific measure. Another implication of the present findings is that the ability to tolerate delay per

se is unaffected by long-term loss of 5-HT of the magnitude found in this study.

It is clear that long-term increases in impulsivity after MDMA cannot be proposed as a mediating behavior in the anxiolytic effects we have previously described. We showed anxiolytic effects at later (day 80) but not earlier (day 7) time points after MDMA administration (Mechan et al. 2002a). Clearly, some other explanation for this pattern of effects is required such as dissociable anxiolytic/anxiogenic effects being dependent upon strain-dependent baseline anxiety levels (Mechan et al. 2002b) and there is some support for this notion (Green and McGregor 2002).

We found some evidence for a small early effect of MDMA on the percentage of correct measure of vigilance. The MDMA-treated group showed some evidence of reduced percentage correct scores on day 7, despite no significant difference between groups on percentage of omissions or premature responses; however, this reduction was not statistically significant using Bonferroni correction. A transient acute effect of MDMA on vigilance might be consistent with studies showing behavioral abnormalities interpreted as attentional changes in animals (Piper et al. 2005), though others have indicated no changes in vigilance defined as changes in sleep/wake cycle patterns (Balogh et al. 2004). More direct tests of this possibility are required. Studies in humans do suggest changes in attentional aspects of cognitive task batteries though this may be limited to complex attention (Gouzoulis-Mayfrank et al. 2000; Yip and Lee 2005). Clearly, further studies using more complex dedicated measures of vigilance are required before a firm conclusion can be drawn with respect to MDMA effects on vigilance.

In summary, we found no evidence for long-term changes in impulsivity after MDMA despite a significant long-term decrease in cortical 5-HT and 5-HIAA. This also indicates that increased impulsivity is not a behavioral mediator of previously demonstrated long-term anxiolytic effects of single dose MDMA in the elevated plus maze in DA rats.

References

Balogh B, Molnar E, Jakus R, Olverman HJ, Kelly PAT, Kantor S, Bagdy G (2004) Effects of a single dose of 3,4-methylenedioxymethamphetamine on circadian patterns, motor activity and sleep in drug-naive rats and rats previously exposed to MDMA. Psychopharmacology 173:296–309

Battaglia G, Brooks BP, Kulsakdinum C, Desouza EB (1988) Pharmacologic profile of MDMA (3,4-Methylenedioxymethamphetamine) at various brain recognition sites. Eur J Pharmacol 149(1–2):159–163

Bizot JC, Le Bihan C, Puech AJ, Hamon M, Thiebot MH (1999) Serotonin and tolerance to delay of reward in rats. Psychopharmacology 146:400–412

Butler GKL, Montgomery AMJ (2004) Impulsivity, risk taking and recreational 'ecstasy' (MDMA) use. Drug Alcohol Depend 76:55–62

Chudasama Y, Passetti F, Rhodes SEV, Lopian D, Desai A, Robbins TW (2003) Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. Behav Brain Res 146:105–119

Colado MI, O'Shea E, Green AR (2004) Acute and long-term effects of MDMA on cerebral dopamine biochemistry and function. Psychopharmacology 173:249–263

Denk F, Walton ME, Jennings KA, Sharp T, Rushworth MFS, Bannerman DM (2005) Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. Psychopharmacology 179:587–596

Gamma A, Brandeis D, Brandeis R, Vollenweider FX (2005) The P3 in 'ecstasy' polydrug users during response inhibition and execution. J Psychopharmacol 19:504–512

Gerbing DW, Ahadi SA, Patton JH (1987) Toward a conceptualization of impulsivity—components across the behavioral and self-report domains. Multivariate Behav Res 22:357–379

Gouzoulis-Mayfrank E, Daumann J, Tuchtenhagen F, Pelz S, Becker S, Kunert HJ, Fimm B, Sass H (2000) Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). J Neurol Neurosurg Psychiatry 68:719–725

Green AR, McGregor IS (2002) On the anxiogenic and anxiolytic nature of long-term cerebral 5-HT depletion following MDMA. Psychopharmacology 164:448–450

Green AR, Mechan AO, Elliott JM, O'Shea E, Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol Rev 55: 463–508

Green AR, O'Shea E, Saadat KS, Elliott JM, Colado MI (2005) Studies on the effect of MDMA ('ecstasy') on the body temperature of rats housed at different ambient room temperatures Br J Pharmacol 146:306–312

Halpern JH, Pope HG, Sherwood AR, Barry S, Hudson JI, Yurgelun-Todd D (2004) Residual neuropsychological effects of illicit 3,4-methylenedioxymethamphetamine (MDMA) in individuals with minimal exposure to other drugs. Drug Alcohol Depend 75: 135–147

Hanson KL, Luciana M (2004) Neurocognitive function in users of MDMA: the importance of clinically significant patterns of use. Psychol Med 34:229–246

Malle BF, Neubauer AC (1991) Impulsivity, reflection, and questionnaire response latencies-no evidence for a broad impulsivity trait. Pers Individ Differ 12:865–871

McCann UD, Ridenour A, Shaham Y, Ricaurte GA (1994) Serotonin neurotoxicity after (±) 3,4-methylenedioxymethamphetamine (MDMA, ecstasy)—a controlled study in humans. Neuropsychopharmacology 10:129–138

McCann UD, Szabo A, Scheffel U, Dannals RF, Ricaurte GA (1998) Neurotoxic effects in human brains after use of 3,4-methylenedioxymethamphetamine ("ecstasy") viewed by positron emission tomography. Lancet 352:1433–1437

McGregor IS, Clemens KJ, Van der Plasse G, Li KM, Hunt GE, Chen F, Lawrence AJ (2003) Increased anxiety 3 months after brief exposure to MDMA ('ecstasy') in rats: association with altered 5-HT transporter and receptor density. Neuropsychopharmacology 28:1472–1484

Mechan AO, Moran PM, Elliott JM, Young AMJ, Joseph MH, Green AR (2002a) 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. Psychopharmacology 159:167–175

Mechan AO, Moran PM, Elliott JM, Young AMJ, Joseph MH, Green AR (2002b) A comparison between Dark Agouti and Sprague-Dawley rats in their behaviour on the elevated plus maze, open field apparatus and activity meters, and their response to diazepam. Psychopharmacology 159:188–195

Moeller FG, Dougherty DM, Barratt ES, Schmitz JM, Swann AC, Grabowski J (2001) The impact of impulsivity on cocaine use and retention in treatment. J Subst Abuse Treat 21:193–198

Morgan MJ (1998) Recreational use of "ecstasy" (MDMA) is associated with elevated impulsivity. Neuropsychopharmacology 19:252–264

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

O'Shea E, Orio L, Escobedo I, Sanchez V, Camarero J, Green AR, Colado MI (2006) MDMA-induced neurotoxicity: long-term effects on 5-HT biosynthesis and the influence of ambient temperature Br J Pharmacol DOI 10.1038/sj.bjp.0706783

Parrott AC (2002) Recreational ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. Pharmacol Biochem Behav 71:837–844

Parrott AC, Sisk E, Turner JJD (2000) Psychobiological problems in heavy 'ecstasy' (MDMA) polydrug users. Drug Alcohol Depend 60:105–110

Petry NM (2001) Substance abuse, pathological gambling, and impulsiveness. Drug Alcohol Depend 63:29–38

Piper BJ, Fraiman JB, Meyer JS (2005) Repeated MDMA ("ecstasy") exposure in adolescent male rats alters temperature regulation, spontaneous motor activity, attention and serotonin transporter binding. Dev Psychobiol 47:145–157

Rodgers J, Buchanan T, Scholey AB, Heffernan TM, Ling J, Parrott A (2001) Differential effects of Ecstasy and cannabis on self-reports of memory ability: a web-based study. Hum Psychopharmacol 16:619–625

Schifano F, Di Furia L, Forza C, Minicuci N, Bricolo R (1998) MDMA ('ecstasy') consumption in the context of polydrug abuse: a report on 150 patients. Drug Alcohol Depend 52:85–90

Schmidt CJ, Taylor VL (1987) Depression of rat-brain tryptophan-hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem Pharmacol 36 (23):4095–4102

Sher KJ, Trull TJ (1994) Personality and disinhibitory psychopathology-alcoholism and antisocial personality disorder. J Abnorm Psychol 103:92–102

Sher KJ, Bartholow BD, Wood MD (2000) Personality and substance use disorders: a prospective study. J Consult Clin Psychol 68:818–829

Semple SJ, Zians J, Grant I, Patterson TL (2005) Impulsivity and methamphetamine use. J Subst Abuse Treat 29:85–93

Thomasius R, Petersen K, Buchert R, Andresen B, Zapletalova P, Wartberg L, Nebeling B, Schmoldt, A (2003) Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users. Psychopharmacology 167:85–96

Travers KR, Lyvers M (2005) Mood and impulsivity of recreational ecstasy users in the week following a "rave." Addiction Research and Theory 13:43–52

Verkes RJ, Gijsman HJ, Pieters MSM, Schoemaker RC, de Visser S, Kuijpers M, Pennings EJM, de Bruin D, Van de Wijngaart G, Van Gerven JMA, Cohen AF (2001) Cognitive performance and serotonergic function in users of ecstasy. Psychopharmacology 153:196–202

Weir E (2000) Raves: a review of the culture, the drugs and the prevention of harm. CMAJ 162:1843–1848

Yip JTH, Lee TMC (2005) Effect of ecstasy use on neuropsychological function: a study in Hong Kong. Psychopharmacology 179:620–628