Administration of MDMA to ethanol-deprived rats increases ethanol operant self-administration and dopamine release during reinstatement

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Abstract
Recreational use of (±)-3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is often associated with other drugs, among which ethanol (EtOH) is one of the most common. However, little is known about how neurochemical sensitization produced by MDMA can modulate EtOH abuse. In this study we used EtOH operant self-administration tasks to investigate the effect of several low doses (0.33, 1.0 and 3.0 mg/kg) of MDMA in Dark Agouti rats. Motor activity was recorded after each MDMA administration. Changes in extracellular dopamine in the nucleus accumbens following a single EtOH injection (1.5 g/kg i.p.) were measured using intracerebral microdialysis in vivo after 1 wk of abstinence from EtOH, in order to mimic the dopaminergic response associated with reinstatement into EtOH consumption. Animals exposed to higher doses of MDMA (1.0 and 3.0 mg/kg) showed significantly enhanced EtOH self-administration during reinstatement and an increased EtOH-induced dopamine efflux. MDMA treatment acutely elevated motor activity after each administration in a dose-dependent manner. These findings suggest that repeated administration of MDMA, a relatively common drug of abuse, even at low doses, can alter subsequent vulnerability to EtOH consumption.

Key words: Addiction, dopamine, ethanol deprivation and reinstatement, MDMA, operant self-administration.

Introduction
Recreational use of (±)-3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is often associated with other drugs, among which ethanol (EtOH) is one of the most common due to its social role and easy availability (Lora-Tamayo et al. 2004; Schifano, 2004).

Immediately after administration, MDMA causes a rapid efflux of dopamine (DA) and serotonin (5-HT) in various brain areas including striatum, and nucleus accumbens (NAc) (Esteban et al. 2001; Gudelsky & Nash, 1996; O’Shea et al. 2005). In addition, MDMA induces a long-lasting reduction in 5-HT concentration and density of 5-HT transporters in the rat brain. The magnitude and duration of this effect may depend on various factors such as dose, number of doses, interval between doses and genetically determined differences in sensitivity in strains (Green et al. 2003).

It is well known that repeated administration of MDMA produces behavioural sensitization and enhanced DA transmission in the NAc (Kalivas et al. 1998). This long-term elevation of DA function in the
NAc may be responsible for increasing the rewarding actions of other psychostimulants like amphetamines or cocaine (Morgan et al., 1997).

Studies in our laboratory have shown that the non-contingent administration of nicotine or the cannabinoid agonist WIN 55,212-2 during EtOH deprivation results in an increase in the operant responding for EtOH during reinstatement when compared with a simple EtOH deprivation (Lopez-Moreno et al., 2004a, b). Both nicotine and WIN 55,212-2 acutely increase dopaminergic transmission in NAc.

Research on the interaction between EtOH and MDMA has mainly been focused on the effects of EtOH on MDMA-induced hyperthermia, locomotor hyperactivity and neurotoxicity (Cassel et al. 2005; Izco et al. 2007) as well as the modulation that acute or sub-chronic MDMA administration exerts on EtOH consumption (Bilsky et al. 1990). However, the effect that repeated MDMA administration might have on subsequent EtOH consumption during a period of EtOH withdrawal has not been previously assessed.

The aim of the present study was to determine the effect of a 5-d treatment with MDMA on EtOH consumption in the rat. MDMA-induced dopamine release was measured by in-vivo intracerebral microdialysis, in an attempt to mimic the neurochemical situation associated with EtOH resumption. Furthermore, locomotor activity was assessed in order to discard the possibility of locomotor sensitization interference with operant responding to EtOH.

Materials and methods

Animals and drugs

Forty adult male Dark Agouti rats (Harlan, Spain) weighing 175–200 g at the beginning of the experiments were housed two per cage in a room with a controlled 12 h reversed light/dark photoperiod (lights on 08:00 hours and a constant temperature/humidity environment (21 ± 2 °C). Food and water were available ad libitum in the home cage. The experiments were conducted under dim red light, between 09:00 and 15:00 hours. All experimental procedures were performed in accordance with the guidelines of the Animal Welfare Committee of the Universidad Complutense de Madrid (following DC86/609/EU).

MDMA (NIDA, Research Triangle Park, USA) was dissolved in saline (0.9% NaCl) and given intraperitoneally (i.p.) in a volume of 1 ml/kg at the doses of 0.33, 1.0 and 3.0 mg/kg. Doses are reported in terms of the base.

EtOH solution was prepared daily as a 10% EtOH v/v solution from 99% EtOH.

Training procedure for oral EtOH self-administration

Fixed ratio-1 (FR-1) training was achieved using a modification of the method described by Samson et al. (1999), which is described extensively in López-Moreno et al. (2004a). Briefly, rats were placed on a water restriction schedule for 2–4 d to facilitate training of lever pressing. During the first 3 d of training, the animals received 0.2% saccharin solution in the dipper. Thereafter, the following sequence on a FR-1 schedule was used: 0.2% saccharin for four sessions, 0.2% saccharin and 2% EtOH for two sessions, 0.16% saccharin and 4% EtOH for two sessions, 0.12% saccharin and 6% EtOH for four sessions, 0.08% saccharin and 8% EtOH for four sessions, 0.04% saccharin and 10% EtOH for four sessions, 0.02% saccharin and 10% EtOH for four sessions and finally 10% EtOH continuously until the establishment of the baseline after at least 6 wk. The chambers were equipped with two retractable levers located on either side of a drinking reservoir (0.1 ml) positioned in the centre of the front panel of the chamber. The levers were counterbalanced to respond as either the active or inactive lever. Animals were divided into four groups all of which received intermittent (Monday–Friday) and limited (30-min sessions) access to EtOH every week. Minimum response threshold for baseline was set at 15 lever presses in a 30-min session. Following this criterion, two animals were excluded from each group for a final configuration of n = 32 (n = 8 per group). The experiment began once baseline had been reached after a 6-wk period of access to EtOH (10% w/v). We used a modification of the EtOH deprivation effect. In our model, ‘Drug during deprivation’, we treat the animals with a drug (in this case with MDMA) during the period of EtOH deprivation. In this way, it is possible to evaluate long-lasting behavioural and neurochemical changes induced by MDMA. Furthermore, non-specific or interfering effects of drugs used concomitantly with the EtOH operant self-administration are avoided (such as motor alterations, or conditioned taste aversion, etc.).

EtOH deprivation and MDMA administration

Animals were then exposed to two EtOH deprivation periods (7 d), each period followed by two consecutive 5-d cycles (weeks 1 and 2) of voluntary operant EtOH self-administration (Fig. 1) (for details see López-Moreno et al. 2004a). During the first deprivation period animals were treated daily with an i.p. injection.
of saline (1 ml/kg) (days 1–5). Reinstatement of EtOH consumption was recorded for two consecutive 5-d cycles in self-administration cages (weeks 1 and 2 of post-deprivation after saline). During the second withdrawal period, animals were treated daily with an i.p. injection of either saline (1 ml/kg) or 0.33, 1.0 or 3.0 mg/kg MDMA (days 1–5). The doses chosen for the MDMA repeated treatment were doses that do not produce neurotoxicity (O'Shea et al. 1998). Motor activity and rewarding effect of MDMA were measured in a conditioned place preference (CPP) apparatus immediately after drug administration. Again, reinstatement of EtOH operant self-administration was observed for two consecutive 5-d cycles (weeks 1 and 2 of post-deprivation after MDMA). Then, a third withdrawal period was carried out in the same conditions as the second, in an attempt to reproduce the conditions associated with the second reinstatement. Intracerebral microdialysis was performed to measure the extracellular concentrations of DA and 5-HT in rat NAc following a challenge dose of ethanol (1.5 g/kg i.p., 20% w/v solution in saline) according to previous studies (for review, see Gonzales et al. 2004).

**Plasma EtOH determination**

Adult Dark Agouti and Wistar rats were given EtOH (0.8 g/kg, 10% w/v solution in water, oral gavage). Blood EtOH levels were determined 30 and 60 min after oral administration of EtOH and then every hour up to 4 h after treatment. Samples of 20 μl of blood were collected from the tail in heparanized capillary tubes, centrifuged at 1500 g for 6 min at 4 °C (Microcentrifuge MK5, model 01400–00, Analox, UK) and injected in an analyser (AM1, Analox, UK). The rationale of the method consists of EtOH being oxidized by the enzyme EtOH oxidase in the presence of molecular oxygen. Therefore, the rate of oxygen consumption is directly proportional to EtOH concentration. Plasma ethanol levels were calculated as mg/dl, using 300 mg/dl ethanol as standard.

**Implantation of microdialysis probes**

On day 7 of the third withdrawal period, rats were anaesthetized with isoflurane (1.5% isoflurane in a mixture of 30% oxygen and 70% nitrous oxide) and fixed in a stereotaxic frame equipped with an anaesthetic mask (David Kopf Instruments, USA) with the tooth bar at −3.3 mm below the interaural zero. A guide cannula was implanted in the right NAc according to the following coordinates: +1.06 cm from the interaural line, −0.06 cm mediolateral and −0.7 cm below the skull (Paxinos & Watson, 1986). Cannulae were secured to the skull as described by Baldwin et al. (1994). On the day of the experiment (day 8), the dialysis probe (membrane length: 2.0 mm × 500 μm; CMA/12, Sweden) was inserted in the guide cannula such that the membrane protruded its full length from the end of the cannula.

**Sample collection and quantification of DA and 5-HT in the dialysate**

Catechol and indole efflux in the brain in vivo was measured by the method described in detail by Colado et al. (1999) Probes were perfused with artificial cerebrospinal fluid (aCSF: 2.5 mm KCl, 125 mm NaCl, 1.18 mm MgCl₂,6H₂O, 1.26 mm CaCl₂·2H₂O) at a rate of 1 μl/min and samples collected from the freely moving animals at 30-min intervals in tubes containing 5 μl of a solution composed of HClO₄ (0.01 m), cysteine (0.2%) and sodium metabisulfite (0.2%). The first 60-min sample was discarded and the next three 30-min baseline samples collected. After EtOH injection, samples were collected every 30 min for 6 h.

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**Fig. 1.** Schedule of drug administration. Grey blocks indicate EtOH deprivation periods (7 d from Monday to Sunday) in which either saline or MDMA was administered. White blocks denote 5-d cycles (week) in which animals had 30 min to voluntary self-administer a 10% EtOH solution in operant chambers. After the third deprivation period, microdialysis was carried out on reinstatement of forced EtOH administration.
DA and 5-HT were measured in the dialysate by HPLC coupled to electrochemical detection. The mobile phase consisted of KH$_2$PO$_4$ (0.05 M), octanesulfonic acid (0.4 mM), EDTA (0.1 mM) and methanol (16%) and was adjusted to pH 3 with phosphoric acid, filtered and degassed. The flow rate was 1 ml/min. The HPLC system consisted of a pump (Agilent 1100 series) linked to an automatic sample injector (Agilent 1100 series), a stainless-steel reverse-phase column (Zorbax Eclipse XDB-C18, 4.6 x 150 mm, 5 µm, Agilent) with a coulometric detector (Coulochrome III, ESA, USA). The working electrode potential was set at 400 mV with a gain of 10 nA for DA and 200 nA for 5-HT. The current produced was monitored using integration software (Chemstation, Agilent).

Measurement of rectal temperature

Immediately before and up to 6 h after EtOH challenge administration, temperature was measured by use of a digital readout thermocouple (BAT-12 thermometer, Physitemp Instruments, USA) with a resolution of 0.1 °C and accuracy of ±0.1 °C attached to a RET-2 rodent sensor which was inserted 2.5 cm into the rectum of the rat, the animal being lightly restrained by holding in the hand. A steady readout was obtained within 10 s of probe insertion.

Data analysis

Data analysis from weekly operant responses and analysis of the day-by-day responses was performed by two-way repeated-measures analysis of variance (ANOVA): before-deprivation (baseline) and post-deprivation (within-subjects factor), and different drug treatment (between-groups factor). Only significant effects ($p$ values $<0.05$) in ANOVA analysis were subjected to Tukey’s honestly significant difference (HSD) test (between-groups factor), and the post-hoc analysis for repeated measures (subprogram of the SPSS statistical software package, version 13.0 for Windows; USA).

Statistical analyses of the temperature and dialysis experiments were performed using the statistical computer package BMDP/386 Dynamic (BMDP Statistical Solutions, Ireland). Data were analysed by repeated-measures ANOVA (program 2V) or, where missing values occurred, an unbalanced repeated-measures model (program 5V) was used. Both used treatment as the between-subjects factor and time as the repeated measure. ANOVA was performed on both pretreatment and post-treatment data. Differences were considered significant at $p<0.05$.

Results

Effect of repeated administration of MDMA during a period of EtOH deprivation on reinstatement to EtOH operant self-administration.

Figure 2 depicts the responses to obtaining EtOH under a FR-1 schedule, i.e. one response equal to one 0.1 ml cup of EtOH solution. In Fig. 2a, these responses were averaged over a week, giving an index of the total EtOH consumption. No significant differences were found among groups at baseline (week 0) and in operant responses after the first deprivation period (weeks 1 and 2 of post-deprivation after saline). Repeated administration of both of the higher MDMA doses (1.0 and 3.0 mg/kg) for five consecutive days (Monday–Friday) during the second EtOH deprivation period, significantly increased EtOH consumption over the first week of reinstatement (week 1 of post-deprivation after MDMA). Instead of weakening in their EtOH consumption as the week progressed, animals maintained this elevated operant responding ratio for the 5 d (Fig. 2d,e). The group treated with the lower MDMA dose (0.33 mg/kg) did not show differences compared with its own response after saline (Fig. 2c). No significant changes were observed at any of the doses in the second week of reinstatement (week 2 of post-deprivation after MDMA) (Fig. 2a).

All rats showed a robust and significant increase in their responding for EtOH during the first day of EtOH reinstatement when compared with their previous baseline (the EtOH deprivation effect). MDMA did not modify this effect, revealing a ceiling effect. The corresponding ANOVAs for Fig. 2 were as follows: Fig. 2a (ANOVA within weeks: $F_{1,28}=41.60$, $p<0.0001$; interaction between weeks and treatment: $F_{3,28}=4.63$, $p<0.01$); Fig. 2b (ANOVA post-deprivation week 1, between subjects: $F_{4,56}=0.34$, n.s.; ANOVA within days: $F_{4,56}=2.56$, $p=0.48$; interaction between days and treatment: $F_{4,56}=2.14$, $p=0.08$); Fig. 2c (ANOVA post-deprivation week 1, between subjects: $F_{1,14}=0.92$, n.s.; ANOVA within days: $F_{4,56}=5.01$, $p=0.002$; interaction between days and treatment: $F_{4,56}=0.12$, $p=0.97$); Fig. 2d (ANOVA post-deprivation week 1, between subjects: $F_{1,14}=14.05$, $p<0.05$; ANOVA within days: $F_{4,56}=3.75$, $p=0.009$; interaction between days and treatment: $F_{4,56}=1.44$, $p=0.23$); Fig. 2e (ANOVA post-deprivation week 1, between subjects: $F_{1,14}=5.12$, $p<0.05$; ANOVA within days: $F_{4,56}=3.36$, $p=0.016$; interaction between days and treatment: $F_{4,56}=5.88$, $p=0.01$). The subsequent post-hoc analysis revealed significant differences only in the groups treated with MDMA (1.0 and 3.0 mg/kg) (Fig. 2d,e), when compared with the vehicle group.
and its own baseline (Tukey’s HSD post-hoc analysis, \( p < 0.05 \)).

The estimation of EtOH intake (g/kg) by Dark Agouti rats is shown in Table 1. These values correspond to the values depicted in Fig. 2 as EtOH responses (Fig. 2a, averaged over a week). Similarly, the weekly average number of lever presses on the inactive lever are shown in Table 2. Note that contrary
to Table 1, where significant differences are found between groups, there were no significant differences in the number of inactive lever presses.

In addition, in order to investigate if different strains of rats (Wistar vs. Dark Agouti rats) show differences in EtOH intake, we analysed EtOH clearance during four consecutive hours after an oral dose of 0.8 g/kg (average EtOH intake in operant responding, see Table 1). Plasma EtOH concentration did not differ between Wistar and Dark Agouti rats (Fig. 3) indicating that there were no significant differences between these strains.

**Table 1.** Ethanol intake (g/kg) per 30-min session in ethanol-operant self-administration chambers averaged over a week

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-deprivation after saline</th>
<th>Post-deprivation after MDMA</th>
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<tr>
<td></td>
<td>Week 0</td>
<td>Week 1</td>
<td>Week 2</td>
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<tr>
<td>Mean ± S.E.M.</td>
<td>0.58 ± 0.45</td>
<td>0.63 ± 0.64</td>
<td>0.58 ± 0.65</td>
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<tr>
<td>Veh 0.33 1.0 3.0</td>
<td>Veh 0.33 1.0 3.0</td>
<td>Veh 0.33 1.0 3.0</td>
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Values are mean ± S.E.M. of eight rats.

**Table 2.** Number of inactive lever presses per 30-min session in ethanol-operant self-administration chambers averaged over a week

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-deprivation after saline</th>
<th>Post-deprivation after MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>1.11 ± 0.24</td>
<td>0.88 ± 0.81</td>
<td>1.19 ± 0.37</td>
</tr>
<tr>
<td>Veh 0.33 1.0 3.0</td>
<td>Veh 0.33 1.0 3.0</td>
<td>Veh 0.33 1.0 3.0</td>
<td>Veh 0.33 1.0 3.0</td>
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Values are mean ± S.E.M. of eight rats.

Effect of repeated administration of MDMA during a period of EtOH deprivation on DA and 5-HT release in rat NAc caused by EtOH administration

Administration of EtOH (1.5 g/kg i.p.), increased extracellular DA levels in the NAc in MDMA-treated animals. Extracellular DA levels following EtOH in the MDMA-treated groups were significantly greater than those observed in the saline-treated rats (Fig. 4d) ($F_{1,9}=12.40$, $p<0.0001$; $F_{1,8}=31.17$, $p<0.0001$; $F_{1,7}=33.06$, $p<0.0001$ for 0.33, 1.0 and 3.0 mg/kg MDMA, respectively, compared with saline-treated animals). In addition, the increase in extracellular DA produced by EtOH in the animals treated with 1.0 and 3.0 mg/kg MDMA was greater than that observed in animals treated with 0.33 mg/kg MDMA ($F_{1,9}=10.03$, $p<0.0005$ and $F_{1,8}=10.24$, $p<0.0004$, respectively).
although they did not differ between each other ($F_{1,7} = 0.18$, n.s.) (Fig. 4a). In contrast, administration of an EtOH challenge (1.5 g/kg i.p.) injection to animals treated with MDMA (0.33, 1.0 or 3.0 mg/kg i.p.) for five consecutive days during EtOH deprivation did not modify the extracellular levels of 5-HT compared with those in animals given saline during the abstinence period and challenged with EtOH ($F_{3,17} = 0.35$, n.s.) (Fig. 4b).

**Effect of MDMA on EtOH-induced changes in rectal temperature**

Administration of MDMA (0.33, 1.0 or 3.0 mg/kg i.p.) during the first 5 d of EtOH deprivation did not modify the effect on body temperature of a subsequent EtOH challenge (1.5 g/kg i.p.) injection compared with animals given saline during the abstinence period ($F_{3,35} = 1.94$, n.s.; Fig. 5). Basal temperatures before EtOH challenge were similar in all groups ($F_{3,37} = 0.00$, n.s.).

**Effect of MDMA on locomotor activity during EtOH abstinence and CPP**

The effects of different MDMA doses on locomotor activity during EtOH deprivation are depicted in Fig. 6. Animals were placed in a CPP apparatus (for description see López-Moreno et al. 2004a) immediately after drug administration and locomotor activity was measured during 30-min conditioning sessions. Cumulative number of photocell crossings during MDMA or saline conditioning for five consecutive days are shown in Fig. 6a. Administration of MDMA...
dose-dependently increased locomotor activity ($F_{3,31} = 21.89$, $p < 0.0001$; ** $p < 0.01$, *** $p < 0.001$ vs. vehicle; ** $p < 0.01$, *** $p < 0.001$ vs. higher MDMA dose). Day-by-day results and 5-min intervals for the first and last conditioning days are shown in Fig. 6b–d, respectively (ANOVA, interaction between days and treatment: $F_{12,112} = 2.69$, $p = 0.003$; between subjects: $F_{3,28} = 21.89$, $p < 0.0001$; within days: $F_{4,112} = 2.29$, $p = 0.064$). This pattern of activity was consistent throughout the 5 d of treatment (Fig. 6b). Finally, MDMA did not produce CPP at any dose (data not shown).

**Discussion**

In the present study we have assessed a previously unexplored aspect of the interaction between MDMA and EtOH: how repeated treatment with low doses of MDMA during EtOH deprivation can affect subsequent reinstatement by rats with a previous history of EtOH consumption. This represents an interesting situation, since recreational use of MDMA is often discontinuous in time (weekends, parties) but concomitant with EtOH consumption (Schifano, 2004).

The major findings of the present study are that MDMA administration during EtOH deprivation increases operant responses for obtaining EtOH on the first week of reinstatement, and that this MDMA treatment produces an increase in efflux of DA into the rat NAc after an EtOH challenge injection.

Repeated EtOH withdrawals have been postulated to increase vulnerability and susceptibility to future relapse episodes (Becker et al. 1997). Furthermore, non-contingent administration of another drug that is able to increase accumbal dopaminergic efflux (nicotine or the cannabinoid agonist WIN 55,212-2) during EtOH deprivation has been shown to increase vulnerability to EtOH in an operant self-administration model (López-Moreno et al. 2004a, b). In line with these previous results, repeated low-dose MDMA (1.0 and 3.0 mg/kg) during an EtOH deprivation period increased operant responding for obtaining EtOH in conditioned place preference apparatus immediately after treatment, and their activity was registered throughout the following 30 min. (a) Depicts total photocell counts for the 5 d. (b) Day-by-day results for the 30-min sessions. (c, d) Motor activity in 5-min intervals during the first and last sessions, respectively (days 1 and 5). Values represent the mean ±S.E.M. of eight rats. Data were first analysed by repeated-measures ANOVA, followed by Tukey’s post-hoc test for contrast between groups. ** $p < 0.01$, *** $p < 0.001$ compared with vehicle; ** $p < 0.01$, *** $p < 0.001$ compared with 3.0 mg/kg MDMA.
Dark Agouti rats on reinstatement. However, in contrast to the effect observed following nicotine or WIN 55,212-2, increased responding was only evident during the first week after MDMA treatment. A possible explanation for this might be that, despite its selection as a reproducible animal model for studying the neurochemical effects of MDMA, the Dark Agouti strain has shown particular reluctance to operantly self-administer EtOH. In previous studies performed on Wistar rats (Lopez-Moreno et al. 2004a, b; Roberts et al. 1999, 2000; Schulteis et al. 1996), the baseline for EtOH responses was spontaneously set at values between 25–30 lever presses, while in this case, average responses for baseline barely reached 20. Moreover, EtOH-prefering rats maintained on an FR-1 schedule of reinforcement for 10% v/v EtOH displayed a higher breakpoint determinant than comparably trained non-EtOH-prefering rats (Ciccióoppo et al. 2001). Thus, operant responding for EtOH seems to be highly dependent on rat strain. However, in this case, despite lower responding, Dark Agouti rats had similar plasma EtOH levels to Wistar rats (Alén et al. 2008). This is due to the lower adult weight of the former strain compared with the latter (an average of 150/160 g lower throughout a chronic experiment of this kind). Thus, the rewarding properties of EtOH and motivation for EtOH consumption appear to be increased in MDMA-treated animals leading to a higher operant responding rate. Although a progressive ratio is normally used to measure motivational aspects of drug craving, FR-1 was maintained throughout the experiment considering the responsiveness of these animals.

In view of the fact that low responding seems unlikely to be responsible for the lack of enhanced EtOH reinstatement in the second week following MDMA exposure a possible explanation is that amphetamine treatment may not lead to long-term neuroadaptations. Behavioural sensitization following repeated administration of MDMA has been reported by several groups (Ball et al. 2006; Kalivas et al. 1998; Ramos et al. 2004, 2005). However, there are discrepant data regarding the required number of repeated injections, the washout period and the maintenance period. This discrepancy in findings could be attributed to differences in rat strain, age, sex and various MDMA-treatment protocols used. Thus, repeated MDMA treatment at a dose of 2.5 mg/kg elicited sensitization of a transient nature while the sensitization elicited by a 5.0 mg/kg dose persisted for a longer period (Modi et al. 2006).

Acute administration of MDMA produces DA release and behavioural sensitization (Spanos & Yamamoto, 1989), and a repeated MDMA regimen produces neurochemical sensitization to drugs such as cocaine, leading to a greater increase in DA release in treated animals compared with saline controls (Morgan et al. 1997). Considering that EtOH exerts its rewarding properties, at least in part, through the same dopaminergic pathways in rats, as well in humans (Boileau et al. 2003; Diana et al. 1993, 1996; Imperato & Di Chiara, 1986; Volkow et al. 2007), it is likely that MDMA sensitization could affect EtOH vulnerability in a similar manner. Thus, we decided to determine, by means of microdialysis in the NAc, the modulation caused by repeated administration of MDMA during EtOH deprivation on DA levels following EtOH challenge. We found that repeated administration of MDMA doses of 1.0 and 3.0 mg/kg significantly increased DA efflux in rat NAc elicited by a challenge injection of 1.5 g/kg EtOH. It is likely that repeated administration of low doses of MDMA is able to elicit increased DA release in the NAc, by producing enduring neuroadaptations in brain regions implicated in the effects of other commonly abused drugs (Kalivas et al. 1998; Mayerhofer et al. 2001). Therefore, the enhancement of EtOH-induced dopaminergic efflux in the rat NAc caused by MDMA administration during EtOH deprivation, might be potentiating the rewarding properties of EtOH in Dark Agouti rats. However, although there are an important number of studies showing that release of DA and 5-HT can be observed after both forced and self-administration paradigms (Di Chiara et al. 2004; O’Shea et al. 2005; Weiss et al. 1996), it is possible to find differences depending on the drug-exposure paradigm. Some studies with drugs such as cocaine and amphetamine have demonstrated that extracellular levels of DA are higher during active than during passive drug administration (for review, see Jacobs et al. 2003).

In contrast, 5-HT did not show significant changes after 1.5 g/kg EtOH injection in any group. Interestingly, on the one hand this result is in agreement with studies which show no significant effect of a 1.5 g/kg EtOH injection in EtOH-experienced mice (e.g. Szumlinski et al. 2007); but contrasts with the slight increase in 5-HT release found in EtOH-experienced Wistar rats after EtOH operant self-administration (Weiss et al. 1996). However, as far as we know these are the first data reported in Dark Agouti rats. It is likely that strain differences could explain our results. Thus, EtOH-prefering mice showed an EtOH-sensitized 5-HT response to EtOH, whereas EtOH-avoiding mice did not show any effect in their 5-HT response (Kapasova & Szumlinski, 2008).

A very well described effect following acute MDMA administration is hyperactivity (Hamida et al. 2007;
O’Shea et al. 2005; Pálenícek et al. 2007). As expected, we observed a dose-dependent increase in motor activity of Dark Agouti rats following MDMA injection. Although co-administration of EtOH and MDMA appears to potentiate locomotor effects of MDMA (Cassel et al. 2004), under our experimental conditions abstinence from EtOH did not affect this parameter at any of the MDMA doses. One main factor led us to discard a motor contribution to differences in active lever pressing: responses on the inactive lever remained unchanged compared with baseline before treatment (Table 2). In addition, all the repeated injections of MDMA were given in an environment different from the operant administration cages (in a CPP apparatus) in order to avoid place conditioning, a determinant feature for motor sensitization (Kalivas et al. 1998). Although the rewarding properties of MDMA have been described to cause place conditioning under certain circumstances (Bilsky et al. 1991; Braida et al. 2005) our treatment did not produce place conditioning at any dose (data not shown). In conclusion, our results indicate that repeated administration of MDMA during EtOH deprivation produces a significantly higher EtOH intake in a voluntary, operant self-administration model for measuring reinstatement, very close to a model of social drinking. Furthermore, a single EtOH injection elicited a dose-dependently greater increase in extracellular DA levels in the NAc of Dark Agouti rats repeatedly pretreated with MDMA compared with those pretreated with saline. Since MDMA is a commonly abused drug and appears to augment, in part, the pharmacological actions of EtOH and since the effects of EtOH on DA levels in the NAc appear to underlie its abuse potential, the present findings suggest that occasional MDMA consumption may increase vulnerability to EtOH consumption.

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Statement of Interest

None.

References


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