

# Rewarding effects of 3,4-methylenedioxymethamphetamine (“Ecstasy”) in dominant and subordinate OF-1 mice in the place preference conditioning paradigm

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## Abstract

We tested the ability of 3,4-methylenedioxymethamphetamine (MDMA) to induce conditioned place preference (CPP) in dominant and subordinate OF-1 mice subjected to cohabitation and repeated sessions of agonistic confrontation, as well as in non-confronted mice. We selected doses of MDMA (2, 6, 10 mg/kg) previously reported to induce CPP in mice and we measured expression of c-Fos evoked by the treatments in non-confronted mice. MDMA induced c-Fos protein in several corticolimbic regions involved in drug-induced reward. Mice were exposed to brief sessions of agonistic confrontation on 5 consecutive days. Determinations of circulating hormones and drug conditioning tests were carried out on completion of the encounters. The results of hormone assays indicated that dominant mice had higher serum concentrations of testosterone, but lower levels of corticosterone, than submissive mice. Post-conditioning tests after drug conditioning (4 injections of MDMA or saline on alternate days) showed that MDMA significantly produced CPP at doses of 2 and 6 mg/kg, but not at 10 mg/kg, an inverted U-shaped pattern of conditioning that was invariable in non-confronted, dominant and subordinate mice. These results demonstrate that the endocrine and behavioural correlates linked to social status and social stress in mice are not paralleled by significant changes in the rewarding efficacy of MDMA in the CPP paradigm under the specific conditions tested.

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**Keywords:** c-Fos; Corticosterone; MDMA; Place preference; Social status; Social stress; Testosterone

## 1. Introduction

The neuroscience of social conflict highlights the presence of distinct neurobiological substrates with phenotype dominant and submissive subjects. Such specific substrates could influence the behavioural responses to drugs and the vulnerability to addiction (Miczek et al., 2004). Accrued evidence in labo-

ratory animals indicated that subordinate subjects experiencing repeated episodes of social defeat were more prone than victorious counterparts to exhibit sensitized responses to drugs and to self-administering addictive substances. Specifically, episodic social stress following aggressive encounters induced sensitization to the locomotor-stimulant effects of amphetamine in defeated rats (Covington and Miczek, 2001; de Jong et al., 2005) and of cocaine in defeated mice (Nikulina et al., 1998). Further, social defeat stress increased cocaine self-administration in rats (Miczek et al., 2004; Covington and Miczek, 2005) and alcohol consumption in mice (Hilakivi-Clarke and Lister, 1992; Kudryavtseva et al., 2006). Social stress and status also influenced morphine place preference in rats in complex fashion (Coventry et al., 1997). Several neurobiological correlates of social defeat stress have been identified, including changes in the density or availability of neurotransmitter receptor (Miller

*Abbreviations:* CPP, conditioned place preference; MDMA, 3,4-methylenedioxymethamphetamine.

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et al., 1987; Morgan et al., 2002) and transporter sites (McKittrick et al., 2000; Isovich et al., 2001) and in the expression of neurotrophic factors in limbic brain regions (Pizarro et al., 2004; Berton et al., 2006). In addition, one key element which was correlated with the behavioural signs of social defeat stress, and with the ensuing establishment of social hierarchy, was a change in the activity of the neurosympathetic system and the hypothalamo-pituitary-adrenal axis, with variations in the levels of testosterone, corticosterone, prolactin, renin, and other hormones (Henry, 1992; Blanchard et al., 1993, 2001).

3,4-Methylenedioxymethamphetamine (MDMA), commonly referred to as ecstasy, is a potent stimulant and psychedelic drug which possesses considerable abuse liability in humans (Morton, 2005; Easton and Marsden, 2006). MDMA stimulated self-administration in rats (Braida and Sala, 2002; Schenk et al., 2003), though not as potently as did other abused drugs. In addition to maintaining self-administration, MDMA was shown to produce place preference after conditioning sessions in a given environment (Salzmann et al., 2003; Robledo et al., 2004). Though MDMA induced apparently weaker rewarding effects in animal models, a fact which contrasts with its abuse potential in humans, such paradigms might be relevant for understanding human abuse of MDMA (Green et al., 2003; Easton and Marsden, 2006) and might throw light into the general basis of self-medication in humans (Self and Nestler, 1998).

It has not been determined whether or not the rewarding efficacy of MDMA is modified in animals which have experienced defeat after agonistic encounters, compared to those which experienced victory and became dominant. In the present studies, we selected doses of MDMA which have been previously used to study CPP in mice and which we showed here as capable of inducing c-Fos expression in the OF-1 mouse forebrain. We used social defeat as a model of social stress to examine the extent to which aggressive interactions and stress hormones could influence MDMA-induced place preference in mice, as a measure of drug-induced reward. We administered the drug to non-confronted mice and to mice which exhibited both the phenotypical changes in hormonal regulation and the dominant/submissive behaviours which typically result from cohabitation and repeated agonistic confrontation.

## 2. Methods

### 2.1. Subjects and drugs

OF-1 male mice ( $N=199$ ) of ca. 40 days of age (30–32 g) were purchased from Criffa-Credo Laboratories (Lyon, France) or Charles River (Barcelona, Spain). Mice were housed singly in plastic cages for 2 weeks prior to any manipulation. The vivarium was a room with 12 h alternating light/dark cycle (lights on at 19:30) and controlled temperature ( $20\pm 1$  °C). Food and water were available ad libitum throughout the experiments. All experiments were performed during the dark phase of the light cycle. 3,4-Methylenedioxymethamphetamine (MDMA) (Sigma-Aldrich, Spain) was dissolved daily in 0.9% saline and administered at doses of 0, 2, 6 and 10 mg/kg (i.p.) at a volume of 0.1 ml/10 g. All experiments were carried out in compliance

with current European directives on animal experimentation (86/609/ECC).

### 2.2. Immunocytochemistry

A group of non-confronted mice ( $n=22$ , Fig. 1) received treatment with 0.9% saline or MDMA (0, 2, 6 and 10 mg/kg) to produce a dose–response curve for c-Fos expression. 1 h after the MDMA or saline challenge mice were deeply anaesthetized with sodium pentobarbital (60 mg/kg) and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, NaKPO<sub>4</sub>). The brains were post-fixed for 24 h, rinsed in 0.1 M PB and transferred to 20% glycerol solution. Free-floating coronal 25  $\mu$ m sections were cut on a Leica cryostat and kept in 0.1 M PB with 0.01% sodium azide until further processing. Sections were exposed to immunocytochemistry for c-Fos, as previously described (Canales, 2004, 2005). Briefly, endogenous peroxidase activity was quenched with 3% H<sub>2</sub>O<sub>2</sub>, and sections were exposed to blocking serum (5% normal goat serum), and incubated overnight at 4 °C in primary antibody against c-Fos (rabbit anti-c-Fos Ab-5, 1:5000, Oncogene). Sections were washed and incubated in secondary antibody (goat anti-rabbit IgG, Vector Laboratories) for 1 h followed by HRP-conjugated streptavidin (1:5000). To reveal antigenic sites, the sections were treated with diaminobenzidine-H<sub>2</sub>O<sub>2</sub> complex with nickel (NiSO<sub>4</sub>) intensification, which produced a standard nuclear black reaction product. Appropriate controls were performed in which the primary antibody was omitted from the protocols. Sections were finally mounted with Permount and cover slipped.

### 2.3. Microscopy and c-Fos quantification

Standard procedures were used for c-Fos quantification (Canales, 2004, 2005). Sections were studied with a Nikon Eclipse E800 microscope with image analysis software (Analysis, Leica). Sections were coded for analysis by a blind observer and codes were only unveiled on completion of the experiments. High resolution photographs were taken of the relevant brain areas under investigation, identified by known landmarks, and c-Fos-positive cells were quantified by light thresholding with the ImageTool 3.0 software. At least 4 sections from each structure were quantified and averaged per animal. Counts were expressed as c-Fos-positive cells/mm<sup>2</sup>. The corticolimbic areas examined included the nucleus accumbens (a portion of ca. 1 mm<sup>2</sup> encompassing both core and shell regions), dorsal striatum, prelimbic cortex, anterior cingulate cortex, bed nucleus of the stria terminalis, central nucleus of the amygdala, and the CA1, CA3 and dentate gyrus regions of the hippocampus.

### 2.4. Social stress protocols and induction of social status

To establish social status weight-matched animals were assigned to one of two groups: a social stress, cohabitation group, and a singly housed, non-confronted group (Fig. 1). In the groups undergoing confrontations ( $n=128$ ) mice were allocated

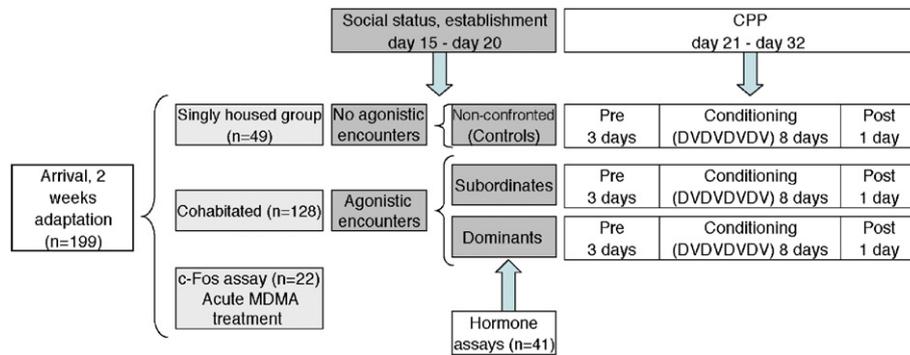


Fig. 1. Schematic representation of the experiments performed showing the different experimental groups and the time during which treatments were administered. See Methods for detailed information. D=drug, V=vehicle.

in pairs in plastic cages ( $20 \times 20 \times 13$  cm); both animals were separated by a barrier of opaque Perspex with small holes through which animals could see and smell, but not touch, each other (Kudryavtseva et al., 1991). In the non-confronted group ( $n=49$ ) mice were housed singly in plastic cages ( $20 \times 10 \times 13$  cm).

For the social stress group, the phase of paired agonistic encounters began 24 h after cohabitation. Mice housed in isolation did not undergo agonistic confrontations. Agonistic encounters were carried out for 5 consecutive days in the same cage in which mice were housed by removing the Perspex partition for 10 min. The cage was transported to a test room illuminated by a red light (40 W). Encounters were preceded by an adaptation period of 1 min before the partition was removed (Kudryavtseva et al., 1991).

### 2.5. Selection of dominant and subordinate animals

The behaviours were videotaped with a camera (Hitachi VM-E535LE, 8 mm). The videotapes were analyzed using a PC computer and a custom-developed programme (Brain et al., 1989) which enables estimations of time allocated to functional

categories of behaviour, including grooming, digging, non-social exploration, exploration from distance, social investigation, threat, attack, avoidance/flee, defensive/submissive behaviour, and immobility. Each of the behaviours was defined by different postures and motor elements. Discrete behaviours were recorded and pooled into the general categories of attack, threat, avoidance/flee and defensive/submissive behaviour. The videotapes were scored by an observer blind to the treatment conditions. A mouse was categorized as dominant if during the agonist encounters it showed behaviours of attack and threat, but not behaviours associated with subordinate status, such as immobility, escape, flight and defence. In turn, a mouse was categorized as subordinate if it scored highly in categories of immobility, escape, flight and defence, but not in categories of threat and attack.

### 2.6. Hormone assays

Once social status was established, groups of non-confronted, dominant and submissive mice were killed to perform testosterone and corticosterone assays ( $n=41$ , Fig. 1). Samples

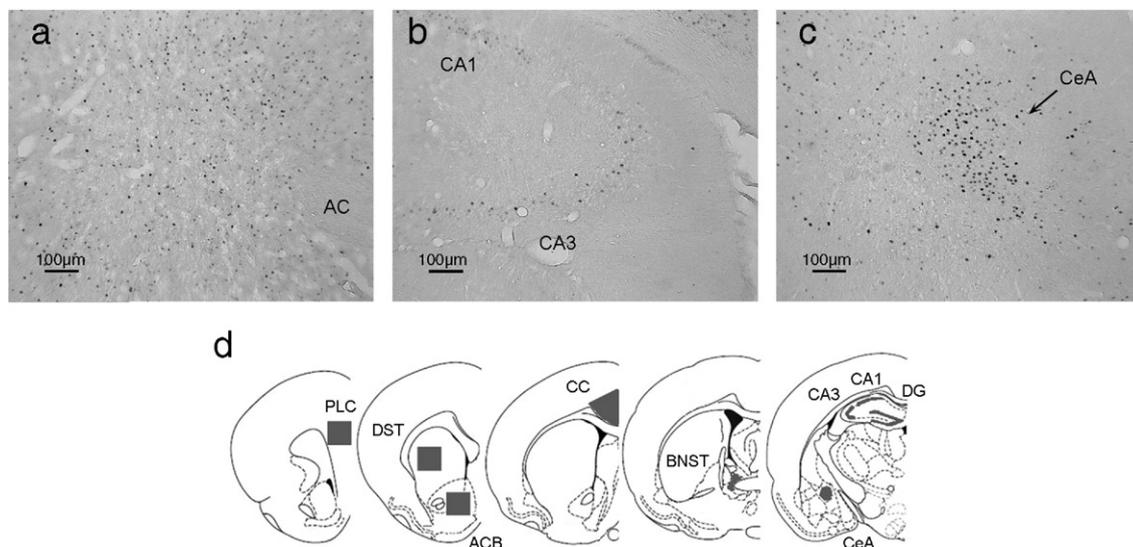


Fig. 2. Sample photomicrographs ( $10 \times$ ) showing induction of c-Fos protein in the nucleus accumbens (a), dorsal hippocampus (b) and central nucleus of the amygdala (c) following challenge with MDMA (10 mg/kg). Control levels of expression were very low by comparison (see Fig. 3). The areas where quantification was carried out appear in gray in the diagrams representing coronal sections through the mouse brain (d). AC=anterior commissure, CeA=central nucleus of the amygdala, PLC=prelimbic cortex, DST=dorsal striatum, ACB=nucleus accumbens, CC=cingulate cortex, BNST=bed nucleus stria terminalis. Scale bar: 100  $\mu$ m.

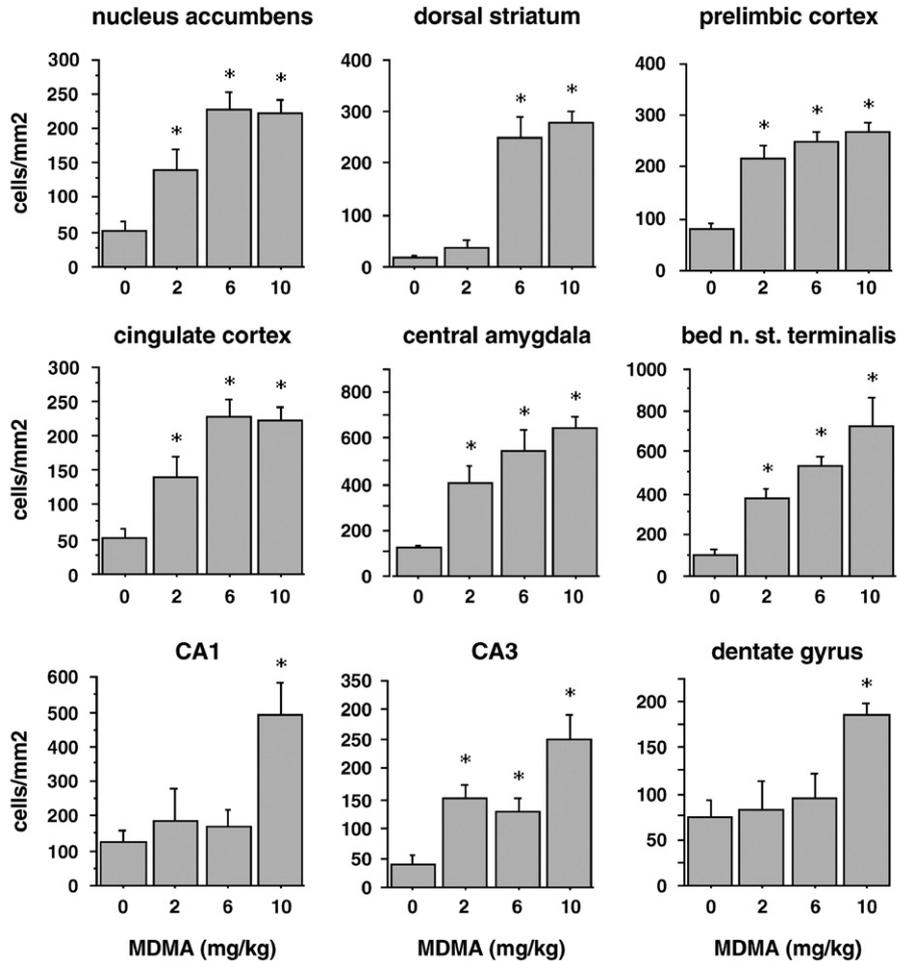


Fig. 3. c-Fos expression induced in mice forebrain following acute MDMA (0, 2, 6, 10 mg/kg) exposure. Significant induction was observed in all regions studied by the high dose of MDMA, but only at selected sites by the low and moderate doses. \*Significantly different from control,  $p < 0.05$  (Newman–Keuls test using sampling error of significant ANOVA as denominator).

were taken 1 h after completion of the last of the 5 sessions of agonistic confrontation (between 9:00 A.M. and 12:00 A.M. and in counterbalanced order). Blood samples were obtained by cardiac puncture and prepared by centrifugation to separate out the serum. Serum was immediately frozen ( $-80^{\circ}\text{C}$ ) until further processing. Testosterone and corticosterone analyses were performed using a gamma Counting 5500 System (Beckman). Serum testosterone (ng/ml) was determined by radioimmunoassay using Coat-A-Count Total Testosterone Kits (Diagnostic Products Corporation, California). The sensitivity was 4 ng/dl. The within and between assay variation coefficients were 3% and 13%, respectively. Testosterone (nmol/l) values were the mean of duplicate determinations. Serum corticosterone (ng/ml) was determined by radioimmunoassay using a commercially available reagent kit (ICN Biomedicals, CostaMesa, CA). Assay sensitivity was 5 pg/tube and within and between assay variation coefficients were 7% and 8%, respectively.

### 2.7. Place preference paradigm

Conditioned place preference (CPP) was assessed in a box ( $30 \times 15 \times 20$  cm) made of aluminium and Perspex, similar to

that described by [Cunningham et al. \(1992\)](#). The place preference apparatus consisted of two conditioning compartments ( $30 \times 15 \times 20$  cm) with different floor configuration. The movement and location of mice were recorded by computerized monitoring software (Cibertec, Madrid, Spain). The walls of the box were made of black Perspex, and the floor consisted of interchangeable halves of two different textures: a floor grid with 3 mm rods mounted 6.4 mm apart in Plexiglas rails, and a stainless steel floor with round perforations of 6.4 mm in diameter. The floor texture was used as distinctive contextual stimuli to establish place conditioning. This type of apparatus has been used previously to assess the rewarding properties of several addictive substances in mice ([Cunningham et al., 1992](#); [Chester and Cunningham, 1998](#)). An unbiased place conditioning method was used based on our own preliminary evidence showing that non-confronted, dominant and submissive OF-1 mice do not show preference for either compartment during pre-conditioning, a fact which we replicated in the present experiments (see the control values in [Fig. 5](#)). Subordinate and dominant mice were assigned to the different drug groups (0, 2, 6, 10 mg/kg MDMA). The protocol consisted of three phases: a “pre-conditioning phase”, performed in 3 consecutive days. During this phase, drug-naïve

mice had free access to both compartments for 15 min, and the time spent in each compartment was recorded. Data from the third day was considered as baseline. A “conditioning phase”, carried out in 8 consecutive days. On the first conditioning day, mice were treated with MDMA (2, 6 or 10 mg/kg) or saline 20 min before being placed individually for 15 min in one of the conditioning environments, without access to the other compartment. On the next conditioning day, mice were given saline in the opposite compartment. The drug-saline sequence alternated during 8 days. A “post-conditioning phase”, performed 24 h after the last conditioning session. Mice received saline injections and were allowed free access to both compartments of the apparatus for 15 min. Differences between times spent in the drug-paired environment during the post-conditioning and pre-conditioning tests were calculated.

### 2.8. Statistical analysis

All analyses were performed using the Statview 5.0 package. Significance levels were set at  $\alpha=0.05$ . c-Fos data was analyzed by ANOVA with one between-subject factor, “treatment”, with four levels (0, 2, 6 and 10 mg/kg of MDMA), followed by Newman–Keuls post-hoc tests. Data for hormone levels were analyzed by ANOVA with one between-subject factor, “Status”, with two levels (dominant and subordinate) followed by Newman–Keuls tests. Behavioural observations were analyzed using one-tail Student’s *t*-tests. Data from the CPP tests (difference between post-conditioning and pre-conditioning times spent in the drug-paired context) were analyzed by ANOVA with two between-subject factors. The two between factors were “Status”, with three levels (non-confronted, dominant and subordinate), and “Treatment”, with four levels (0, 2, 6 and 10 mg/kg of MDMA). Significant interactions were further explored with Newman–Keuls test comparisons. Preference was defined as a significant increase in the time spent in the drug-

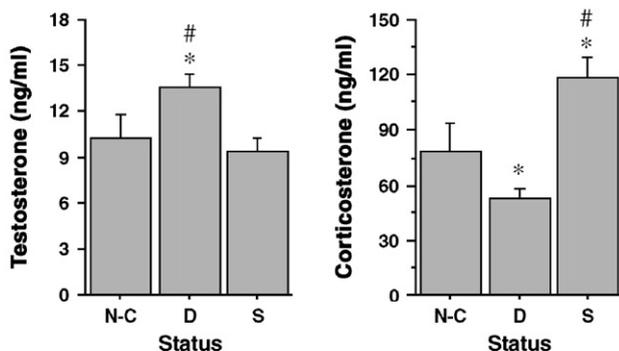


Fig. 4. Circulating levels of testosterone and corticosterone in non-confronted mice and in mice which underwent social confrontation. Dominant mice (D) exhibited higher levels of testosterone than both non-confronted (N-C) or subordinate mice (S) (left). By contrast, dominant mice had lower levels of corticosterone than both non-confronted and subordinate mice; levels of corticosterone of submissive mice were significantly elevated compared to those of non-confronted mice (right). \*Significantly different from control,  $p<0.05$ ; #significantly different from subordinate,  $p<0.05$  (Newman–Keuls test using sampling error of significant ANOVA as denominator).

Table 1

Accumulated scores in the four categories of social dominance/subordination

	Behavioural categories	
	Dominant	Subordinate
<i>1st day</i>		
Attack	65.76+5.86	9.26+2.40
Threat	72.44+8.02	13.21+2.90
Avoidance/flee	2.86+0.65	59.52+11.33
Defensive/submissive	2.99+0.82	58.58+5.85
<i>5th day</i>		
Attack	60.63+3.81**	0.50+0.05
Threat	62.89+6.79**	0.75+0.10
Avoidance/flee	0.40+0.10	58.96+5.43**
Defensive/submissive	0.20+0.10	55.92+3.13**

Discrete behaviours were recorded during the 10 min confrontation. Values indicate means+S.E.M. for behaviours displayed by all pairs undergoing confrontation. Behaviours were pooled into the general categories of attack, threat, avoidance/flee and defensive/submissive behaviour. Quantification was carried out after the first (day 1), indicated for reference, and after the last aggressive (day 5) encounter, used for statistical analysis. Comparisons of mean values obtained after the 5th day of confrontation indicated significant effects for the variables attack [ $t=21.077$ ,  $p<0.0001$ ], threat [ $t=13.010$ ,  $p<0.0001$ ], avoidance/flee [ $t=27.418$ ,  $p<0.0001$ ] and defensive/submissive [ $t=20.856$ ,  $p<0.0001$ ]. \*\* $p<0.01$ .

paired floor during post-conditioning. All data are shown as mean±S.E.M.

## 3. Results

### 3.1. Induction of c-Fos expression in mouse forebrain following MDMA treatment

The results of c-Fos measurements indicated that forebrain induction following MDMA exposure in mice was dose-dependent in most areas studied (Figs. 2 and 3). Induction was statistically significant in all regions investigated. In the prefrontal cortex, MDMA evoked c-Fos expression in the

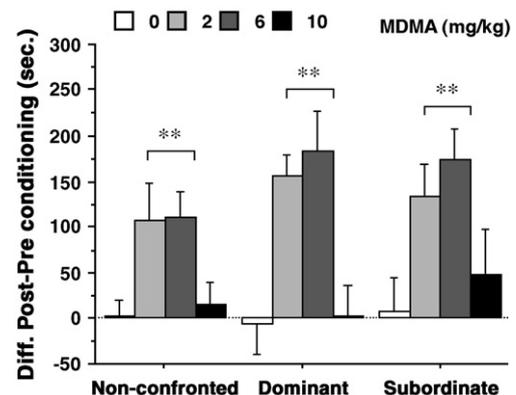


Fig. 5. Differences between post-conditioning and pre-conditioning times spent in the drug-paired environment in the CPP test following exposure to MDMA (0, 2, 6, 10 mg/kg). ANOVA and post-hoc comparisons showed that the doses of 2 and 6 mg/kg of MDMA significantly induced CPP in all experimental groups. \*\*Significantly different from saline,  $p<0.01$  (Newman–Keuls test using sampling error of significant ANOVA as denominator).

prelimbic cortex (PLC) [ $F(3,18)=19.901$ ,  $p<0.001$ ] and the cingulate cortex (CC) [ $F(3,18)=7.028$ ,  $p<0.003$ ]. In the striatum, MDMA induced significant levels of c-Fos in the nucleus accumbens (NAc) [ $F(3,18)=13.749$ ,  $p<0.001$ ] and in the dorsal striatum (DS) [ $F(3,18)=29.527$ ,  $p<0.001$ ]. High levels of c-Fos expression were observed in the central nucleus of the amygdala (CeA) [ $F(3,18)=12.194$ ,  $p<0.001$ ] and in one of its main projection targets, the bed nucleus of the stria terminalis (BNST) [ $F(3,18)=12.292$ ,  $p<0.001$ ]. Only the dose of 10 mg/kg of MDMA induced significant levels of c-Fos expression in the CA1 area [ $F(3,18)=5.654$ ,  $p<0.007$ ] and in the dentate gyrus (DG) of the hippocampus [ $F(3,18)=6.122$ ,  $p<0.005$ ], whereas induction was dose-dependent in the CA3 region [ $F(3,18)=13.749$ ,  $p<0.001$ ]. These results suggested that the doses selected for MDMA were able to produce significant levels of activation in several key corticolimbic nuclei implicated in drug-induced reward and encoding of spatial information. Thus this dose range was considered appropriate for the place conditioning studies.

### 3.2. Serum testosterone and corticosterone levels following acquisition of social status

We sampled serum testosterone and corticosterone from non-confronted mice and from mice subjected to agonistic confrontation ( $n=41$ , Fig. 1). ANOVA [ $F(2,38)=5.451$ ,  $p<0.008$ ] and post-hoc comparisons indicated that mice categorized as dominants showed significantly greater concentrations of testosterone than subordinate littermates and non-confronted mice (Fig. 4). On the contrary, ANOVA [ $F(2,36)=13.066$ ,  $p<0.001$ ] and post-hoc comparisons revealed that dominant mice had significantly lower corticosterone levels than submissive and non-confronted mice. Also, subordinate mice had significantly higher concentrations of corticosterone than non-confronted mice (Fig. 4). Thus these observations demonstrate that the paradigm of cohabitation and social encounter used in the present experiments led to a double dissociation of dominant and submissive mice in terms of serum levels of both corticosterone and testosterone.

### 3.3. Behavioural observations following agonistic encounters

During sessions of agonistic confrontation, mice quickly and invariably established social status manifested in clearly asymmetric behaviours. Following 5 days of aggressive confrontation mice consolidated patterns of responding (aggression vs. submission) already evident in the first agonistic session. Quantification is shown after the first and last agonistic session (Table 1).

### 3.4. MDMA-induced place preference in confronted and non-confronted mice

The results of the CPP experiments with MDMA in isolated, dominant and subordinate mice were clear-cut. MDMA evoked CPP with similar strength and at the same doses in all experimental groups. The ANOVA showed no effects of Status [ $F(2,124)=0.897$ ,  $p<0.410$ ], and no interaction of Status with Treatment [ $F$

(6,124)=0.440,  $p<0.851$ ]. There was, however, a highly significant effect of the Treatment [ $F(3,124)=14.831$ ,  $p<0.001$ ]. This effect was further explored with post-hoc tests which showed that conditioning with doses of 2 and 6 mg/kg, but not with 10 mg/kg, of MDMA significantly increased the time spent in the drug-paired compartment during the test (Fig. 5). The lack of interaction Status  $\times$  Treatment in the ANOVA clearly indicated that the effects of MDMA in the CPP test were independent of the social categories in which subjects had been allocated and, by implication, of their hormonal status. We performed an overall ANOVA with repeated measures (with 2 levels, pre-conditioning and post-conditioning values) and two between factors (Status and Treatment), which yielded similar results (a significant Treatment effect, a significant interaction with the repeated measures factor, but no interaction of the main effects with the Status factor).

## 4. Discussion

The results of the present experiments included several key observations. We studied MDMA-induced CPP in non-confronted, dominant and subordinate mice which could be dissociated on the basis of behavioural parameters and hormone levels phenotypically linked to social hierarchy and chronic stress. The observations in the CPP paradigm used here demonstrated that the rewarding efficacy of MDMA was independent of social status and social stress, with only low and moderate doses being capable of inducing significant CPP in both non-confronted mice and mice exposed to aggressive social interactions.

The present experiments are the most complete study to date on c-fos protein expression induced by MDMA in mouse forebrain. Acute injections of MDMA in non-confronted mice produced an increase in c-Fos protein expression in distinct telencephalic regions whose activation reflects the rewarding efficacy of several neuroactive compounds, confirming and extending previous observations in mice (Salzmann et al., 2003; Navarro et al., 2004) and rats (Dragunow et al., 1991; Erdtmann-Vourliotis et al., 1999; Stephenson et al., 1999). Previous data showed that MDMA-induced CPP was linked to extracellular regulated kinase activation and c-fos and egr-1 expression. Inhibition of this signalling cascade suppressed both CPP and c-Fos induction by MDMA (Salzmann et al., 2003). In the present studies, low and moderate acute doses of MDMA significantly elevated c-Fos induction in the NAc, prefrontal cortex, CeA, BNST and CA3, whereas the high dose of MDMA increased c-Fos expression at all sites investigated. These results suggested that c-Fos inducibility by MDMA in mouse forebrain, which followed a dose-dependent pattern, might not be univocally linked to the ability of MDMA to induce CPP, at least in non-confronted mice. It is unclear why the high dose of MDMA, which elicited strong c-Fos activation in frontal cortex, striatum, amygdala and hippocampus following acute challenge, was inefficacious at inducing CPP in these mice. Inverted U-shaped dose-effect curves have been found for other drugs, such as alcohol (Philpot et al., 2003) and buprenorphine (Tzschentke, 2004) in the CPP paradigm. With regards to

MDMA, several explanations for this effect are possible. First, the strong motor-stimulant and hallucinogenic properties of high doses of MDMA (Cole and Sumnall, 2003) may interfere with the processing and encoding of spatial information relevant to the context. Second, previous studies have shown that high doses of MDMA (>8 mg/kg) have anxiogenic-like properties in situations of social interaction (Navarro et al., 2004). Such high doses might induce aversive effects or mixed rewarding and aversive effects, affecting CPP as a result.

Experiments carried out so far investigating the effects of MDMA in the CPP paradigm have yielded disparate results. In CD-1 mice only high doses of MDMA have been documented to produce CPP (Salzmann et al., 2003; Robledo et al., 2004). Differences in experimental designs, which may result in carry-over effects from drug to vehicle conditioning (Tzschentke, 2004), age-related effects (Philpot et al., 2003) and, more importantly, genotypic variations between species or strains of mice (Belzung and Barreau, 2000) can all influence drug-induced place conditioning. Also, housing conditions differed in the present experiments (singly housed or cohoused) from other studies (group housed) (see Salzmann et al., 2003; Robledo et al., 2004), which might have increased the sensitivity of the mice, thereby shifting the dose response to MDMA to the left. The observations presented here, though not contributing directly to resolve these inconsistencies, were the first to show CPP conditioning in the OF-1 strain of mice in response to MDMA treatment.

Chronic stress resulting from asymmetric social interaction has been associated with changes in endocrine regulation. Neurohumoral correlates of social stress include the sympathetic adrenal medullary and hypothalamic pituitary adrenal responses. Repeated defeat provoked adrenocorticotrophic hormone release and rise in corticosterone levels, whilst testosterone levels increased with success in agonistic social encounters (Henry, 1992; Blanchard et al., 1993, 2001). We used a model of social interaction which clearly identified mice as dominant or submissive on the basis of their agonistic and non-agonistic behaviours (Kudryavtseva et al., 1991). To further ascertain such categorization, measurements of serum hormone concentrations of testosterone and corticosterone demonstrated that high-rank mice had higher testosterone, but sharply lower corticosterone, levels than low-rank mice, confirming therefore prior observations in analogous situations.

Previous evidence demonstrated that the experience of social defeat enhanced the sensitivity to addictive substances, such as cocaine (Morgan et al., 2002; Miczek et al., 2004), amphetamine (Covington and Miczek, 2001; de Jong et al., 2005) and alcohol (Hilakivi-Clarke and Lister, 1992; Kudryavtseva et al., 2006). In considering such evidence, the results presented demonstrating that MDMA was equipotent across a dose range at inducing CPP in non-confronted, dominant and subordinate mice were striking. These findings raise important questions on the neural substrates mediating MDMA-induced reward, as measured by CPP, which were unaffected by social ranking and the experience of episodic social stress. Central to the neuropharmacology of MDMA is the reuptake and release of serotonin (Morton, 2005; Easton and Marsden, 2006). Chronic

social stress was shown to correlate with changes in central serotonin transmission such that subordinate subjects showed increased 5-HIAA/5-HT ratios in a number of brain areas, and alterations of 5-HT<sub>1A</sub> receptor binding at some sites (Blanchard et al., 1991, 1993; Summers et al., 1998). We did not examine serotonergic neurotransmission or serotonin receptor sites in the present experiments. However, had changes in this neurotransmitter system been produced in the mice as a result of agonistic confrontation, they certainly did not have any impact on MDMA-induced CPP.

MDMA also has micromolar potency for muscarinic M<sub>1</sub>, alpha-2 adrenergic and histamine H<sub>1</sub> receptors (Battaglia et al., 1988; Fischer et al., 2000), and produces the release of dopamine and acetylcholine at several brain sites (Nair and Gudelsky, 2006). Dopamine is a neurotransmitter which is likely to play a major role in social defeat stress and the psychopharmacological effects of abused drugs. Expression of brain-derived neurotrophic factor in the mesolimbic dopamine pathway was shown to be essential in mice for the development of social aversion linked to repeated defeat (Berton et al., 2006). Moreover, data accrued using a variety of different procedures supports the involvement of dopamine in the rewarding effects of both stimulant and opiate drugs (Phillips and Fibiger, 1987; Bardo, 1998). MDMA evoked dopamine release in the nucleus accumbens and other dopaminergic regions (see Green et al., 2003). However, the implication of dopamine receptors in MDMA-induced CPP is insufficiently documented. Other transmitter systems, including neuropeptides, might contribute to the rewarding effects of MDMA. Recent evidence suggested that the endocannabinoid system regulated both MDMA-induced place preference (Brida et al., 2005) and MDMA intracerebral self-administration (Brida and Sala, 2002). The relationships of these potential substrates of MDMA-induced reward with the neurochemical effects of social stress have not been explored. Notwithstanding the neurochemical complexity of the effects induced by MDMA, the data presented here suggested that the mechanisms underlying MDMA-induced CPP and reward remained unmodified after the experiences of social defeat or victory and the acquisition of social status in mice.

## 5. Conclusion

Social stress has been previously shown to influence the behavioural effects of a number of abused drugs. However, the present experiments demonstrated that mice which experienced social defeat and exhibited behavioural and hormonal changes associated to that experience did not respond differently to MDMA in the CPP paradigm we designed, when compared to mice which were victorious and mice which did not undergo social confrontation. However, we do not rule out the possibility that the use of alternative experimental conditions in the CPP paradigm, such as fewer conditioning trials or analysis of delayed post-conditioning tests, may reveal group differences in response to MDMA. In summary, the observations presented indicate that the rewarding effects of MDMA, as assessed in the CPP paradigm described, are not dependent on social status and are not modulated by social stress. In this regard, MDMA

critically differs from the drugs of abuse so far investigated in this model.

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