Available online at www.sciencedirect.com
ScienceDirect
Progress in Neuro-Psychopharmacology & Biological Psychiatry 31 (2007) 191–199
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

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

G. Rodriguez-Alarcón [a], J.J. Canales [b,c,*], A. Salvador [a]

[a] *Department of Psychobiology, University of Valencia, Valencia, Spain*
[b] *Laboratory of Biopsychology and Comparative Neuroscience, Cavanilles Institute, University of Valencia, Paterna, Valencia, Spain*
[c] *CEU Institute on Drugs and Addictive Behaviour, University Cardenal Herrera, Alfara del Patriarca, Valencia, Spain*

Received 27 May 2006; received in revised form 24 August 2006; accepted 25 August 2006
Available online 4 October 2006

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.

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 laboratory 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.

* Corresponding author. Laboratory of Biopsychology and Comparative Neuroscience, Cavanilles Institute, University of Valencia, Poligono de la Coma s/n Paterna-46980 Valencia, Spain. Tel.: +34 96 354 3768; fax: +34 96 354 3670.
E-mail address: juan.canales@uv.es (J.J. Canales).

0278-5846/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.pnpbp.2006.08.018

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±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, $NaKPO_4$). The brains were post-fixed for 24 h, rinsed in 0.1 M PB and transferred to 20% glycerol solution. Free-floating coronal 25 μm 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_2O_2, 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_2O_2 complex with nickel ($NiSO_4$) 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^2. The corticolimbic areas examined included the nucleus accumbens (a portion of ca. 1 mm^2 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