Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuropharmacology 50 (2006) 884–896
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Partial lesion of the serotonergic system by a single dose of MDMA results in behavioural disinhibition and enhances acute MDMA-induced social behaviour on the social interaction test

Romeo D. Ando [a], Anita Benko [a], Linda Ferrington [b], Eszter Kirilly [a], Paul A.T. Kelly [b], Gyorgy Bagdy [a,c,*]

[a] Laboratory of Neurochemistry and Experimental Medicine and Hungarian Academy of Science, Group of Neuropsychopharmacology and Department of Vascular Neurology, Semmelweis University, National Institute of Psychiatry and Neurology, Huvosvolgyi ut 116, 1021 Budapest, Hungary
[b] Division of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2XU, UK
[c] Group of Neuropsychopharmacology, Hungarian Academy of Science and Semmelweis University, Budapest, Hungary

Received 8 July 2005; received in revised form 21 November 2005; accepted 16 December 2005

Abstract

The acute effects of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) on anxiety-related behaviours were studied using indices of social interaction in Dark Agouti (DA) both drug naive rats and those pretreated with MDMA (15 mg/kg i.p.) 3 weeks earlier. The functional neuroanatomy of these MDMA effects was visualised using 2-deoxyglucose imaging of local cerebral glucose use (LCMRglu), whilst MDMA-induced serotonergic neurotoxicity was measured by radioligand binding with [^{3}H]paroxetine. Acute MDMA alone markedly decreased most typical elements of social interaction but increased adjacent lying, a behaviour that also contains social elements. In animals pre-exposed to MDMA, decreased [^{3}H]paroxetine binding indicated serotonergic terminal depletion, and in these animals significant increases in locomotor activity, exploratory behaviour and aggressive behaviour were found. Both behavioural effects and also the metabolic activation induced by acute MDMA were potentiated in rats previously exposed to the drug. In conclusion, a single dose of MDMA caused marked changes in social behaviour acutely that might be interpreted either as a decrease or increase in anxiety. Three weeks after MDMA a behavioural disinhibition similar to psychomotor agitation, a symptom connected to depression or mania, and a sensitization to the acute effects of MDMA are apparent in both the behavioural and brain metabolic effects of the drug.

Keywords: 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy); Depression; Impulsivity; Anxiety; Social behaviour; Local cerebral glucose utilization

1. Introduction

The illicit psychostimulant drug ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is a widely abused, recreational amphetamine-derivative, popular amongst many young people throughout the world. MDMA administration causes a rapid release of monoamines, mainly serotonin (5-HT) and dopamine, giving rise to a series of behavioural responses that are consistent with the psychopharmacological profile of the drug (Green et al., 1995, 2003). Human users report increased emotional and sensory sensitivity, euphoria, psychomotor agitation, strong feelings of love and empathy towards others, restlessness, and increased physical energy (Vollenweider et al., 1998, 2002). However, acute adverse effects are not uncommon, and increased anxiety and panic attacks have been also reported (Pallanti and Mazzi, 1992; Whitaker-Azmitia and Aronson, 1989).

There is extensive evidence from most experimental animal species that MDMA causes dose-dependent, selective and

* Corresponding author. Laboratory of Neurochemistry and Experimental Medicine and Hungarian Academy of Science, Group of Neuropsychopharmacology and Department of Vascular Neurology, Semmelweis University, National Institute of Psychiatry and Neurology, Huvosvolgyi ut 116, 1021 Budapest, Hungary. Tel.: +36 1 3915407; fax: +36 1 3915408.
E-mail address: bag13638@mail.iif.hu (G. Bagdy).

0028-3908/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2005.12.010

persistent damage to serotonergic neurons. A few days after initial exposure reductions in 5-HT and 5-HIAA levels are found in parallel with reductions in the density of serotonergic terminals (Sprague et al., 1998). Serotonin is a neurotransmitter implicated in many behavioural and psychological functions and both long term and acute alterations in 5-HT function play a role in modulating, amongst other things, anxiety-related behaviours (Griebel, 1995; Iversen, 1984). Therefore, the possibility of long-lasting MDMA-induced serotonergic dysfunction in human users and as a consequence the emergence of psychiatric problems in both present and past users, are of particular concern. Although causality between MDMA ingestion and psychiatric illnesses is difficult to establish in human studies, it appears that heavy ecstasy users, at least, are more susceptible to disorders like anxiety, depression and impulsiveness (Butler and Montgomery, 2004; Green, 2004; Parrott, 2000, 2001; Parrott et al., 2000). In contrast, there are also data that controlled ecstasy use may have some therapeutic potential in post-traumatic stress disorder, and in particular may be of use in the support of rape and cancer victims (Green, 2004).

Previous studies aimed at investigating the long-term effects of exposure to neurotoxic doses of MDMA upon anxiety have yielded contradictory results but have indicated that the acute effects of MDMA on anxiety depend greatly on many factors, including the experimental test and the species. In Wistar rats, MDMA treatment consistently resulted in increases in anxiety which were associated with serotonergic terminal depletion several weeks after MDMA treatment. In contrast, in the Dark Agouti rat strain, a model of human CYP2D6 deficiency, MDMA decreased anxiety and increased locomotor activity in the elevated plus maze test several weeks after administration, although neither the acute nor the long-term behavioural effects of MDMA in this strain have been tested using social interaction tests or other relevant anxiety tests. This is of particular importance because in Wistar rats, in keeping with the human entactogenic profile of the drug, anxiolytic effects were described acutely in the social interaction test, but anxiogenic properties were found in other tests. Acute effects of MDMA in the social interaction test in Dark Agouti rats were, however, not tested. Furthermore, one may presume that acute effects of MDMA might be altered after significant serotonergic damage but, to our knowledge, no studies to date have compared the acute effects of MDMA in the social interaction test in drug naive rats and rats previously exposed to a neurotoxic dose of the drug.

In our study, we aimed to investigate the acute and long-term effects of MDMA in Dark Agouti rats in the social interaction (SI) anxiety test. In parallel experiments, the metabolic mapping properties of 2-deoxyglucose autoradiographic imaging, which we and others have used previously to study acute effects of MDMA, were used to identify the functional neuroanatomy that might possibly underpin long-lasting behavioural responses to MDMA. Particular attention was paid to those areas of the brain known to be constituent parts of the anxiety pathway, mediating both anxiety and impulsive behaviour, and to detect where in the brain the functional significance of serotonergic terminal depletion might be most clearly manifest. MDMA-induced serotonergic terminal depletion was assessed by measuring [^{3}H]paroxetine binding to 5-HT uptake sites in the brain.

2. Materials and methods

2.1. Animals

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health "Principles of Laboratory Animal Care" (NIH Publications No. 85-23, revised 1985), as well as specific national laws (the Hungarian Governmental Regulation about animal studies, December 31, 1998 and the United Kingdom Animals (Scientific Procedures) Act). Permission was also obtained from local ethical committees. Male Dark Agouti rats (Harlan, Olac Ltd, Shaw's Farm, Blackthorn, Bicester, Oxon, UK, aged 4–5 weeks at arrival) were used in the experiments. The animals (four per cage) were kept under controlled environmental conditions (temperature at 21 ± 1 °C, and a 12 h light–dark cycle starting at 06.00 h). Standard food and water were freely available.

2.2. Drug administration

(±)3,4-Methylenedioxymethamphetamine hydrochloride (MDMA, certified reference compound, purity >99.5%) was provided by Sanofi-Synthelabo-Chinoin, Hungary. The drug was dissolved in 0.9% NaCl at a dose equivalent to 15 mg/kg free base and was injected intraperitoneally in a volume of 1 ml/kg. Control animals received an injection of 0.9% NaCl in a volume of 1 ml/kg.

2.3. Social interaction test

Behavioural testing was conducted under two different conditions, familiar or unfamiliar arena (Table 1). In both testing situations, rats received MDMA or saline pretreatment 3 weeks before the test. At the first treatment animals were 7 weeks old.

To investigate the acute effects of the drug in the familiar arena condition, rats received either MDMA or saline injection on the test day, while in the unfamiliar arena only saline injection was administered on the test day (Table 1). A square open field arena (60 × 60 × 40 cm) marked in 10 × 10 cm compartments by lines on the floor was used to monitor social interaction as described previously (To et al., 1999). When familiarization was used, rats were pre-exposed three times to the test condition (including injections with saline) on the days preceding the test. On the test days, rats were injected with MDMA or saline and 20 min after drug administration two animals were placed together in a low light (5 lx, red light) arena in an adjacent darkened room for 7.5 min. Each rat was tested for social interaction with an unknown test partner that did not differ by more than 10 g in weight. Both members of any given pair had the same prior familiarisation experience and the same drug treatment. At the end of each test the box was thoroughly wiped with detergent and dried. All pairs were tested between 09:00 and 13:00 h.

The behaviour of the animals was recorded on video tape and was scored manually at a later time using a computer program designed for the purpose.

Table 1
Drug treatment design in the social interaction test

SI test	Day −21	Day 1, test day	Pairs of animals
Familiar arena	MDMA	MDMA	6
	MDMA	SAL	6
	SAL	MDMA	6
	SAL	SAL	6
Unfamiliar arena	MDMA	SAL	5
	SAL	SAL	5

Treatments: MDMA, 15 mg/kg i.p.; SAL, 0.9% NaCl. Testing occurred on day 1.

To generate the final results, average scores were calculated from the raw scores of two experienced observers. The following behaviours were included in total social interaction: sniffing partner, anogenital sniffing, peaceful following, grooming partner, under-crawling, over-climbing, chasing, aggressive grooming, dominant posture, submissive posture, biting, boxing, kicking, pushing, wrestling. Self-grooming, number of line crossings (a measure of locomotor activity) and rearing were also scored. These behaviours including ambulation, self grooming, rearing, reversing, sniffing air, walls and floor were summarised as non-social activities. Behaviours including chasing, aggressive grooming, dominant posture, submissive posture, biting, boxing, kicking, pushing, and wrestling were defined as aggressive social interaction (Kantor et al., 2000). The sum of peaceful and aggressive social interaction was defined as total social interaction.

Adjacent lying, which was previously reported to be a very frequent behavioural element after acute MDMA administration (Morley et al., 2005; Morley and McGregor, 2000), was also scored during the familiar arena testings. Adjacent lying was defined as a situation when animals lie next to each other (within a distance of 1 cm from skin to skin). The sum of passive and active adjacent lyings are given in Section 3 and the figures. Adjacent lying including active elements was more frequent, especially after acute MDMA treatment. Adjacent lying was considered active when animals displayed any elements of activity towards each other, including, e.g. correcting their position to maintain the contact, but clear social interaction was not present. Passive adjacent lying was defined as the lack of any behavioural signs towards each other.

2.4. Measurement of local cerebral glucose utilization and [^{3}H]paroxetine autoradiographic binding

The measurement of local cerebral glucose utilisation (LCMRglu) performed using the fully quantitative 2-[^{14}C]deoxyglucose (Sokoloff et al., 1977) autoradiographic technique in rats, 21 days after pretreatment with either MDMA or saline. The measurement protocols were in complete accordance with the methodology as originally published (Sokoloff et al., 1977) and as previously detailed from this laboratory (Kelly et al., 1995), with the addition of an in-dwelling i.p. cannula inserted on the day of the experiment to allow the atraumatic, acute injection of MDMA (Quate et al., 2004). Briefly, on the final experimental day, rats from both pretreatment groups were injected with MDMA (15 mg/kg) via the in-dwelling intraperitoneal cannula to produce two groups: saline pretreatment + acute MDMA ($n = 7$) and MDMA pretreatment + acute MDMA ($n = 7$). Similarly, rats were injected acutely with saline to provide two further groups; saline pretreatment + acute saline ($n = 7$) and MDMA pretreatment + acute saline ($n = 7$). Fifteen minutes after the acute injection, the measurement of LCMRglu was initiated with the intravenous injection of 2-[^{14}C]deoxyglucose (30 μCi in 0.75 ml saline) administered at a constant rate over the first 30 s of the experiment. At the end of the measurement period the rats were killed, the brains immediately dissected out intact, and rapidly frozen in precooled 2-methylbutane (−45 °C). Frozen brains were sectioned in a cryostat for the preparation of autoradiograms.

Sections adjacent to those used for autoradiographic measurement of LCMRglu were thaw-mounted onto gelatin covered glass slides and stored at −70 °C for subsequent [^{3}H]paroxetine autoradiographic binding analysis of 5-HT uptake sites according to the protocol described by De Souza and Kuyatt (1987), but with the addition of two 5 min prewashes in buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, pH 7.7 at 23 °C) to remove ^{14}C-tracers from the tissue (De Souza and Kuyatt, 1987; Sharkey et al., 1991). Sections were then incubated in the same buffer containing a saturating concentration (250 pM) of [^{3}H]paroxetine (specific activity 23.1 Ci/mmol; New England Nuclear). Non-specific binding was defined in adjacent sections by [^{3}H]paroxetine binding in the presence of 4 μM citalopram. Following incubation, the sections were washed in buffer, dipped in deionised water and rapidly dried under a stream of cold air. The slides, together with a set of [^{3}H]containing standards (Amersham Microscales) were apposed to x-ray film (Amersham Hyperfilm) in a light-tight cassette and stored at −70 °C for 4–6 weeks. The films were processed according to the manufacturer's instructions.

Analysis of 2-[^{14}C]deoxyglucose and [^{3}H]paroxetine autoradiograms was performed using a computer-based image analysis system (MCID/M5+). Local tissue isotope concentrations were derived from the optical density of autoradiographic images of brain tissue, relative to appropriate ^{14}C- or ^{3}H-containing standards. LCMRglu was calculated using the operational equation for the technique (Sokoloff et al., 1977), and radioligand binding was calculated from the tissue concentrations and the specific activity of the [^{3}H]paroxetine. Regions of interest for analysis in the 2-deoxyglucose experiments were chosen on the basis of putative involvement in processing of anxiety and/or fear, whilst regions of interest for analysis in radioligand binding experiments were chosen on the basis of normal 5-HT terminal densities. Data were analysed using Student's *t*-test for grouped data with acceptable levels of significance set at $P < 0.05$.

2.5. Statistical methods

Data obtained from the social interaction test were analysed using one- or two-way analysis of variance (ANOVA), and the Tukey test was used for post-hoc comparisons. Groups consisted of 10–12 animals in the social interaction test (Table 1). Each rat was tested only once. Autoradiographic data were analysed using Student's *t*-test for grouped data with acceptable levels of significance set at $P < 0.05$. Where multiple pair-wise comparisons were required, the Bonferroni correction factor was applied. Data in the following figures and text are expressed as mean ± SEM.

3. Results

3.1. Social interaction test

3.1.1. Acute effects of MDMA in drug-naive rats

MDMA decreased the time of regular total social interaction compared to vehicle control (acute treatment effect, $F_{1,44} = 36.63$, $P < 0.001$, Fig. 2a). In contrast, the time of adjacent lying was markedly increased ($F_{1,44} = 25.06$, $P < 0.001$, Fig. 1), and if we included adjacent lying in the social interaction behaviour, this sum was also markedly increased by MDMA (acute treatment effect, $F_{1,44} = 8.434$, $P < 0.01$, Fig. 2b). Adjacent lying included also some active elements towards each other, including, e.g. correcting their position to maintain the contact, and some, but not typical sniffing, or turning their head towards each other, but typical clear social interaction was not present. There was also a significant decrease in locomotor activity (Fig. 1c; treatment effect, $F_{1,44} = 49.252$, $P < 0.001$) as indicated by the number of line-crossings compared to vehicle treated animals. In MDMA treated rats self-grooming (treatment effect, $F_{1,44} = 32.690$, $P < 0.001$) and rearing (treatment effect, $F_{1,44} = 173.653$, $P < 0.001$) were absent (Fig. 1d and 1e, respectively).

3.1.2. Long-term effects of MDMA

In the familiar arena condition, 3 weeks after MDMA (15 mg/kg), there was no significant change in the time of total social interactions (pretreatment effect, $F_{1,44} = 3.088$, $P = 0.086$, Fig. 2b), similarly, there was no change in time of social interactions when adjacent lying was excluded ($F_{1,44} = 1.054$, $P = 0.310$, Fig. 2a). Adjacent lying was also unaltered ($F_{1,44} = 2.031$, $P = 0.16$, Fig. 1a,c).

There was, however, significant change in social behaviour. Namely, aggressive behaviour was not present in the VEH-VEH group (Figs. 1c and 3a,c), but significant, albeit small amount was observed in the MDMA-VEH group (pretreatment effect for the time of aggressive behaviours; $F_{1,44} = 5.246$, $P = 0.026$, Figs. 1c and 3a,c; pretreatment effect for the number of aggressive behaviours, $F_{1,44} = 4.859$,

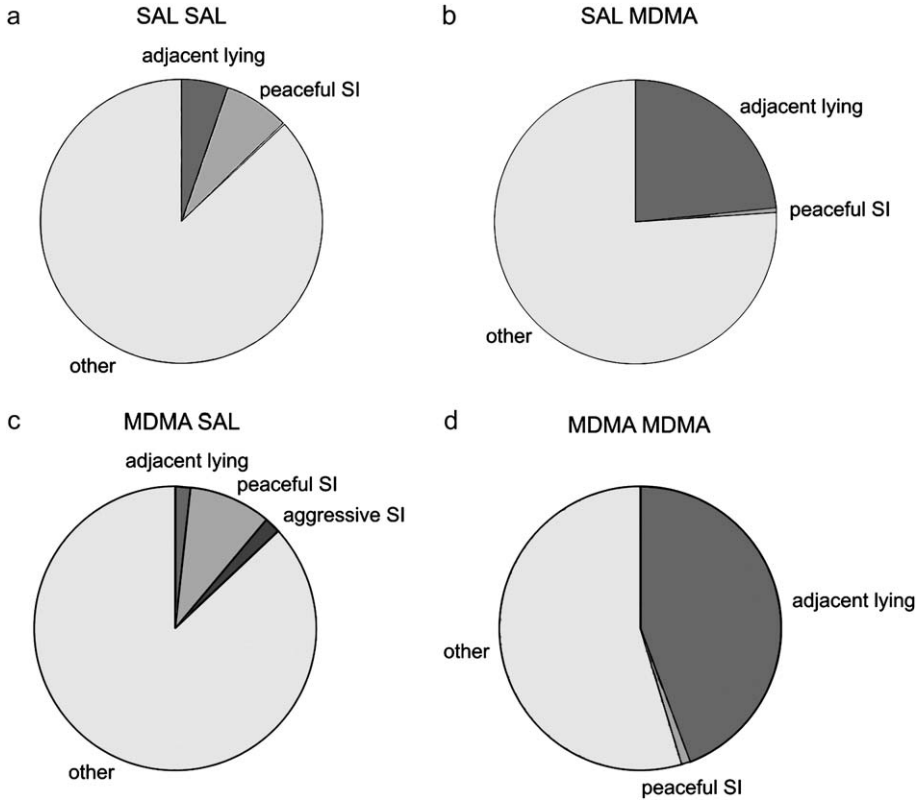

Fig. 1. The ratio of the duration of behavioural elements in familiar arena in MDMA-treated and vehicle-treated rats after acute treatment, in drug-naive animals and in animals exposed to MDMA 3 weeks earlier ($n = 12$). The charts represent the entire 7.5-min period of the SI test. Peaceful interaction was defined as the total social interaction minus aggressive social interaction. Adjacent lying contained also active social elements which were, however, different from typical social interaction.

$P = 0.033$). This aggressive behaviour consisted of dominant posture, chasing and aggressive grooming, while overt aggressive behaviours, like biting, boxing and kicking were not present. This increase in aggressive behaviour 3 weeks after MDMA was not significant in animals tested in the unfamiliar arena (Fig. 3b,d).

MDMA significantly increased rearing (pretreatment effect, $F_{1,44} = 14.803$, $P < 0.001$, Fig. 2d) and line crossings compared to the vehicle control. In the latter case, although the pretreatment effect was not significant, pretreatment × treatment interaction was significant (pretreatment effect, $F_{1,44} = 1.232$, $P = 0.274$, pretreatment × treatment interaction $F_{1,44} = 6.483$, $P = 0.015$). In the unfamiliar arena condition there were no significant changes in these behaviours compared to the control 3 weeks after the MDMA treatment (data not shown).

3.1.3. Acute effects of MDMA in rats previously exposed to the drug

The acute effects of MDMA in MDMA-pretreated animals were, in general, qualitatively similar when compared to the vehicle-pretreated group. The acute effects of MDMA were not decreased in MDMA-pretreated animals in either behaviours. Moreover, the acute effects of MDMA were potentiated in the case of total time of social behaviour including adjacent lying (interaction effect, $F_{1.44} = 4.042$, $P = 0.0505$, Fig. 2b), the total number of non-social activities ($F_{1,44} = 12.191$, $P = 0.0011$, Fig. 2f) and line crossings ($F_{1,44} = 6.483$, $P = 0.015$, Fig. 2c). In addition, significant (pretreatment × treatment) interactions were also found in both measures of aggression (time, $F_{1,44} = 5.246$, $P = 0.026967$ and number, $F_{1,44} = 4.859$, $P = 0.0329$, Fig. 3a,c, respectively), although aggression was not observed after acute MDMA in either saline or MDMA-pretreated animals.

3.2. Autoradiographic mapping of cerebral function and pharmacology

In animals pretreated with MDMA 3 weeks previously, LCMRglu was generally decreased from saline pretreated control (Table 2). Significant decreases were observed in 17 of the 29 anatomically diverse brain areas included in the analysis, ranging from −25% in medial amygdala to 14% in nucleus accumbens but including elements of neocortex (prefrontal cortex, −20%), hippocampus (CA2, −24%), and the extrapyramidal motor system (substantia nigra, −22%). In parallel to these changes in functional activity, [^{3}H]paroxetine binding to 5-HT uptake sites was also significantly decreased (Table 3). The most marked effects were observed in parietal (−80%) and entorhinal cortex (−74%), hippocampal CA1 (−68%) and mediodorsal thalamus (−69%).

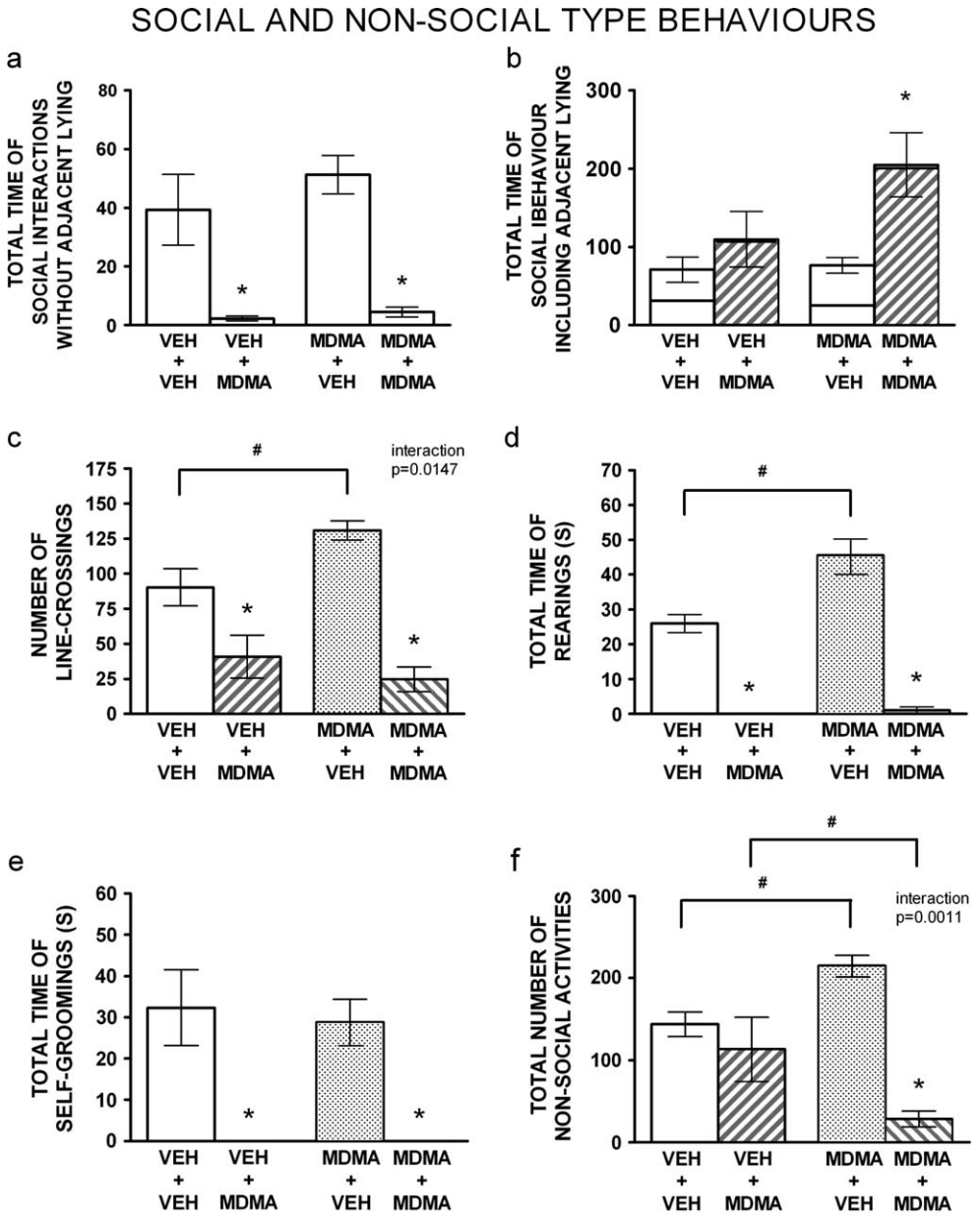

Fig. 2. Acute effects of MDMA in familiar arena upon time of social interaction without adjacent lying (a) total time of social interaction, with adjacent lying (b), line-crossings (c), rearings (d), self-groomings (e) and non-social activities (f) in drug-naive animals and in animals exposed to MDMA 3 weeks earlier ($n = 12$). In case of (b), bars are divided: lower parts denote adjacent lying while upper parts denote the remaining, typical social interactions (values of (a)). Pretreatment–treatment interactions of ANOVA are significant where noted. *Significant difference compared to corresponding acute vehicle treated group ($P < 0.05$). #Significant difference compared to corresponding vehicle-pretreated group ($P < 0.05$).

In contrast to the persistent (chronic) effects of a single exposure to MDMA, the same dose of drug produced marked increases in LCMRglu in previously drug-naive animals which were similar to those reported by us previously. However, in rats previously exposed to the drug, the effects of MDMA were significantly potentiated in most, but not all, brain areas analysed (Figs. 4 and 5); exceptions included parietal cortex, hippocampal CA1, globus pallidus, and lateral amygdala. Although some of the apparent potentiation could be accounted for by the fact that the baseline for comparison was lower in MDMA-pretreated rats, this is insufficient to explain the effect in those areas where acute MDMA had no significant effect in drug naive rats (e.g. entorhinal cortex) or where the drug produced significant decreases in drug naive rats, but a significant increase in MDMA-pretreated rats (e.g. frontal cortex).

4. Discussion

The Dark Agouti rat is a useful experimental tool with which to model MDMA exposures that are pertinent to the human experience of the drug. Similarly to many other drugs, the effects of MDMA vary according to the extent of metabolism. MDMA is metabolised principally by the cytochrome P450 isoenzyme, 2D6 or debrisoquine hydroxylase, an enzyme

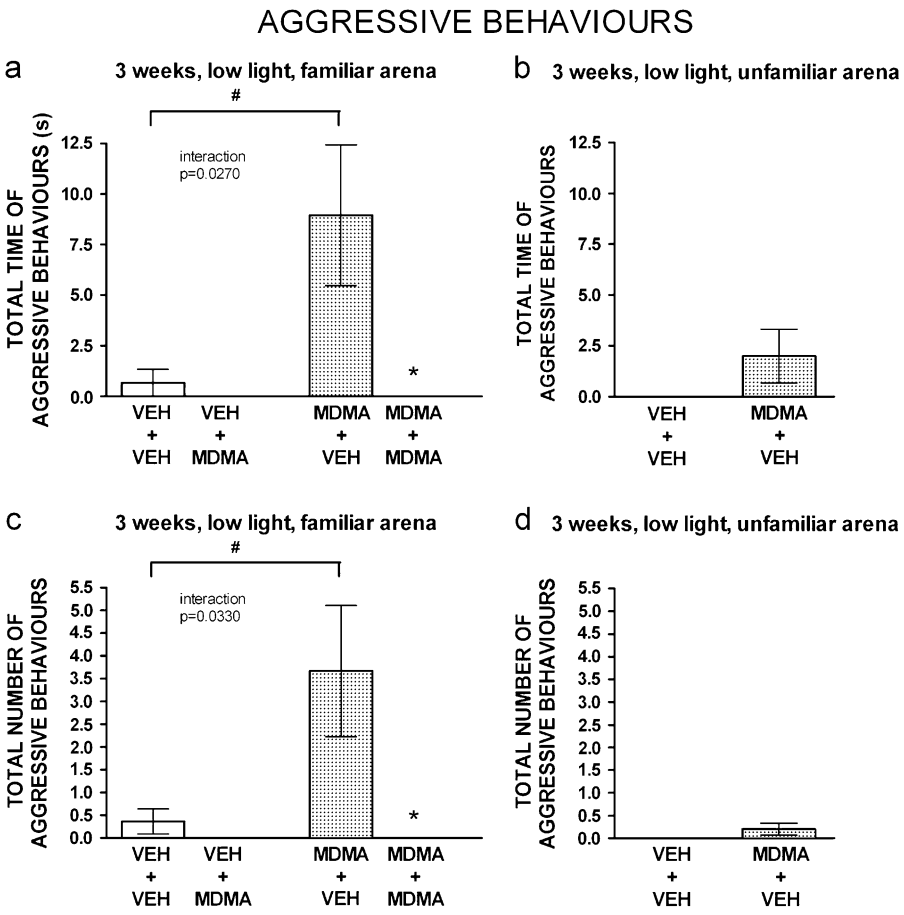

Fig. 3. Effects of MDMA on aggressive behaviours in familiar arena. Acute effects of MDMA upon aggressive behaviours in drug-naive animals and in animals exposed to MDMA 3 weeks earlier (a,c) ($n = 12$). Aggressive behaviours in animals exposed to MDMA 3 weeks earlier in the unfamiliar arena (b,d) ($n = 12$). * Significant difference compared to corresponding acute vehicle pretreated group ($P < 0.05$). #Significant difference compared to corresponding vehicle-pretreated group ($P < 0.05$).

saturated by MDMA. This isoenzyme is considered to be polymorphic, which means that there are allelic differences in the gene coding for CYP2D6. According to this polymorphism, 5–9% of the Caucasian population is considered to be a poor metaboliser phenotype, which means a lower capacity of metabolism of several drugs, including MDMA. Indeed, there is evidence that ''poor metabolisers'' of debrisoquin may have lower metabolic capacity and higher plasma levels of MDMA (de la Torre et al., 2005). Such persons might represent a genetically defined human sub-population in which clinical complications (including lethal side effects) are more likely to occur.

An analogous isoenzyme, CYP2D1, also exists in rats. The Dark Agouti rat strain possesses decreased microsomal CYP2D1 isoenzyme activity, which plays an important role in the metabolism of MDMA and thus, the Dark Agouti rat is a poor or moderate metaboliser of the drug compared to other rat strains, e.g. the Sprague–Dawley or Wistar. The pharmacokinetics of MDMA in Dark Agouti rats are thought to be similar to vulnerable human users, the ''poor metabolisers''. Thus, the Dark Agouti rat provides a model of a genetically defined human sub-population in which clinical complications may be more likely to occur.

The treatment regimen that we used in this study comprised a single, 15 mg/kg dose of MDMA, which is reported to be selectively neurotoxic to the serotonergic axon terminals (Balogh et al., 2004; O'Shea et al., 1998; Quate et al., 2004). Applying the principles of interspecies scaling, this dose range in rats might be considered roughly equivalent to the dose of an average MDMA tablet in humans. Although the validity of such interpolation has been questioned (de la Torre and Farre, 2004), following a dose of 15 mg/kg, plasma levels of MDMA in the Dark Agouti strain are known to fall within a similar range to that found in persons with acute MDMA intoxication (Colado et al., 1995).

There may however be some disadvantages to using Dark Agouti rats in studies such as those reported here. It has been suggested previously that the Dark Agouti strain exhibits high baseline anxiety levels. Thus, Dark Agouti rats spend less time in the open arms of the elevated plus maze, avoid the middle zone in the open field test, and show low locomotor activity (Mechan et al., 2002a) in comparison to Sprague–Dawley rats. Similar patterns were observed also in this study. Indeed, previous data from our laboratory (Kantor et al., 2000) has shown that Sprague–Dawley and Wistar rats show less anxiety and exhibit much higher locomotor and exploratory

Table 2
Local cerebral metabolic rate of glucose utilisation ($lCMR_{glu}$) in saline and MDMA (15 mg/kg, i.p.) pretreated animals 3 weeks previously

Brain area	LCMRglu (μmol/100 g/min)		
	Saline pretreatment + acute saline ($n = 7$)	MDMA pretreatment + acute saline ($n = 7$)	% change
Neocortex			
Anterior cingulate	115 ± 4	91 ± 2*	−19
Posterior cingulate	52 ± 4	48 ± 1	−8
Frontal	107 ± 5	97 ± 5	−9
Prefrontal	115 ± 5	92 ± 1*	−20
Somatosensory	111 ± 4	99 ± 3	−11
Temperoparietal	110 ± 5	108 ± 5	−2
Entorhinal	75 ± 2	60 ± 2*	−20
Dorsal hippocampus			
Subiculum	76 ± 4	69 ± 2	−9
CA1	62 ± 3	56 ± 3	−10
CA2	55 ± 2	42 ± 3*	−24
CA3	66 ± 2	57 ± 1*	−14
Dentate gyrus	85 ± 4	67 ± 2*	−21
Extrapyramidal motor areas			
Caudate nucleus	91 ± 3	76 ± 3*	−16
Globus pallidus	47 ± 2	42 ± 2	−11
Substantia nigra	51 ± 3	40 ± 1*	−22
Subthalamic nucleus	77 ± 4	67 ± 3	−13
Medial septal nucleus	48 ± 2	44 ± 2	−8
Lateral septal nucleus	60 ± 3	50 ± 2*	−17
Medial amygdala	53 ± 5	40 ± 2*	−25
Lateral amygdala	68 ± 7	66 ± 3	−3
Mediodorsal thalamic nucleus	101 ± 4	83 ± 3*	−18
Hypothalamic paraventricular nucleus	54 ± 2	45 ± 2*	−17
Hypothalamic ventromedial nucleus	49 ± 4	49 ± 1	0
Nucleus accumbens	84 ± 4	72 ± 2*	−14
Ventral tegmental area	44 ± 2	39 ± 2	−11
Periaqueductal grey	60 ± 2	48 ± 2*	−20
Locus coeruleus	62 ± 2	49 ± 2*	−21
Dorsal raphé nucleus	80 ± 3	66 ± 2*	−18
Median raphé nucleus	87 ± 3	70 ± 2*	−20

Data presented as mean ± SEM. Percentage changes are differences from mean saline control. *Significantly different from saline control group.

Table 3
[^{3}H]Paroxetine binding in animals exposed to either saline or MDMA (15 mg/kg) 3 weeks previously

Brain area	Saline ($n = 7$)	MDMA ($n = 7$)	% Change
Neocortex			
Anterior cingulate	66 ± 7	27 ± 4*	−59
Frontal	100 ± 15	65 ± 6*	−35
Prefrontal	147 ± 19	65 ± 13*	−56
Somatosensory	107 ± 13	45 ± 10*	−58
Temperoparietal	77 ± 16	15 ± 5*	−80
Entorhinal	168 ± 15	43 ± 8*	−74
Dorsal hippocampus			
Subiculum	130 ± 8	55 ± 10*	−58
CA1	123 ± 11	39 ± 9*	−68
CA2	144 ± 11	52 ± 9*	−64
CA3	106 ± 10	49 ± 11*	−54
Dentate gyrus	116 ± 9	46 ± 12*	−60
Extrapyramidal motor areas			
Caudate nucleus	57 ± 11	50 ± 6	−12
Substantia nigra	229 ± 19	225 ± 20	−2
Mediodorsal thalamic nucleus	127 ± 18	40 ± 12*	−69
Septal nucleus	74 ± 4	51 ± 3*	−31
Amygdala	210 ± 15	134 ± 10*	−36
Dorsal raphé nucleus	321 ± 28	310 ± 25	−3
Median raphé nucleus	170 ± 16	173 ± 21	2

Data are presented as mean binding density (fmol/mg tissue) ± SEM. *Significantly different from saline control group.

behaviour under identical conditions (vehicle treatment, low light, familiar arena) than the Dark Agouti rats in this study. However, despite the clearly different behavioural pattern in this strain, as compared to the Wistar rat (Green and McGregor, 2002), the effects of MDMA on anxiety-like behaviours using the social interaction test have not previously been studied in Dark Agouti strain.

4.1. Acute effects of MDMA in the social interaction test

In the present study, an important acute effect of MDMA was the increase of adjacent lying and as a consequence, increase in social behaviour. Since adjacent lying contained some active elements that were directed towards the partner, it could be interpreted as a special social behaviour.

In recent studies, adjacent lying has been shown to be present after acute MDMA in the SI test in Wistar rats. In these studies, significant decreases in anogenital sniffing, rearing and marked increase in adjacent lying were found after a lower dose of MDMA (5 mg/kg). These results were interpreted as an increase in social interaction (Morley et al., 2005) and it was suggested that MDMA has anxiolytic and prosocial properties, similarly to the empathogenic effects reported in humans. Similar decreases in anogenital sniffing, rearing and marked increase in adjacent lying were found in our studies at the dose of 15 mg/kg in Dark Agouti rats. Thus, the conclusion of increase in social interaction after MDMA is in agreement also with the results of our study.

It is notable, however, that the increase in overall social behaviour attributed to increase in adjacent lying, while most typical measures of social interaction test were even decreased after MDMA (e.g., anogenital sniffing, grooming partner). Similar observation, namely, markedly decreased social interaction was described in the Charles–Foster rats after lower doses (5 and 10 mg/kg) of MDMA 30 min after MDMA administration (Bhattacharya et al., 1998). Elevated anxiety-like effects were also described in mice in a variety of anxiety tests, including the SI test (Maldonado and Navarro, 2000, 2001; Navarro and Maldonado, 1999).

Inconsistent results were described also in other anxiety tests, acute anxiolytic and anxiogenic effects being reported. Acute anxiogenic effects have been previously reported in a number of studies. Anxiogenic effects were described in the elevated plus maze and open field anxiety tests (Bhattacharya et al., 1998). Whilst Ho et al. (2004) found an anxiogenic

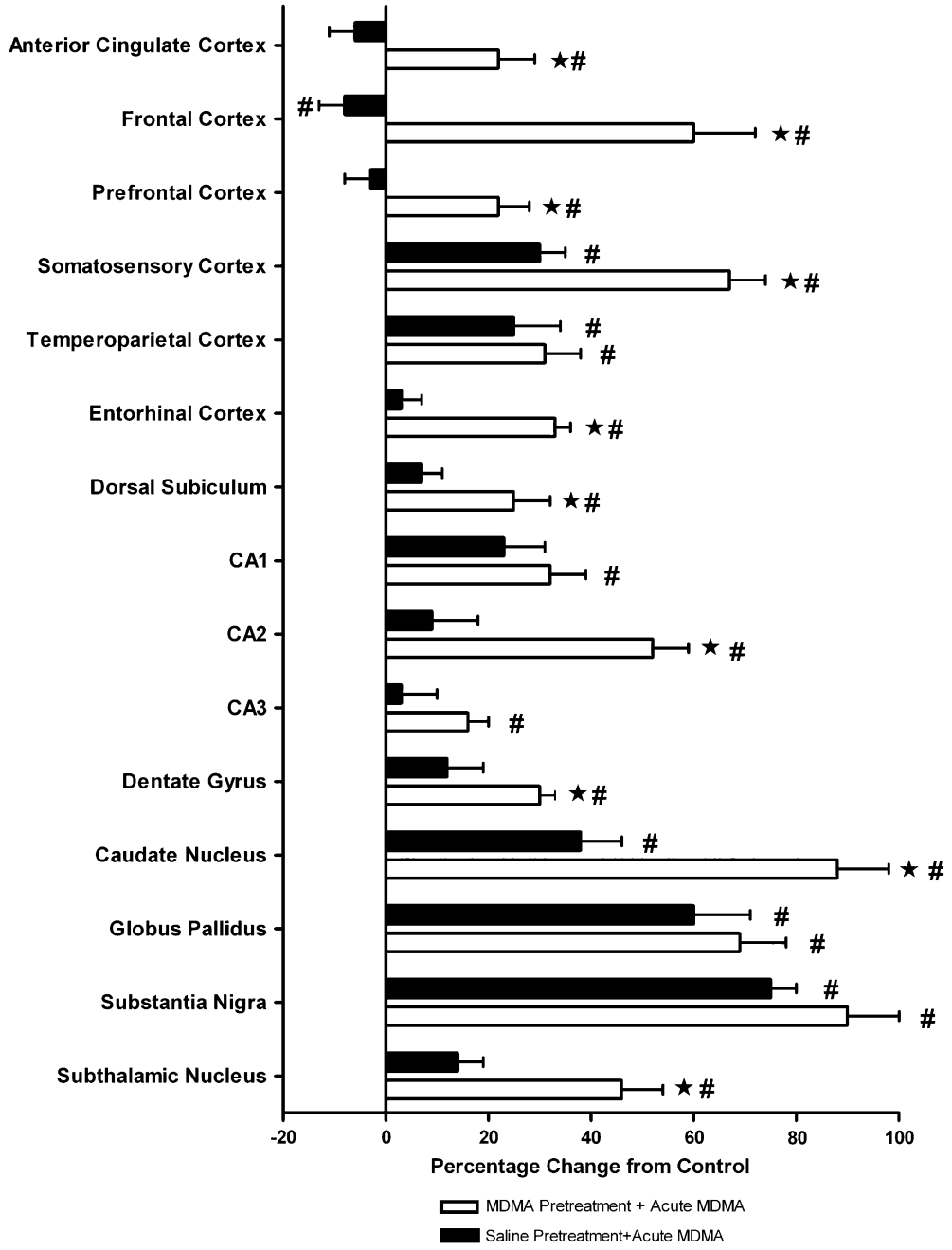

Fig. 4. Acute effects of MDMA on local cerebral glucose utilization in saline and MDMA-pretreated rats in neocortex, hippocampus and extrapyramidal areas. Data are expressed in mean ± SEM, percent change from control ($n = 12$). #Significant difference compared to corresponding acute vehicle treated group ($P < 0.05$). *Significant difference compared to corresponding saline-pretreated group ($P < 0.05$).

effect at lower doses of MDMA (7.5 mg/kg), at the dose of 15 mg/kg (the dose used in our study) MDMA exerted strong hyperlocomotion on the plus maze test, and thus these results did not clearly suggest a specific reduction in anxiety-like behaviours. Sumnall et al. (2004) also found that after a single 10 mg/kg treatment, in the Lister-hooded rat MDMA caused a clear anxiogenic response on the plus maze test. Using different indices of anxiety, it was found that 5 mg/kg MDMA was clearly anxiogenic and not anxiolytic (Morley and McGregor, 2000).

It is reasonable that these behavioural effects, namely that MDMA increased adjacent lying and decreased typical social behaviours, cannot be interpreted in this test solely as specific measures of anxiety, but rather as a complex behavioural phenomena. It is likely that changes in the perception of the environment, described also by human users may alter behaviour in rats. Also, cognitive processes and the reaction of the animals may be altered as well, and thus, the behaviour elicited by MDMA may not be interpreted clearly along the axis of increased or decreased anxiety.

Some reduction in line crossings and marked decrease in exploratory behaviour was observed after MDMA in our study. The reduction of locomotor activity and exploratory behaviour has been observed after administration of serotonergic

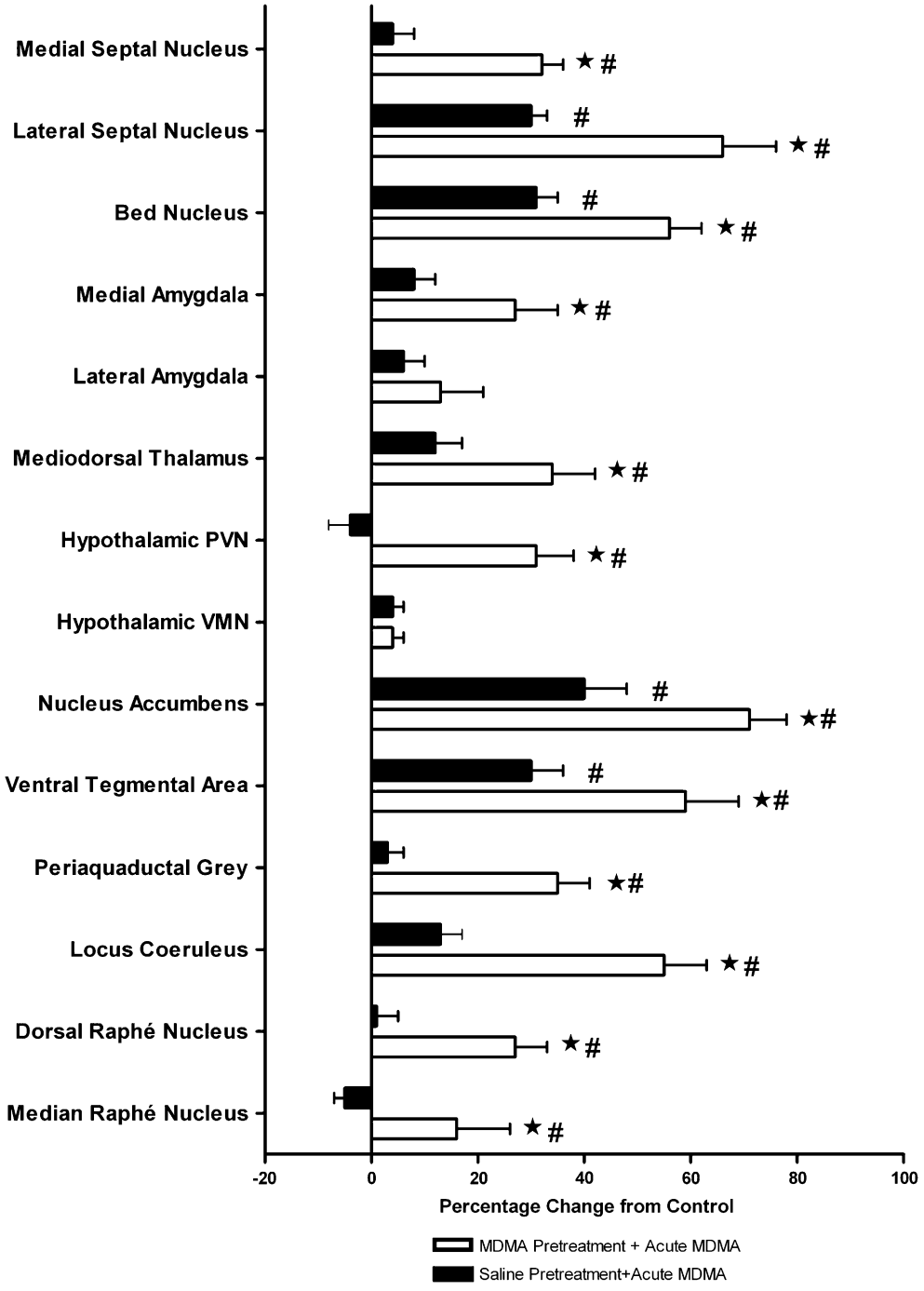

Fig. 5. Acute effects of MDMA on local cerebral glucose utilization in saline and MDMA-pretreated rats in brain areas related also to anxiety-like and social behaviours. Data are expressed in mean ± SEM, percent change from control. #Significant difference compared to corresponding acute vehicle treated group ($P < 0.05$). *Significant difference compared to corresponding saline-pretreated group ($P < 0.05$).

agonists such as LSD (Mittman and Geyer, 1991), m-CPP, and the SSRIs fluoxetine and sertraline (Bagdy et al., 2001). Since these effects of the SSRIs and m-CPP could be reversed by subtype-selective 5-HT_{2C} receptor antagonists, this effect may also be mediated by the activation of 5-HT_{2C} receptors (Bagdy et al., 2001). Effects of MDMA-induced serotonin syndrome might, however, also interfere with acute locomotor effects. Significant, albeit relatively weak tail winding, flat body posture and head weaving were observed after MDMA administration. Furthermore, it is likely that reduction of line crossings after MDMA was, at least partially, a consequence of marked increase in adjacent lying.

From an anatomical perspective the proposed afferent arm of the anxiety pathway conveys anxiogenic stimuli to the dorsal thalamus, and thence via sensory motor cortex on to the entorhinal, frontal, and cingulate cortex. The efferent pathways involving the amygdala, locus coeruleus, hypothalamus periaquaductal grey and striatum mediate autonomic,

neuroendocrine and motor responses associated with fear and anxiety. An activation of these central pathways, thought to comprise the functional neuroanatomy of anxiety and fear (Charney and Deutch, 1996), was confirmed by our studies in which functional imaging of acute MDMA effects indicated regionally specific increases in LCMRglu. In keeping with our previous studies (Balogh et al., 2004; Sharkey et al., 1991), especially marked increases were found in regions associated with locomotor function (e.g. caudate nucleus, substantia nigra, and globus pallidus), but also in the lateral septum and bed nucleus and the extra handling and saline pretreatment necessitated by the current experimental design did not appear to affect the outcome. Interestingly, functional activation of the latter has been found to be strongly associated with increased anxiety (Singewald et al., 2003), and although smaller in magnitude, an increase was also found in the locus coeruleus, which plays a key role in the mediation of anxiogenesis. However, it may be overly simplistic to interpret as anxiogenic any such metabolic activation of the neuroanatomical constituents that make up brain pathways involved in anxiety. It is equally possible that basal levels of activation represent the anxious state and that increased metabolic activity reflects an inhibition the expression of anxious behaviours. Thus appropriate interpretation of the metabolic mapping profile in these studies is substantially enhanced by the parallel behavioural studies and represents one of the strengths of this experimental design.

Taken together, these results indicate that MDMA may produce a complex behaviour in social environment in rats, which includes decreased exploration and locomotor activity with changes in social behaviour. It is also important to emphasise that the effect of MDMA on anxiety varies considerably depending on species, strain, and dosage regimen, and is critically dependent on the exact test of anxiety used and its environmental parameters. The largely positive human experience of acute MDMA may suggest that the drug has anxiolytic properties, and data from our studies may parallel some of these effects. Although acute adverse effects were also described in humans, such as panic attacks (Davison and Parrott, 1997; Whitaker-Azmitia and Aronson, 1989), this type of behaviour was also present in Dark Agouti rats, but only at higher doses (Bagdy et al., personal communications).

4.2. Chronic effects of MDMA

Our study shows that if rats pretreated with a single 15 mg/kg dose of MDMA 3 weeks previously were placed into a low anxiety environment (low light, familiar arena) there was an increase in the duration and number of aggressive episodes, as well as in the number of line crossings, rearings, and non-social activities. In contrast, MDMA failed to cause any increase in either the time of total or peaceful social interaction (regardless of whether adjacent lying was included or not) or the time of self-grooming in the long term.

These results suggest that significant increases were found only in those parameters of the social interaction test, which can be related rather to locomotion and the general activity of the animal (namely, rearing, line-crossings and non-social activities). In contrast, no changes were found in those behaviours (time of total social interaction or self grooming), which are more specific measures of anxiety in the social interaction test. Furthermore, when animals were exposed in the present study to an environment causing a high level of anxiety (unfamiliar arena), these behavioural effects were not present. Possibly, these results would suggest that MDMA exposure does not alter anxiety-like behaviours in the SI test 3 weeks after the drug.

Using a variety of anxiety tests, including the social interaction test, the majority of previous studies investigating chronic behavioural changes following MDMA exposure have described increased anxiety in the long term in both Wistar and Lister-hooded rat strains (Bull et al., 2003, 2004; Fone et al., 2002; Ho et al., 2004; McGregor et al., 2003a; McGregor et al., 2003b; Morley et al., 2001; Thompson et al., 2004). Reduced anxiety was found on the plus maze after repeated intermittent dosing of MDMA in Sprague-Dawley rats (Piper and Meyer, 2004), but more interestingly, Mechan et al. found that in an experimental model similar to ours (male Dark Agouti rats, single neurotoxic 15 mg/kg dose of MDMA), MDMA increased open arm entry several weeks after the treatment (Mechan et al., 2002b). Nevertheless, open arm entry, a parameter that inversely correlates with anxiety in the elevated plus maze, is much lower in control, vehicle treated Dark Agouti rats compared to Wistar and Sprague-Dawley animals, suggesting an increased basal anxiety in this strain. According to these observations, it has been proposed that the diametrically opposite changes that are found in different strains could be explained by differences in intrinsic levels of anxiety (Green and McGregor, 2002). The high intrinsic anxiety of Dark Agouti rats, as described earlier, and the chronic anxiolytic-like effect of MDMA are in accordance with the results described by Mechan et al (Mechan et al., 2002b).

In an alternative interpretation provided for the anxiolytic-like effect of MDMA observed in the Dark Agouti strain, it was suggested that effects of MDMA may be explained by increase in impulsivity and reactivity (Harro, 2002). The rationale for this interpretation was that para-chloroamphetamine, another serotonergic neurotoxin with similar neurotoxic profile, caused a temporary reduction in immobility in the forced swimming test after partial depletion of serotonin (Haidkind et al., 2004), and this phenomenon was explained as an increase in ‘‘reactive’’ and ‘‘impulsive’’ behaviour (Haidkind et al., 2004; Harro, 2002; Harro et al., 2001). Analysis of this MDMA-induced aggressive social behaviour in our study showed that elements of overt aggressive behaviour, like biting, boxing, kicking were not present. MDMA induced, however, dominant posture, chasing or aggressive grooming. In contrast, to these findings, the same 21 day pretreatment with MDMA failed to increase aggression in the resident-intruder test, a typical test for measuring aggression (Kirilly et al., 2006). It has to be mentioned, however, that residents are kept in social isolation in the above test, and lack of increase in aggression 3 weeks after MDMA may be a likely

consequence of the effect of social isolation on brain 5-HT metabolism (Kirilly et al., 2006). Although our results are in accordance with the hypothesis of increase in reactivity, conclusion of increased impulsivity must be stated with caution.

We found significant increases in line crossings, rearing and other activities related to exploratory behaviour 21 days after MDMA, and a similar effect might affect interpretation of results obtained by Mechan et al (Mechan et al., 2002b). Indeed, under some circumstances, especially in some anxiety tests where all parameters are linked to motor activity, e.g., elevated plus maze, the increases in locomotion may be interpreted as anxiolytic-like activity. Furthermore, in another previous study, we found increased motor activity 2 weeks after MDMA administration, especially at the time of circadian phase-shift, lights off (Balogh et al., 2004).

An alternative explanation for the data presented here, namely the increase in the duration and number of aggressive episodes, the increase in the number of line crossings, rearings, and non-social activities, might be that it is a phenomenon similar to mood disorders rather than anxiolytic-type behaviour. Irritated mood, psychomotor agitation, frequent disruption of ongoing regular behaviour, irritability, aggression, change in social functions are well known symptoms of mania, hypomania or mixed states related to bipolar depression described also by DSM-IV. This state might be a consequence of serotonergic damage (e.g., decrease in paroxetine binding in the prefrontal cortex and hippocampus), and/or long-term changes in metabolic activities in areas crucial for monoamine neurotransmission (e.g., decrease in LCMRglu in dorsal raphe, locus coeruleus and prefrontal cortex), or decrease in the expression of 5-HTT mRNA in the dorsal raphe (Kirilly et al., 2005).

In most brain areas involved in the generation of fear and anxiety, e.g., prefrontal cortex, medial amygdala, hippocampal fields CA2 and CA3, periaqueductal grey, locus coeruleus, and dorsal raphe, significant decreases in LCMRglu were found 3 weeks after MDMA. This parallels our previous observation that LCMRglu is reduced in areas of the brain subserving motor function (26). Accompanying these decreases in LCMRglu were widespread and substantial reductions in [^{3}H]paroxetine binding to 5-HT transporter sites, indicative of a profound loss of 5-HT terminals throughout the forebrain, but most markedly in areas of neocortex. Although it is tempting to invoke a direct causational link between the decrease in 5-HT terminals and decreases in brain metabolism, it is possible that the latter reflect compensatory mechanisms activated to ameliorate the effects of reduced serotonergic influence. Moreover, although our 2-deoxyglucose data directly parallel those described in man using PET imaging (Buchert et al., 2001), one interpretation of this apparent decrease in functional activity could be that it represents a paradoxical, disinhibitory effect. However it is also possible that the decreased LCMRglu actually represents a lowering of the threshold for response in these areas, so that when placed into an anxiety-inducing environment they are more likely to express behaviours, such as impulsivity, which would not normally be expressed overtly. Some evidence that this indeed might be the case can be derived from the enhanced response to acute MDMA exposure in these MDMA-pretreated rats (see later discussion). Whatever the underlying mechanism, this paradox has been described previously, and long-term decrease in serotonergic tone have been shown to lead to either anxiogenic or anxiolytic effects (Briley et al., 1990; Green and McGregor, 2002; Hall et al., 1999). Thus it appears likely that these two neurochemical changes (decrease in brain metabolism and depletion of 5-HT) may lead to a complex outcome depending on environmental factors, previous experience, and genetic factors that may exert their effects through the regulation of the actual activity of brain structures involved in fear and anxiety.

In general terms it would appear that the acute effects of MDMA upon LCMRglu parallel changes found in immediate early gene expression (e.g. the activity-regulated cytoskeleton-associated protein, Arc) - that is, acute MDMA treatment increases both LCMRglu and Arc (Beveridge et al., 2004). However, using the same experimental treatment design as we have used here, but with a slightly lower dose of MDMA, the same authors found no significant change in Arc expression in MDMA pretreated rats, with the exception of hippocampal CA1 where significant increase was observed. This is in contrast to the decreased LCMRglu that we measured 21 days after MDMA treatment. However, the Arc response measured in these animals appeared to be very dependent upon the extent of handling immediately prior to sacrifice.

4.3. Acute effects of MDMA in rats previously exposed to MDMA

In rats previously exposed to the drug, MDMA exerted similar or even enhanced acute effects compared to drug-naive rats. Interestingly, behavioural measures, which were increased by acute MDMA in drug-naive rats, were even more increased, while those which were decreased or tended to decrease in drug-naive animals, were further decreased in animals exposed to MDMA earlier.

Namely, adjacent lying was even further increased in MDMA-pretreated animals, and the decreases in non-social activities and line crossings by acute MDMA that were not significant or small in drug-naive animals, were markedly decreased in animals pretreated with MDMA previously.

It has been reported that a previous non-neurotoxic MDMA treatment does not alter the behavioural effects of MDMA (Morley and McGregor, 2000), however, behavioural sensitisation has been reported after neurotoxic doses of MDMA (Kalivas et al., 1998).

In our study, increases in LCMRglu after acute MDMA injection were apparently potentiated in animals previously exposed to the drug. In general, as discussed above, the reason for this apparent enhancement of the acute response was the lower baseline against which the data were compared, that is, attenuated brain glucose metabolism in rats pre-exposed to MDMA. Therefore, acute MDMA injection increased brain metabolism up to similar levels, irrespective of whether animals were drug-naive previously pretreated with MDMA.

However, in a small number of areas, e.g. frontal cortex, the second MDMA injection clearly had an effect on brain metabolism which was both qualitatively and quantitatively dissimilar in saline (reduced) MDMA (increased) pretreated rats. Moreover, in a previous study in which the experimental design made use of the same control group for acute MDMA treatments in both drug naive and drug pre-exposed groups, a similar enhancement was evident in those areas of the brain involved in motor function (Balogh et al., 2004). These subtle differences in the metabolic response of the brain to acute MDMA might imply some difference in brain function between the two acute groups. The relevance of this phenomenon is the behavioural sensitisation found in our study and also some others as described above.

In conclusion, a previous drug treatment does not abolish, rather, it potentiates either the acute behavioural effects measured in the social interaction test, or the functional activation in several pathways as indicated by increased LCMRglu. In the absence of serotonergic terminals in the forebrain in MDMA-pretreated rats, it is tempting to question whether it is indeed 5-HT released by acute MDMA treatment that is responsible for the observed effects and not, for example, the other monoamine neurotransmitters. There is, however, preliminary evidence that despite the profound depletion of terminals, the remaining serotonergic systems in the brain adapt to accommodate the loss by increasing sensitivity to available 5-HT (Ferrington et al., 2005; Quate et al., 2004).

5. Conclusion

MDMA administration elicits a complex behavioural phenomenon, with increased social behaviour and adjacent lying which is a typical behavioural element after acute MDMA administration. We have found evidence for behavioural disinhibition several weeks later, and also a sensitisation to the acute effects of the drug. These changes are consistent with metabolic changes in the brain, but environmental factors have an essential role in the manifestation of these effects.

Acknowledgements

This study was supported by the Fifth Framework Programme of the European Community, QLG3-CT-2002-00809, by the Sixth Framework Programme, LSHM-CT-2004-503474 the Hungarian Research Fund Grant T020500, the Ministry of Welfare Research Grant 058/2003, the Fund Management of Ministry of Education OMFB 01926/2002 and PhD Fellowship Program of the Semmelweis University, Ministry of Education, Hungary.

References

Bagdy, G., Graf, M., Anheuer, Z.E., Modos, E.A., Kantor, S., 2001. Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A receptor antagonist WAY-100635. Int. J. Neuropsychopharmacol. 4, 399–408.

Balogh, B., Molnar, E., Jakus, R., Quate, L., Olverman, H.J., Kelly, P.A., 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 (Berl) 173, 296–309.

Beveridge, T.J., Mechan, A.O., Sprakes, M., Pei, Q., Zetterstrom, T.S., Green, A.R., Elliott, J.M., 2004. Effect of 5-HT depletion by MDMA on hyperthermia and Arc mRNA induction in rat brain. Psychopharmacology (Berl) 173, 346–352.

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

Briley, M., Chopin, P., Moret, C., 1990. Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. Psychopharmacology (Berl) 101, 187–189.

Buchert, R., Obrocki, J., Thomasius, R., Vaterlein, O., Petersen, K., Jenicke, L., Bohuslavizki, K.H., Clausen, M., 2001. Long-term effects of 'ecstasy' abuse on the human brain studied by FDG PET. Nucl. Med. Commun. 22, 889–897.

Bull, E.J., Hutson, P.H., Fone, K.C., 2003. Reduced social interaction following 3,4-methylenedioxymethamphetamine is not associated with enhanced 5-HT 2C receptor responsivity. Neuropharmacology 44, 439–448.

Bull, E.J., Hutson, P.H., Fone, K.C., 2004. Decreased social behaviour following 3,4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT2A receptor responsivity. Neuropharmacology 46, 202–210.

Butler, G.K., Montgomery, A.M., 2004. Impulsivity, risk taking and recreational 'ecstasy' (MDMA) use. Drug Alcohol Depend. 76, 55–62.

Charney, D.S., Deutch, A., 1996. A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorders. Crit. Rev. Neurobiol. 10, 419–446.

Colado, M.I., Williams, J.L., Green, A.R., 1995. The hyperthermic and neurotoxic effects of 'Ecstasy' (MDMA) and 3,4 methylenedioxyamphetamine (MDA) in the Dark Agouti (DA) rat, a model of the CYP2D6 poor metabolizer phenotype. Br. J. Pharmacol. 115, 1281–1289.

Davison, D., Parrott, A.C., 1997. Ecstasy (MDMA) in recreational users: self-reported psychological and physiological effects. Hum. Psychopharmacol. 12, 221–226.

De Souza, E.B., Kuyatt, B.L., 1987. Autoradiographic localization of [^{3}H]-paroxetine-labeled serotonin uptake sites in rat brain. Synapse 1, 488–496.

Ferrington, L., McBean, D.E., Olverman, H.J., Leith, J.L., Kelly, P.A.T. Previous exposure to 3,4-methylenedioxymethamphetamine (MDMA) alters cerebral metabolic response to 8-OH-DPAT in Dark Agouti rats. pA2 online, in press.

Fone, K.C., Beckett, S.R., Topham, I.A., Swettenham, J., Ball, M., Maddocks, L., 2002. Long-term changes in social interaction and reward following repeated MDMA administration to adolescent rats without accompanying serotonergic neurotoxicity. Psychopharmacology (Berl) 159, 437–444.

Green, A.R., 2004. MDMA: fact and fallacy, and the need to increase knowledge in both the scientific and popular press. Psychopharmacology (Berl) 173, 231–233.

Green, A.R., Cross, A.J., Goodwin, G.M., 1995. Review of the pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy"). Psychopharmacology (Berl) 119, 247–260.

Green, A.R., McGregor, I.S., 2002. On the anxiogenic and anxiolytic nature of long-term cerebral 5-HT depletion following MDMA. Psychopharmacology (Berl) 162, 448–450.

Green, A.R., Mechan, A.O., Elliott, J.M., O'Shea, E., Colado, M.I., 2003. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Pharmacol. Rev. 55, 463–508.

Griebel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol. Ther. 65, 319–395.

Haidkind, R., Eller, M., Kask, A., Harro, M., Rinken, A., Oreland, L., Harro, J., 2004. Increased behavioural activity of rats in forced swimming test after partial denervation of serotonergic system by parachloroamphetamine treatment. Neurochem. Int. 45, 721–732.

Hall, F.S., Devries, A.C., Fong, G.W., Huang, S., Pert, A., 1999. Effects of 5,7-dihydroxytryptamine depletion of tissue serotonin levels on extracellular

serotonin in the striatum assessed with in vivo microdialysis: relationship to behavior. Synapse 33, 16–25.

Harro, J., 2002. Long-term partial 5-HT depletion: interference of anxiety and impulsivity? Psychopharmacology (Berl) 164, 433–434.

Harro, J., Tonissaar, M., Eller, M., Kask, A., Oreland, L., 2001. Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: effects on behavior and monoamine neurochemistry. Brain Res. 899, 227–239.

Ho, Y.J., Pawlak, C.R., Guo, L., Schwarting, R.K., 2004. Acute and long-term consequences of single MDMA administration in relation to individual anxiety levels in the rat. Behav. Brain Res. 149, 135–144.

Iversen, S.D., 1984. 5-HT and anxiety. Neuropharmacology 23, 1553–1560.

Kalivas, P.W., Duffy, P., White, S.R., 1998. MDMA elicits behavioral and neurochemical sensitization in rats. Neuropsychopharmacology 18, 469–479.

Kantor, S., Anheuer, Z.E., Bagdy, G., 2000. High social anxiety and low aggression in Fawn-Hooded rats. Physiol. Behav. 71, 551–557.

Kelly, P.A., Ritchie, I.M., Collins, F.M., 1995. Cerebrovascular consequences of repeated exposure to NG-nitro-L-arginine methyl ester. Br. J. Pharmacol. 116, 2771–2777.

Kirilly, E., Benko, A., Ferrington, L., Ando, R.D., Kelly, P.A., Bagdy, G., 2006. Acute and long-term effects of a single dose of MDMA on aggression in Dark Agouti rats. Int. J. Neuropsychopharmacol. 9, 63–76.

Kirilly, E., Tóth, Z.E., Gerber, G., Palkovits, M., Bagdy, G., 2005. 3,4-Methylenedioxymethamphetamine (MDMA) modulates serotonin transporter mRNA expression in the rat brainstem. Society for Neuroscience 35th Annual Meeting, November 12–16, 2005, Washington DC, P111.12.

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

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

McGregor, I.S., Clemens, K.J., Van der Plasse, G., Li, K.M., Hunt, G.E., Chen, F., Lawrence, A.J., 2003a. 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.

McGregor, I.S., Gurtman, C.G., Morley, K.C., Clemens, K.J., Blokland, A., Li, K.M., Cornish, J.L., Hunt, G.E., 2003b. Increased anxiety and ''depressive'' symptoms months after MDMA (''ecstasy'') in rats: drug-induced hyperthermia does not predict long-term outcomes. Psychopharmacology (Berl) 168, 465–474.

Mechan, A.O., Moran, P.M., Elliott, M., Young, A.J., Joseph, M.H., Green, R., 2002a. 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 (Berl) 159, 188–195.

Mechan, A.O., Moran, P.M., Elliott, M., Young, A.J., Joseph, M.H., Green, R., 2002b. 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 (Berl) 159, 167–175.

Mittman, S.M., Geyer, M.A., 1991. Dissociation of multiple effects of acute LSD on exploratory behavior in rats by ritanserin and propranolol. Psychopharmacology (Berl) 105, 69–76.

Morley, K.C., Arnold, J.C., McGregor, I.S., 2005. Serotonin (1A) receptor involvement in acute 3,4-methylenedioxymethamphetamine (MDMA) facilitation of social interaction in the rat. Prog. Neuropsychopharmacol. Biol. Psychiatry.

Morley, K.C., Gallate, J.E., Hunt, G.E., Mallet, P.E., McGregor, I.S., 2001. Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine (''ecstasy''). Eur. J. Pharmacol. 433, 91–99.

Morley, K.C., McGregor, I.S., 2000. (+/−)-3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') increases social interaction in rats. Eur. J. Pharmacol. 408, 41–49.

Navarro, J.F., Maldonado, E., 1999. Behavioral profile of 3,4-methylenedioxymethamphetamine (MDMA) in agonistic encounters between male mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 23, 327–334.

O'Shea, E., Granados, R., Esteban, B., Colado, M.I., Green, A.R., 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.

Pallanti, S., Mazzi, D., 1992. MDMA (Ecstasy) precipitation of panic disorder. Biol. Psychiatry 32, 91–95.

Parrott, A.C., 2000. Human research on MDMA (3,4-methylene- dioxymethamphetamine) neurotoxicity: cognitive and behavioural indices of change. Neuropsychobiology 42, 17–24.

Parrott, A.C., 2001. Human psychopharmacology of ecstasy (MDMA): a review of 15 years of empirical research. Hum. Psychopharmacol. 16, 557–577.

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

Piper, B.J., Meyer, J.S., 2004. Memory deficit and reduced anxiety in young adult rats given repeated intermittent MDMA treatment during the periadolescent period. Pharmacol. Biochem. Behav.

Quate, L., McBean, D.E., Ritchie, I.M., Olverman, H.J., Kelly, P.A., 2004. Acute methylenedioxymethamphetamine administration: effects on local cerebral blood flow and glucose utilisation in the Dark Agouti rat. Psychopharmacology (Berl) 173, 287–295.

Sharkey, J., McBean, D.E., Kelly, P.A., 1991. Alterations in hippocampal function following repeated exposure to the amphetamine derivative methylenedioxymethamphetamine (''Ecstasy''). Psychopharmacology (Berl) 105, 113–118.

Singewald, N., Salchner, P., Sharp, T., 2003. Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. Biol. Psychiatry 53, 275–283.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O., Shinohara, M., 1977. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28, 897–916.

Sprague, J.E., Everman, S.L., Nichols, D.E., 1998. An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. Neurotoxicology 19, 427–441.

Sumnall, H.R., O'Shea, E., Marsden, C.A., Cole, J.C., 2004. The effects of MDMA pretreatment on the behavioural effects of other drugs of abuse in the rat elevated plus-maze test. Pharmacol. Biochem. Behav. 77, 805–814.

Thompson, M.R., Li, K.M., Clemens, K.J., Gurtman, C.G., Hunt, G.E., Cornish, J.L., McGregor, I.S., 2004. Chronic fluoxetine treatment partly attenuates the long-term anxiety and depressive symptoms induced by MDMA ('Ecstasy') in rats. Neuropsychopharmacology 29, 694–704.

To, C.T., Anheuer, Z.E., Bagdy, G., 1999. Effects of acute and chronic fluoxetine treatment of CRH-induced anxiety. Neuroreport 10, 553–555.

de la Torre, R., Farre, M., 2004. Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. Trends Pharmacol. Sci. 25, 505–508.

de la Torre, R., Farre, M., Mathuna, B.O., Roset, P.N., Pizarro, N., Segura, M., Torrens, M., Ortuno, J., Pujadas, M., Cami, J., 2005. MDMA (ecstasy) pharmacokinetics in a CYP2D6 poor metaboliser and in nine CYP2D6 extensive metabolisers. Eur. J. Clin. Pharmacol. 61, 551–554.

Vollenweider, F.X., Gamma, A., Liechti, M., Huber, T., 1998. Psychological and cardiovascular effects and short-term sequelae of MDMA (''ecstasy'') in MDMA-naive healthy volunteers. Neuropsychopharmacology 19, 241–251.

Vollenweider, F.X., Liechti, M.E., Gamma, A., Greer, G., Geyer, M., 2002. Acute psychological and neurophysiological effects of MDMA in humans. J. Psychoactive Drugs 34, 171–184.

Whitaker-Azmitia, P.M., Aronson, T.A., 1989. ''Ecstasy'' (MDMA)-induced panic. Am. J. Psychiatry 146, 119.