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Neural correlates of MDMA (''Ecstasy'')-induced social interaction in rats

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The popular drug 3,4 methylenedioxymethamphetamine (MDMA, ''Ecstasy'', ''the Love Drug'') produces feelings of love and closeness in humans and induces analogous prosocial and antiaggressive effects in laboratory animals. Here we examined the specific brain regions that may be involved in these prosocial effects. Male Wistar rats were pretreated with a moderate dose of MDMA (5 mg/kg) or vehicle and then either kept alone in a familiar test chamber for 60 min (groups MDMA-ALONE and VEHICLE-ALONE) or allowed to engage in social interaction in the familiar test chamber with an unfamiliar same-sex conspecific for 60 min (groups MDMA-SOCIAL and VEHICLE-SOCIAL). Rats in the MDMA-SOCIAL group showed much greater overall social interaction than rats in the VEHICLE-SOCIAL group, with microanalysis revealing increased general investigation of other rats but decreased anogenital sniffing. Analysis of neural activation across 39 brain regions using Fos immunohistochemistry showed the following results: (1) VEHICLE-SOCIAL and VEHICLE-ALONE groups did not differ in Fos expression, indicating that a social context *per se* did not affect Fos expression, (2) MDMA-treated groups showed significantly increased Fos expression relative to VEHICLE treated groups in 30 brain regions, (3) the MDMA-SOCIAL group showed augmented Fos expression relative to the MDMA-ALONE group in six brain regions including the caudate-putamen (medial), medial preoptic area, paraventricular thalamic nucleus, central amygdala, ventromedial hypothalamic nucleus, and the medial amygdala (posterodorsal), and (4) the MDMA-SOCIAL group (but not the MDMA-ALONE group) showed augmented Fos expression relative to the VEHICLE groups in the nucleus accumbens, ventral tegmental area and periaqueductal grey. These results indicate that a moderate dose of MDMA given in a social context causes considerably greater brain activation than the same dose given to solitary rats. This activation involves specific neural circuits that are known to regulate affiliative behavior, perhaps by modulating the incentive value of social stimuli. A possible role for the neuropeptide oxytocin in mediating the prosocial effects of MDMA is discussed.

INTRODUCTION

The popular drug MDMA (3,4 methylenedioxy-methamphetamine, ''Ecstasy'', ''the Love Drug'') engenders increased empathy, sensual perceptions and a desire to communicate and interact with others, resulting in an increased reinforcing impact of social situations (Baylen & Rosenberg, 2006;

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Cole & Sumnall, 2003; Dumont & Verkes, 2006; Sumnall, Cole, & Jerome, 2006; Vollenweider, Gamma, Liechti, & Huber, 1998). MDMA can also increase social interaction in rats (Morley & McGregor, 2000) and decreases aggression in fish (Capurro et al., 1997) and mice (Maldonado & Navarro, 2001; Navarro & Maldonado, 1999; Navarro, Rivera, Maldonado, Cavas, & de la Calle, 2004). Given that heightened social reward is a major reason for MDMA use (Soellner, 2005), there is an interest in determining the neural substrates of these prosocial effects.

When given low doses of MDMA (2.5–5 mg/kg), rats display significantly elevated levels of social interaction including particularly high levels of passive physical contact, termed ''adjacent lying'' (Ando et al., 2006; Morley, Arnold, & McGregor, 2005; Thompson, Callaghan, Hunt, Cornish, & McGregor, 2007). This prosocial effect is potentiated at high ambient temperatures (Cornish et al., 2003) suggesting that it is not simply a thermoregulatory ''huddling'' response to perceived cold. The prosocial effects of MDMA in rats are mimicked by the 5-HT_{1A} agonist 8-OH-DPAT and are attenuated by both the 5-HT_{1A} receptor antagonist WAY 100,635 and the oxytocin antagonist tocinoic acid. This has lead to the hypothesis that MDMA-induced release of 5-HT in the paraventricular and supraoptic nuclei of the hypothalamus stimulates 5-HT_{1A} receptors to cause oxytocin release, and that this released oxytocin, most likely acting at distal extrahypothalamic brain targets, stimulates the prosocial behaviors seen with MDMA (Thompson et al., 2007).

To further explore MDMA prosocial effects, the present study examined the brain regions activated when MDMA is given in a social context relative to a non-social one. Our previous work using Fos immunohistochemistry has shown that MDMA, given to solitary rats, activates a widespread network of cortical, limbic and brainstem sites, and that these effects are potentiated by both high ambient temperature (Hargreaves, Hunt, Cornish, & McGregor, 2007) and increasing MDMA dose (Stephenson, Hunt, Topple, & McGregor, 1999). The activated network includes brain regions that are considered as critical substrates for social interaction, social memory and sexual and maternal behavior, including the medial preoptic area, medial amygdala, ventromedial hypothalamus, nucleus accumbens and ventral tegmental area, as well as the oxytocin-containing cells of the supraoptic and paraventricular nuclei of the hypothalamus (Hargreaves et al., 2007; Stephenson et al., 1999; Thompson et al., 2007).

To evaluate the possible involvement of these regions in MDMA social effects, we compared brain activation in rats given MDMA while alone with that seen in rats given MDMA in the presence of another MDMA-treated rat. We reasoned that differences in neural activation seen between these two groups might point to the critical neural substrates mediating the prosocial effects of MDMA, while comparison of vehicle-treated rats in social versus alone conditions would allow elucidation of the basic neural correlates of social interaction *per se*.

MATERIALS AND METHODS

Subjects

The subjects were 36 inbred male Albino Wistar rats bred in our own facility and weighing on average 345 g ($\pm$ 5.9 g) at the start of testing. The rats were housed in groups of 6 per cage during all phases of the experiment. The colony room temperature was set at 22°C with a 12 h reverse light cycle (lights off at 08:30); food and water were available *ad libitum*. All behavioral testing was conducted during the dark cycle at an ambient temperature of 28°C which has previously been shown to potentiate MDMA-induced social interaction (Cornish et al., 2003). The experiment was approved by the University of Sydney Animal Ethics Committee.

Drug treatment

($\pm$)3,4-Methylenedioxymethamphetamine hydrochloride was purchased from the Australian Government Analytical Laboratories (Pymble, NSW, Australia). MDMA was dissolved in 0.9% saline and injected intraperitoneally at a dose of 5 mg/kg at a volume of 1 ml/kg. MDMA doses are expressed in terms of the weight of the hydrochloride salt. Control subjects received an equivalent injection of vehicle (0.9% saline).

Apparatus

The test chamber consisted of a square, black, Perspex box ($52 \times 52 \times 40$ cm) dimly lit with red

light (40 W). A miniature video camera was placed vertically above the box that sent a signal into a VCR in a neighboring room, allowing test sessions to be recorded. The test chamber was cleaned with 50% ethanol and dried between each test to remove any odour cues present.

Behavioral testing

The experiment involved a 2×2 factorial design with rats allocated to one of four treatment groups: VEHICLE-ALONE ($n = 7$) VEHICLE-SOCIAL ($n = 10$), MDMA-ALONE ($n = 7$) and MDMA-SOCIAL ($n = 12$). The larger number of rats in the two SOCIAL groups allowed social interaction data to be collected from pairs of rats and compared across these two groups.

Rats were handled daily for 7 days prior to testing and were habituated to intraperitoneal injection procedures on each of these days. On each of the 3 days prior to the test day, all rats were individually habituated to the testing room and the social interaction test chamber for 30 min.

On the test day, pairs of rats were administered either vehicle or MDMA and were then immediately placed in individual holding cages ($40 \times 25 \times 15$ cm opaque box lined with bedding) in the testing room for 20 min. The testing room had been preheated to 28°C. Following this 20 min period, rats were placed in the test chamber for 60 min. Rats in groups VEHICLE-ALONE and MDMA-ALONE spent the entire 60 min test phase in the test chamber by themselves with no other rat present in the room. Rats in the VEHICLE-SOCIAL and MDMA-SOCIAL groups were placed in the test chamber for 60 min with an unfamiliar conspecific administered the same drug treatment. For the two SOCIAL groups, members of each pair came from different home cages and were matched for body weight.

Social interaction in the two SOCIAL groups was video recorded and subsequently scored by a trained observer who was blind to treatment group allocation. The behaviors scored across the 60 min session were as previously reported (Thompson et al., 2007)—*adjacent lying*: duration of side-by-side contact or ''huddling'', including climbing under/over conspecific; *anogenital sniffing*: duration of sniffing the conspecific's anogenital area; *investigation*: duration of sniffing the conspecific apart from anogenital sniffing. A *total social interaction* score was also obtained by summing the scores of these individual behaviors for each pair of rats. The non-social variable of *rearing*: standing on hind legs, was also scored.

At the conclusion of the 60 min test session and 80 min following drug treatment, rats were injected with 120 mg/kg sodium pentobarbital. They were then perfused with 200 ml of phosphate buffered saline (PBS) immediately followed by 400 ml of cold 4% paraformaldehyde buffered with 0.1M PBS (pH 7.3). Transcardial perfusion was performed with a Gilson perfusion pump at a flow rate of 48 ml/min.

Immunohistochemistry

Following perfusion the brains were removed and post-fixed overnight in 4% paraformaldehyde buffered with 0.1 M PBS (pH 7.3). The brains were then cut into three coronal blocks and were placed in 15% sucrose and phosphate buffer (PB) solution for 24 h, followed by 30% sucrose PB for 48 h. The entire brain (excluding olfactory bulbs) was then sliced in 40 μm coronal sections in a cryostat at –17°C. Brain slices were placed sequentially into four vials containing PB. One vial was used immediately for tissue processing and the other three vials were placed in freezing solution and stored for backup use if required.

Tissue processing was as described previously (McGregor, Hargreaves, Apfelbach, & Hunt, 2004). Briefly, free floating tissue sections were washed in 1% hydrogen peroxide in PB for 30 min. Tissue was then washed in 3% normal horse serum (NHS) in PB for 30 min. Sections were placed in vials with 3 ml of the primary c-Fos antibody (rabbit polyclonal; reacts with c-Fos p62 of mouse, rat, and human; non-crossreactive with FosB, Fra-1, or Fra-2; Santa Cruz Biotechnology, Santa Cruz) at a dilution of 1:2000 in phosphate buffered horse serum (PBH- 0.1% bovine serum albumium, 0.2% Triton X-100, 2% NHS in PB) and stored for 72 h at 4°C.

The tissue was washed for 60 min in PB, and incubated in 3 ml of 1:500 biotinylated antirabbit secondary antibody made in goat (Vector Laboratories) for 2 h. Sections were again washed in PB for 30 min and then incubated in Extravadin-horseradish peroxidase (Sigma; dilution of 1:1000 in PBH) for 90 min. Tissue was run through three 30 min washes. To visualize the horseradish peroxidase activity, tissue was placed in a nickel diaminobenzidine and glucose oxidase reaction. The reaction was terminated after 10 min by washing with PB. The processed tissue was

mounted onto gelatinized slides, then cleaned and dehydrated by placing slides through ascending concentrations of water, ethanol, and xylene before cover slipping.

Quantification of Fos

Fos-positive neurons in each region of interest (Figure 1) were manually counted using an Olympus BX-40 microscope by an experimenter who was blind to group assignment, using a graticule and according to the methods previously described (Stephenson et al., 1999). Only very dark brown or black labelled circular and oval shaped features that fell inside the graticule were counted. Sections were counted using a $\times 20$ objective (0.5 mm $\times$ 0.5 mm graticule area) unless stated otherwise (see Table 1).

Data analysis

A series of *t*-tests were used to compare VEHICLE-SOCIAL and MDMA-SOCIAL rats on the various behaviors assessed during social interaction testing (total social interaction, adjacent lying, anogenital sniffing, and general investigation). A one-way ANOVA was used to identify overall group differences in Fos counts in each brain region. When significant overall group effects were observed in Fos counts, Newman Keuls post-hoc tests were used to allow pairwise comparisons between groups. All data were analyzed using Prism 4 for Macintosh (GraphPad Software, San Diego, CA) with significance levels set at 0.05 for all tests.

RESULTS

Social interaction test

Results for the behavioral variables measured in VEHICLE-SOCIAL and MDMA-SOCIAL groups in the social interaction test are presented in Figure 2. Rats in the MDMA-SOCIAL group showed significantly higher levels of total social interaction than VEHICLE-SOCIAL rats ($t(9) = 2.63$, $p < .05$). Microanalysis showed that rats in the MDMA-SOCIAL group exhibited significantly increased levels of general investigation ($t(9) = 5.28$, $p < .001$) and much lower levels of anogenital investigation ($t(9) = 5.92$, $p < .001$) than VEHICLE-SOCIAL rats. MDMA-SOCIAL rats also showed significantly less rearing than VEHICLE-SOCIAL rats ($t(9) = 2.56$, $p < .05$). MDMA treatment increased adjacent lying, although this effect only approached statistical significance ($t(9) = 2.20$, $p = .0553$).

Fos immunohistochemistry

Fos counts for the 39 regions investigated in the four treatment groups are presented in Table 1. Fos expression did not differ between treatment groups in 9 of the 39 regions. No significant differences were observed between the VEHICLE-ALONE and VEHICLE-SOCIAL treatment groups in any region examined. In 21 of the regions examined, the MDMA-ALONE and MDMA-SOCIAL groups both exhibited significantly higher levels of Fos expression than either the VEHICLE-ALONE or VEHICLE-SOCIAL groups.

An additional nine regions were uniquely affected in the MDMA-SOCIAL group. In six of these nine regions, which included the caudate-putamen (medial), the medial preoptic area, the paraventricular thalamic nucleus, the central amygdala, the ventromedial hypothalamic nucleus and the medial amygdala (posterodorsal), Fos expression was significantly greater in the MDMA-SOCIAL group compared to the MDMA-ALONE group. In the other three regions (nucleus accumbens (shell), anterior ventral tegmental area and dorsomedial periaqueductal grey), differences in Fos expression were observed between the MDMA-SOCIAL group and the two VEHICLE groups but no differences were observed between the MDMA-ALONE group and these VEHICLE groups.

Photomicrographs of Fos expression in four specific regions of interest—the medial preoptic area, the central amygdala, the medial amygdala (posterodorsal) and the Barrel fields of the somatosensory cortex—are presented in Figure 3, 4, 5 and 6 respectively.

DISCUSSION

Behavioral results

As previously reported (Cornish et al., 2003; Morley et al., 2005; Morley & McGregor, 2000; Thompson et al., 2007), MDMA increased social

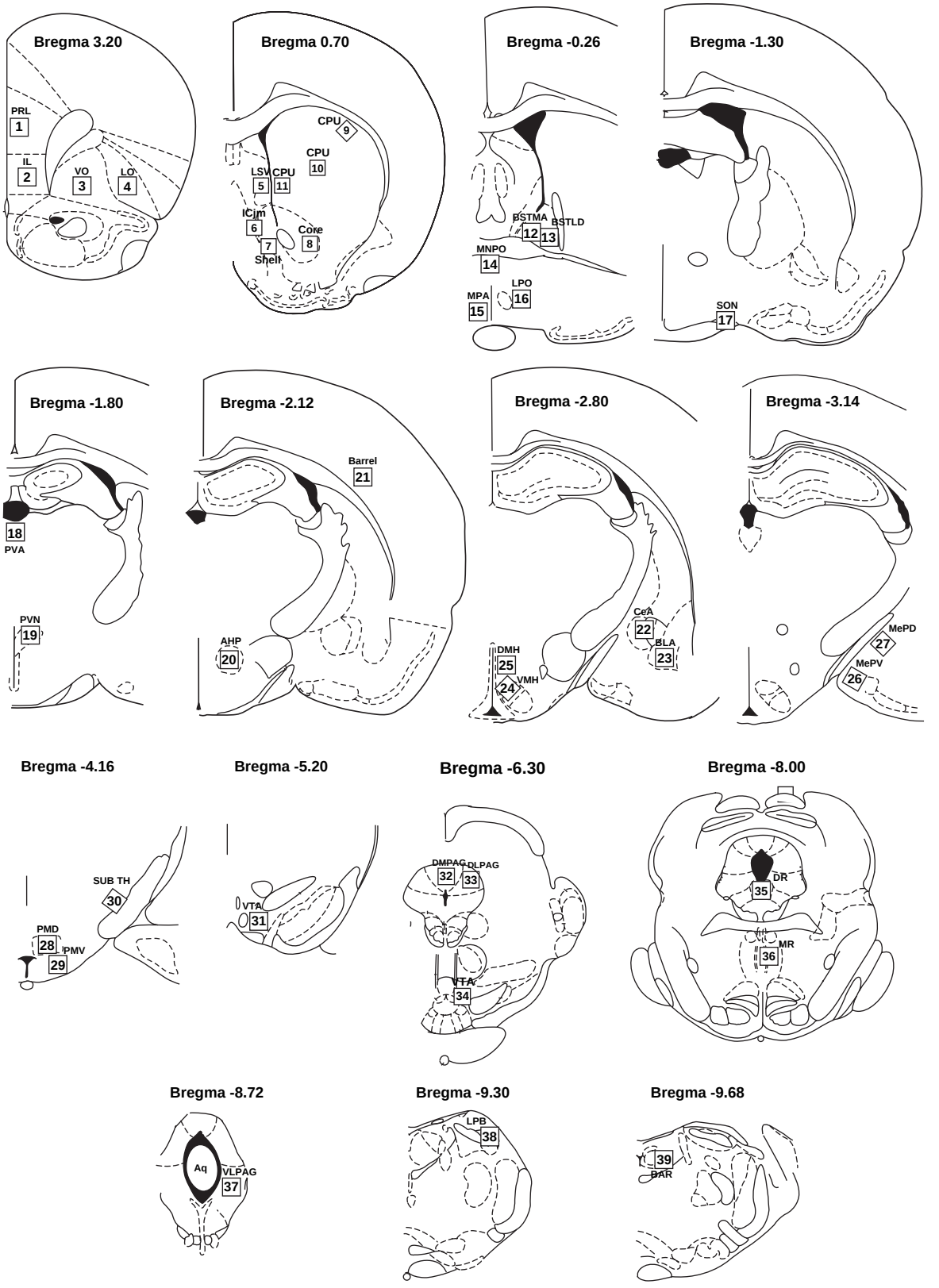

Figure 1. Schematic showing the location of each brain region where Fos immunopositive neurons were quantified. Diagrams of coronal sections taken from the Paxinos and Watson rat brain atlas (Paxinos & Watson, 1998). Note that numbers for each region correspond to brain regions identified in Table 1.

TABLE 1
Mean number (SEM) of Fos positive cells

Region (see Figure 1)	*Bregma*	*VEH-ALONE (n = 7)*	*VEH-SOCIAL (n = 10)*	*MDMA-ALONE (n = 7)*	*MDMA-SOCIAL (n = 12)*
Areas where social interaction differentially affected MDMA-induced Fos expression					
7. Nucleus accumbens (shell)	0.70	4.9 (1.1)	4.9 (0.6)	8.7 (1.1)	14.8 (3.3)[ab]
9. Caudate-putamen (medial)	0.70	3.4 (1.0)	3.4 (0.9)	31.1 (5.9)[ab]	45.9 (6.2)[abc]
15. Medial preoptic area	−0.26	3.9 (1.2)	10.4 (1.4)	6.9 (1.5)	17.4 (2.6)[abc]
18. Paraventricular thalamic nucleus	−1.80	4.3 (1.6)	7.6 (1.8)	29.6 (6.0)[ab]	50.0 (5.3)[abc]
22. Central amygdala	−2.80	3.6 (1.4)	5.0 (1.4)	37.1 (5.8)[ab]	52.4 (5.2)[abc]
24. Ventromedial hypothalamic nucleus	−2.80	0.4 (0.2)	1.7 (0.9)	8.3 (2.4)[ab]	12.7 (1.3)[abc]
27. Medial amygdala (posterodorsal)	−3.14	9.6 (2.2)	6.9 (1.5)	14.1 (1.9)[b]	21.3 (2.8)[abc]
31. Ventral tegmental area (anterior)	−5.20	1.0 (0.7)	0.4 (0.3)	2.0 (0.7)	2.6 (0.5)[ab]
32. Periaqueductal gray (dorsomedial)	−6.30	4.7 (1.5)	4.4 (0.7)	7.4 (1.3)	9.0 (1.3)[ab]
Areas where social interaction had no effect on MDMA-induced Fos expression					
1. Prelimbic cortex	3.20	12.4 (2.4)	12.1 (2.3)	43.4 (6.7)[ab]	40.3 (4.7)[ab]
3. Orbital cortex (ventral)	3.20	4.3 (1.4)	10.0 (3.7)	24.6 (5.3)[ab]	20.5 (3.1)[ab]
4. Orbital cortex (lateral)	3.20	2.1 (1.2)	6.4 (1.9)	19.7 (4.3)[ab]	17.2 (2.6)[ab]
5. Lateral septal nucleus (ventral)	0.70	31.0 (8.2)	27.4 (4.5)	60.0 (9.2)[ab]	47.1 (4.9)[b]
6. Island of Calleja major	0.70	4.6 (1.0)	3.2 (0.7)	19.0 (3.8)[ab]	24.7 (5.4)[ab]
8. Nucleus accumbens (core)	0.70	0.9 (0.3)	1.5 (0.6)	11.4 (3.8)[ab]	8.8 (1.6)[ab]
10. Caudate-putamen (central)	0.70	0.0 (0.0)	0.0 (0.0)	26.3 (4.6)[ab]	23.5 (2.9)[ab]
11. Caudate-putamen (dorsolateral)	0.70	0.1 (0.1)	0.1 (0.1)	9.3 (1.6)[ab]	7.8 (1.3)[ab]
13. BNST (laterodorsal)	−0.26	5.0 (1.2)	3.4 (1.0)	18.7 (8.2)[ab]	16.5 (2.6)[ab]
14. Median preoptic nucleus	−0.26	10.7 (2.2)	13.4 (3.1)	29.3 (5.4)[ab]	28.3 (3.5)[ab]
17. Supraoptic nucleus	−1.30	0.9 (0.3)	1.3 (0.5)	9.4 (1.4)[ab]	11.3 (2.3)[ab]
19. Paraventricular hypothalamic nucleus	−1.80	3.7 (1.9)	4.9 (1.0)	48.0 (9.7)[ab]	57.3 (6.4)[ab]
21. Barrel field (6a and 6b)	−2.12	6.1 (1.2)	7.1 (2.2)	27.7 (5.2)[ab]	27.8 (3.1)[ab]
23. Basolateral amygdala	−2.80	2.4 (0.8)	1.5 (0.6)	5.0 (1.2)[b]	4.8 (0.8)[b]
25. Dorsomedial hypothalamic nucleus	−2.80	10.0 (2.3)	9.3 (1.8)	23.0 (3.6)[ab]	30.2 (3.3)[ab]
26. Medial amygdala (posteroventral)	−3.14	9.7 (2.5)	11.8 (2.6)	22.3 (3.1)[ab]	22.5 (3.9)[ab]
34. Ventral tegmental area (posterior)	−6.30	0.0 (0.0)	0.3 (0.2)	7.3 (2.3)[ab]	8.2 (1.5)[ab]
35. Dorsal raphe nucleus	−8.00	1.1 (0.6)	1.0 (0.5)	7.6 (2.4)[ab]	7.3 (1.6)[ab]
37. Periaqueductal gray (ventrolateral)	−8.72	6.1 (2.2)	8.0 (2.4)	18.1 (4.8)[ab]	17.9 (2.2)[ab]
38. Lateral parabrachial nucleus	−9.30	2.7 (1.1)	2.6 (1.3)	27.5 (3.5)[ab]	25.3 (2.8)[ab]
39. Barrington's nucleus	−9.68	0.9 (0.5)	0.0 (0.0)	2.7 (0.7)[ab]	2.3 (0.4)[ab]
Areas where there was no significant MDMA-induced Fos expression					
2. Infralimbic cortex	3.20	15.4 (2.5)	12.9 (2.8)	19.7 (4.6)	23.7 (3.3)
12. BNST (medial)	−0.26	6.4 (1.8)	7.1 (1.7)	11.3 (2.8)	12.2 (1.7)
16. Lateral preoptic area	−0.26	6.6 (1.8)	5.0 (1.2)	9.6 (1.6)	7.2 (1.2)
20. Anterior hypothalamus	−2.12	16.6 (6.1)	13.8 (2.5)	22.6 (4.4)	25.0 (3.1)
28. Pre-mammilary nucleus (dorsal)	−4.16	5.1 (0.8)	4.2 (1.5)	7.1 (2.3)	6.7 (2.0)
29. Pre-mammilary nucleus (ventral)	−4.16	4.9 (1.9)	7.9 (1.2)	11.9 (3.1)	13.1 (2.7)
30. Subthalamic nucleus	−4.16	0.0 (0.0)	0.1 (0.1)	3.1 (1.5)	1.1 (0.5)
33. Periaqueductal gray (dorsolateral)	−6.30	5.3 (1.8)	8.0 (1.7)	8.6 (1.4)	11.0 (1.6)
36. Median raphe nucleus	−8.00	1.4 (1.0)	0.2 (0.2)	0.7 (0.7)	0.8 (0.5)

Notes: Data are mean (SEM). [a]$p < .05$ vs VEH-ALONE; [b]$p < .05$ vs VEH-SOCIAL; [c]$p < .05$ vs MDMA-ALONE. A ×40 objective was used to count regions 6, 17, and 39. All other regions were counted under a ×20 objective.

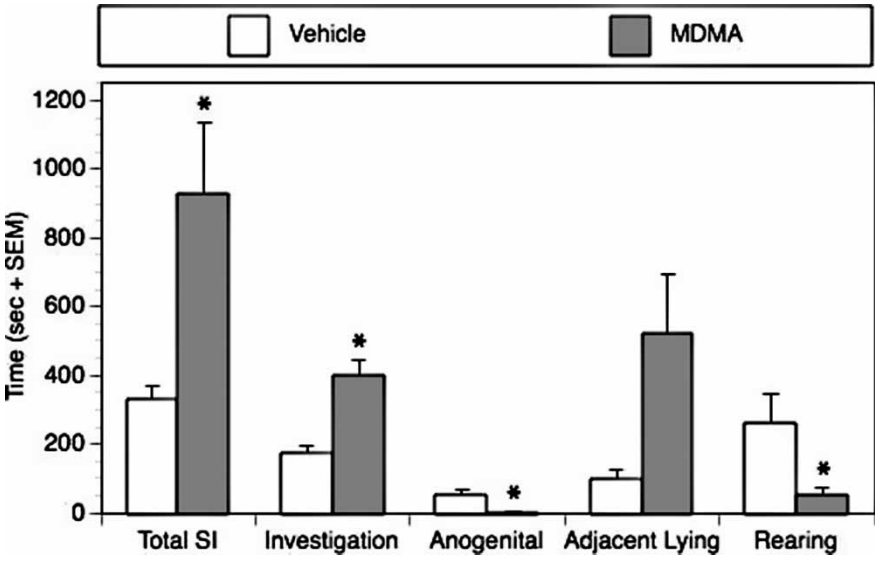

Figure 2. Various social and nonsocial behaviors quantified during the 60 min test session in the VEHICLE-SOCIAL ($n = 5$ pairs) and MDMA-SOCIAL ($n = 6$ pairs) groups. $^*p < .05$, comparing the two groups.

interaction in pairs of rats meeting for the first time. As in these previous studies, this prosocial effect largely comprised elevated levels of general investigation and adjacent lying, while anogenital sniffing was significantly reduced. Rearing behavior was also reduced, in agreement with previous reports (Morley et al., 2005; Thompson et al., 2007). The marginally significant nature of the adjacent lying effect in the present study is somewhat surprising, but this most likely reflects the relatively small number of pairs (five vehicle and six MDMA) used for assessment of social interaction.

Fos expression: Overview

The present study found increased Fos expression in 30 brain regions in rats administered MDMA (5 mg/kg). These effects replicate the findings of previous studies from our laboratory, with widespread MDMA-induced Fos activation in cortical, limbic, striatal, hypothalamic and brainstem sites (Hargreaves et al., 2007; Stephenson et al., 1999; Thompson et al., 2007). Newly characterized areas of MDMA-induced Fos expression in the current study include the Barrel fields of the somatosensory cortex as well as the ventral and lateral orbital cortex.

Interestingly, no differences in Fos expression were observed between rats in the VEHICLE-ALONE and VEHICLE-SOCIAL groups. The neural activation induced by social behavior in rats is a relatively understudied area. In one of the few relevant previous studies, Salchner, Lubec, & Singewald (2004) reported increased Fos expression in numerous brain regions following social interaction in both young and aged rats. However, close scrutiny of this study indicates that social interaction testing was conducted in an unfamiliar testing environment, and that comparisons of Fos expression in social rats were made with control rats that were not exposed to the novel testing environment. Thus the Fos expression in that study could easily reflect exposure to a novel testing environment rather than social interaction *per se*. Our use of a familiar testing environment in the present study may therefore account for the lack of significant differences between VEHICLE-ALONE and VEHICLE-SOCIAL groups reported here.

The distribution of brain Fos expression following a low 5 mg/kg dose of MDMA has previously been reported by our group in solitary rats (Stephenson et al., 1999). Interestingly, the current study shows neural activation in MDMA-ALONE rats in a number of additional regions with 5 mg/kg MDMA relative to our previous study, including the caudate-putamen (central and dorsolateral), the median preoptic nucleus and the anterior ventral tegmental area. In a recent study these regions all displayed greater Fos expression to MDMA (10 mg/kg) when the drug was given at a high ambient temperature of 30°C relative to a lower temperature of 19°C (Hargreaves et al., 2007). This suggests that the

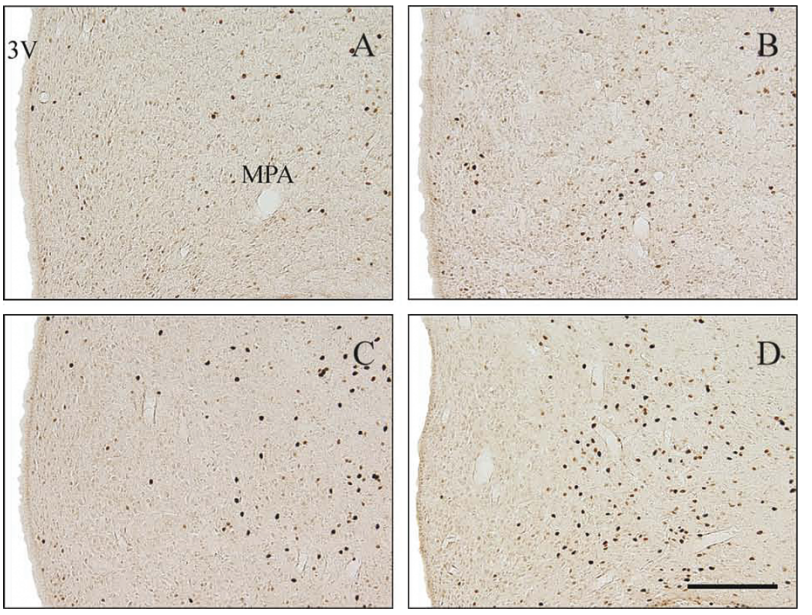

Figure 3. Photomicrographs showing Fos expression in the medial preoptic area of a representative rat from each treatment group. (A) VEHICLE-ALONE, (B) VEHICLE-SOCIAL, (C) MDMA-ALONE, (D) MDMA-SOCIAL. 3V = 3rd ventricle; MPA = medial preoptic area. Scale bar = 200 μm.

high ambient temperature used in the present study (28°C) may be the key factor mediating the additional regional Fos activation seen with 5 mg/kg MDMA relative to the earlier report of Stephenson et al. (1999).

Social interaction augments MDMA-induced Fos expression

A subset of 9 of the 30 regions activated by MDMA had either (a) significantly greater Fos expression in the MDMA-SOCIAL than the MDMA-ALONE group or (b) significantly greater Fos expression in the MDMA-SOCIAL group but not the MDMA-ALONE group relative to the two VEHICLE groups. These 9 regions therefore appear to be more markedly activated by MDMA under social conditions. These regions include the medial caudate-putamen, the medial preoptic area, the nucleus accumbens (shell), the paraventricular thalamic nucleus, the central nucleus of the amygdala, the medial amygdala (posterodorsal), the ventromedial hypothalamic nucleus, the anterior ventral tegmental area and the periaqueductal grey (dorsomedial).

Given the demonstrated role that oxytocin plays in the prosocial effects of MDMA (Thompson et al., 2007), it is interesting to note that many of these structures showing socially augmented Fos expression with MDMA contain moderate to high oxytocin receptor densities and are regions that have been previously implicated in social, sexual and parental behaviors (Gimpl & Fahrenholz, 2001; Vaccari, Lolait, & Ostrowski, 1998). For example, oxytocin acting in the medial amygdala plays a key role in rodent social recognition processes (Ferguson, Aldag, Insel, & Young, 2001; Lim & Young, 2006) while oxytocinergic neural circuits involving the central amygdala and medial preoptic area are fundamental to the expression of maternal and other social behaviors (Keverne & Curley, 2004). Infusions of oxytocin into the central amygdala can serve to reverse the social interaction deficits caused by chronic administration of phencyclidine (Lee et al., 2005) and a variety of manipulations of amygdalar activity can lead to social withdrawal in rats (Truitt et al., 2007). Similarly, the ventromedial hypothalamus, which contains a particularly high concentration of oxytocin receptors (Vaccari et al., 1998) has extensive connections with the medial preoptic area, medial amygdala and bed nucleus of the stria terminalis. This hypothalamic-extended amygdala network is implicated in a range of social, maternal, reproductive and aggressive behaviors (Newman, 1999).

Additionally, the nucleus accumbens shell and anterior ventral tegmental area, which were preferentially activated here by MDMA under

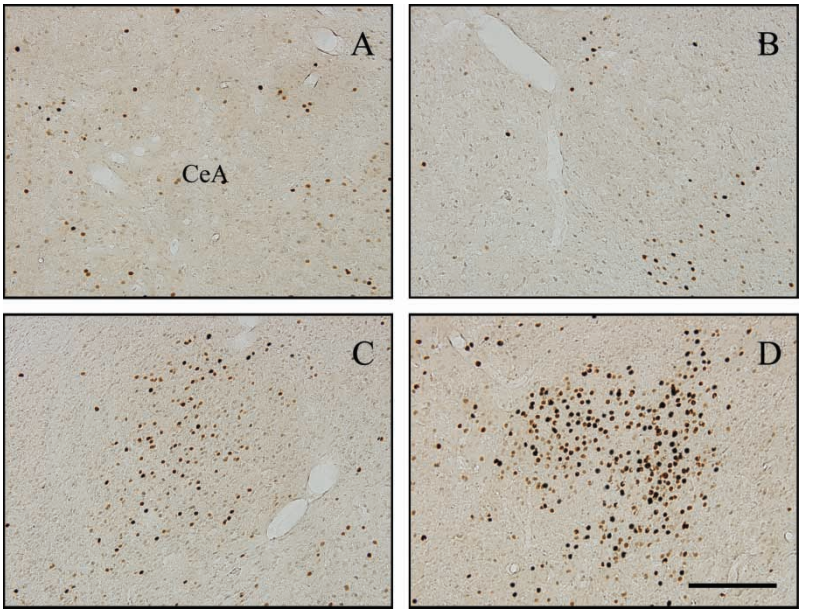

Figure 4. Photomicrographs showing Fos expression in the central amygdala of a representative rat from each treatment group. (A) VEHICLE-ALONE, (B) VEHICLE-SOCIAL, (C) MDMA-ALONE, (D) MDMA-SOCIAL. CeA = central amygdala. Scale bar = 200 μm.

social conditions, are key components of the mesolimbic dopamine system which play a critical role in reinforcement processes (Everitt & Robbins, 2005). This pathway is implicated in the rewarding properties of maternal, sexual and social interactions in a variety of species (Insel, 2003; Keverne & Curley, 2004; Young & Wang, 2004). Dopamine D_2 receptors and oxytocin

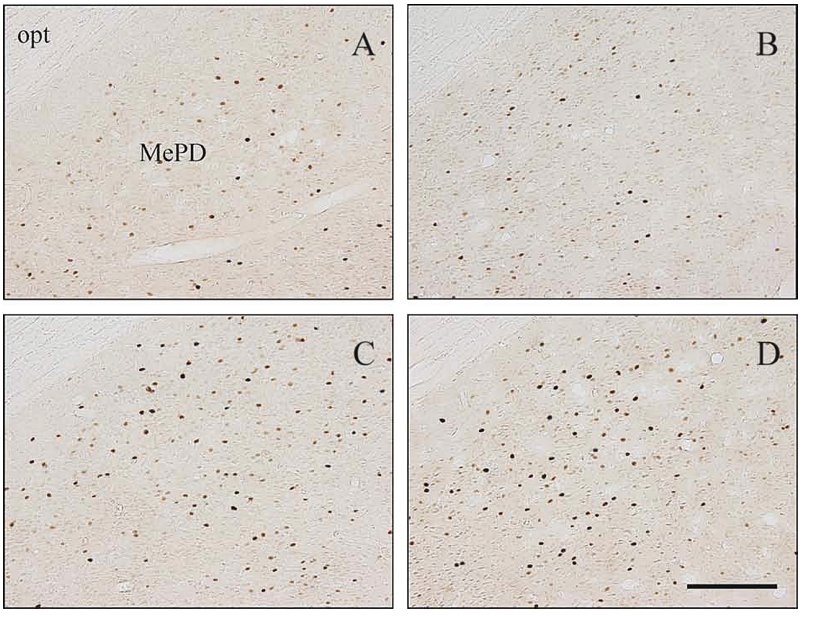

Figure 5. Photomicrographs showing Fos expression in the medial amygdala (posterodorsal) of a representative subject from each treatment group. (A) VEHICLE-ALONE, (B) VEHICLE-SOCIAL, (C) MDMA-ALONE, (D) MDMA-SOCIAL. opt = optic tract; MePD = medial amygdala (posterodorsal). Scale bar = 200 μm.

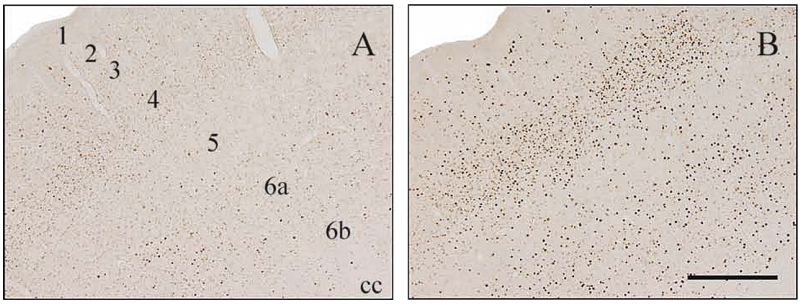

Figure 6. Photomicrographs showing Fos expression in the Barrel fields of the somatosensory cortex (6a and 6b) of representative VEHICLE and MDMA-treated rats. (A) VEHICLE-SOCIAL, (B) MDMA-SOCIAL. 1–6b = respective layers of Barrel cortex; cc = corpus callosum. Scale bar = 500 μm.

acting in the nucleus accumbens shell, but not the core, play a vital role in the formation of pair bonds in monogamous voles, while D_1-like receptors are important for pair bond maintenance (Aragona et al., 2006; Young & Wang, 2004).

The magnified Fos expression in the nucleus accumbens seen with ''social'' MDMA might be taken to imply that the drug accentuates the incentive value of social and somatosensory contact cues, leading to a facilitation of affiliative behaviors (e.g. adjacent lying). Interestingly, the 5-HT_{1A} agonist 8-OH-DPAT, which also stimulates adjacent lying behaviors and facilitation of social interaction in Wistar rats (Thompson et al., 2007), induces burst firing patterns in dopaminergic neurons within the ventral tegmental area (Arborelius et al., 1993; Lejeune & Millan, 2000) suggesting a shared prosocial mechanism with MDMA that may involve 5-HT_{1A} receptor and oxytocin modulation of mesolimbic circuitry. Given the importance of mesolimbic dopamine–oxytocin interactions in the formation of partner preferences (Insel, 2003; Young, Lim, Gingrich, & Insel, 2001), it is interesting to speculate whether MDMA and 8-OH-DPAT might produce enduring preferences for the conspecifics that are interacted with during the drug state.

Human brain imaging studies of the neural basis of maternal and romantic love suggest that the ''loving'' state involves a push–pull mechanism whereby activity in affiliative oxytocinergic and dopaminergic circuits is augmented while activity in brain regions mediating negative emotions and negative social judgments is suppressed (Zeki, 2007). In particular, accumbal, striatal and hypothalamic regions appear critical to the state of romantic love while the periaqueductal grey, an additional oxytocinergic region (Vaccari et al., 1998), is preferentially activated in maternal love. It is interesting to note then that the nucleus accumbens, medial striatum and periaqueductal grey were all regions activated to a greater extent by MDMA under social conditions. Activation of these circuits in the ''loving'' state may underlie the increased empathy and emotional closeness experienced by MDMA users (Bartels & Zeki, 2004). Users report that MDMA stimulates more of a sensual than a sexual experience (Passie, Hartmann, Schneider, Emrich, & Kruger, 2005), suggesting that ''love'' elicited by MDMA may have both romantic and maternal components.

Human fMRI studies have revealed decreases in amygdala activation that are associated with both maternal and romantic love (Bartels & Zeki, 2000, 2004). Consistent with this, a single PET study has also demonstrated decreased left amygdala activation in humans after administration of MDMA (Gamma, Buck, Berthold, Liechti, & Vollenweider, 2000). MDMA-released oxytocin may well be involved in these effects as oxytocin has been previously shown to attenuate both social fear and amygdala activation to anxiogenic social stimuli (Kirsch et al., 2005). The MDMA-induced amygdala Fos expression seen in the present study, clearly accentuated under social conditions, is consonant with these findings. It is important to note that Fos expression cannot readily speak to ''activation'' or ''deactivation'' in a region since Fos expression in inhibitory GABAergic neurons would inhibit a region despite such Fos expression being labeled Fos ''activation''.

Regions where social interaction did not augment MDMA-induced Fos expression

It is also instructive to consider the regions in which no specific increases in Fos expression were observed in the MDMA-SOCIAL group compared to the other groups. Interestingly, the supraoptic nucleus and paraventricular nucleus of the hypothalamus, key locations of oxytocin-synthesizing neurons in the brain, were similarly activated in rats in the MDMA-SOCIAL and MDMA-ALONE groups. That rats in the MDMA-SOCIAL group did not show augmented Fos expression in these areas suggests the possibility that MDMA causes similar oxytocin release under social and solitary conditions, and that this only results in accentuated neural activity in distal oxytocin receptor containing structures when social interaction with another conspecific is possible.

MDMA users report that tactile stimulation from others is especially reinforcing under the influence of the drug, leading to MDMA being sometimes known as an ''entactogen'' (Cole & Sumnall, 2003; Sumnall et al., 2006). We therefore thought it instructive to consider Fos expression in the Barrel fields of the somatosensory cortex, a region that relays tactile information from the whiskers of the rat (Girotti et al., 2006). We show for the first time that this region was significantly activated by MDMA but did not differ between MDMA-SOCIAL and MDMA-ALONE rats. The orbitofrontal cortex has been implicated in the pleasurable components of touch in humans (Rolls et al., 2003). However, again, Fos-expression in both the lateral orbital cortex and ventral orbital cortex was similarly increased in MDMA-SOCIAL and MDMA-ALONE groups. Thus a social-specific neural correlate of MDMA's entactogenic properties remains to be elucidated.

CONCLUSIONS

The present study highlights a number of brain regions that may be involved in MDMA-induced facilitation of social behavior in the rat and, by implication, may help explain the unique subjective and behavioral effects of MDMA in humans. In particular the caudate-putamen (medial), medial preoptic area, paraventricular thalamic nucleus, central amygdala, ventromedial hypothalamic nucleus, and medial amygdala (posterodorsal) may be key areas that are involved in MDMA's unique prosocial effects. A role for the mesolimbic dopamine system is also suggested. However, given the exceedingly complex nature of social behavior and our primitive knowledge of its neural bases, the exact contribution of these regions to social behavior in general, and MDMA stimulation of sociability in particular, requires further elucidation. The fact that a number of these regions contain oxytocin receptors suggests that further analysis of the role of this neuropeptide, acting at central sites, in MDMA prosocial effects may well be fruitful.

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