

# $\alpha$ 1-Adrenergic receptors mediate the locomotor response to systemic administration of ( $\pm$ )-3,4-methylenedioxymethamphetamine (MDMA) in rats

Jennifer Selken, David E. Nichols\*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, IN 47907, United States

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## Abstract

The recreational drug 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) increases locomotor activity when administered to rats. Although the published pharmacology of MDMA has focused almost exclusively on the roles of serotonin and dopamine, *in vitro* studies indicate that MDMA induces serotonin and norepinephrine release with equal potency. The present experiments tested the hypothesis that blockade of  $\alpha_1$ -adrenoceptors with systemic or local administration of the antagonist prazosin would attenuate the locomotor response to systemic administration of ( $\pm$ )-MDMA. Pretreatment with systemic prazosin (0.5 mg/kg) or microinjections into either the prefrontal cortex or ventral tegmental area completely blocked the locomotor stimulant effects of 5 mg/kg ( $\pm$ )-MDMA, assessed using a computerized Behavioral Pattern Monitor. Prazosin was more potent in blocking the locomotor stimulant effects of ( $\pm$ )-MDMA than a 2 mg/kg dose of (+)-amphetamine that produced a similar locomotor activity increase. These results indicate that activation of  $\alpha_1$ -adrenoceptors in both the prefrontal cortex and ventral tegmental areas modulates the locomotor response to MDMA.

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**Keywords:** MDMA; Locomotor activity; Prazosin;  $\alpha_1$ -Adrenoceptor; Rats

## 1. Introduction

MDMA (3,4-methylenedioxymethamphetamine; ecstasy) is a substituted amphetamine derivative that has become a popular recreational drug (Strote et al., 2002; Vollenweider et al., 2002). Neurochemically, MDMA elevates central extracellular monoamine levels by releasing terminal stores through a mechanism that involves a reversal of reuptake transporters. *In vitro* data indicate that MDMA is equally potent at increasing the outflow of both [ $^3$ H]5-HT (serotonin) and [ $^3$ H]NE (norepinephrine) from rat brain synaptosomes, and somewhat less potent in releasing [ $^3$ H]DA (dopamine) (Steele et al., 1987; Johnson et al., 1991; Rothman et al., 2001). The effect of MDMA on 5-HT and NE also has been demonstrated in hippocampal brain slices (Fitzgerald and Reid, 1993).

In spite of the potent ability of MDMA to induce release of NE, previous studies of the mechanism of action of MDMA

have focused almost exclusively on its ability to affect brain serotonergic and dopaminergic systems (e.g. Cole and Sumnall, 2003; Green et al., 2003; Simantov, 2004). Recently, however, Sprague et al. (2003) have shown that  $\alpha_1$ -adrenoceptors are involved in the hyperthermic response to MDMA, and Fantegrossi et al. (2004) reported that the  $\alpha_1$ -adrenoceptor antagonist prazosin blocked the hyperthermia and locomotor increase induced by MDMA in mice. No microdialysis data have been reported to indicate whether MDMA enhances central extracellular NE concentrations.

In rats, acute administration of MDMA produces hyperlocomotion (Gold et al., 1988; Callaway and Geyer, 1992a,b; Callaway et al., 1992, 1990; McNamara et al., 1995). Gold et al. (1988) described this motor response as being more similar to hallucinogen-induced locomotor activity than that induced by amphetamine. The main focus of investigations into neurochemical mechanisms underlying MDMA-induced hyperactivity has been on the contribution of serotonergic neurotransmission, despite the observation that the locomotor response to MDMA is sensitive to 6-OHDA (6-hydroxydopamine)-induced lesions of

\* Corresponding author. Tel.: +765 494 1461; fax: +765 494 6790.

E-mail address: [drdave@pharmacy.purdue.edu](mailto:drdave@pharmacy.purdue.edu) (D.E. Nichols).

the nucleus accumbens (NAc) (Gold et al., 1989) and dopamine receptor antagonists (Kehne et al., 1996).

In studies of drug-induced increases in locomotor activity, local administration of prazosin into the prefrontal cortex (PFC) decreased the locomotor response to amphetamine and morphine (Blanc et al., 1994; Darracq et al., 1998; Drouin et al., 2001), indicating that NE may modulate stimulant-induced locomotor activity through activation of  $\alpha_1$ -adrenoceptors in this region.  $\alpha_1$ -Adrenoceptors in the VTA also facilitate the ability of stimulant drugs to regulate the mesolimbic system. Amphetamine modulates burst activity of VTA neurons and induces DA release in the NAc through the activation of VTA  $\alpha_1$ -receptors (Pan et al., 1996; Shi et al., 2000). This evidence supports a role for tegmental  $\alpha_1$ -adrenoceptors in the neurochemical enhancing effects of psychostimulant drugs, but to our knowledge, it is not yet known whether activation of  $\alpha_1$ -adrenergic receptors in the VTA produces a behavioral effect.

Other studies have shown that systemic administration of prazosin can attenuate the locomotor response to cocaine, the NMDA receptor antagonist MK-801, and the DA reuptake inhibitor GBR 12783 (Arnt, 1995; Dickinson et al., 1988; Drouin et al., 2002a; Harkin et al., 2001; Mathe et al., 1996; Snoddy and Tessel, 1985; Svensson et al., 1995; Wellman et al., 2002). Thus, the experiments described herein tested the hypothesis that activation of  $\alpha_1$ -adrenoceptors in the prefrontal cortex and ventral tegmental area of the rat modulates the locomotor response to MDMA.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats (Harlan Labs, Indianapolis, IN) weighing approximately 300–350 g at the time of testing were housed in groups of 2–3 with food and water available *ad libitum*. For the microinjection studies, animals were housed individually in clear Plexiglas shoe box cages after cannula implantation. A 12/12 h light/dark cycle was used with lights on at 0700. All experiments were conducted between 0900 and 1700. Animals used in these studies were maintained in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals as amended August 2002, and experiments were carried out in accordance with the protocols approved by the Purdue Animal Care and Use Committee Care.

### 2.2. Apparatus

The Behavioral Pattern Monitor (Photobeam Activity System with Flex Field; San Diego Instruments, Inc., San Diego, CA) used for this experiment consisted of eight rectangular “flex field” chambers. Each chamber measured 38 cm high  $\times$  30 cm wide  $\times$  60 cm long and was comprised of four clear Plexiglas sides set atop an opaque Plexiglas base that measured 44 cm  $\times$  72 cm. The bottom frame was located 3 cm from the base and contained eight photo beams along its length and four across the ends. The sides of each chamber were

covered with a black curtain and illumination came from a 15 W bulb hanging directly above the chamber. The Flex Field apparatus collected individual photo beam breaks in “real time.” The field of each chamber was partitioned into 32 equal measured zones, and the number of entries an animal made into a particular zone was recorded through an interface that relayed data to a personal computer in an adjacent room. The number of entries into the 20 zones located along the walls was defined as peripheral activity and the number of entries into the 12 centrally-located zones was recognized as central activity.

### 2.3. Surgery

After one week of handling and acclimation to the lab environment, animals were stereotaxically implanted with bilateral 23-gauge guide cannulae (Plastics One, Roanoke, VA.) into the PFC ( $A=+2.2$  mm,  $L=\pm 0.5$  mm,  $V=-3.2$  mm, from bregma) or the VTA ( $A=-6.0$  mm,  $L=\pm 1.0$  mm,  $V=-5.5$  mm, from bregma). Stylets were inserted into the guide cannulae and animals were allowed one week to recover before beginning the microinjection experiments.

### 2.4. Microinjections

During the microinjection procedure, animals were gently restrained by hand, the stylet was removed from the guide cannula and replaced with an internal injection cannula (Plastics One, Roanoke, VA) attached by Teflon tubing to a 250  $\mu$ L Hamilton syringe (Fisher Scientific, Chicago, IL). A syringe pump (Instech model 2000, Instech Laboratories, Inc., Plymouth Meeting, PA) was used to infuse bilaterally a 0.5  $\mu$ L volume containing 0.25  $\mu$ g (0.6 nmol) of prazosin at a rate of a 0.5  $\mu$ L/min/side into either the PFC or VTA. Following injection, the cannula was left in place for approximately 30 s to prevent backflow of fluid.

### 2.5. Histology

At the completion of the behavioral experiments, animals were overdosed with Nembutal (Abbott Labs, North Chicago, IL) and transcardially perfused with 4% formaldehyde. Brain sections 40  $\mu$ m thick were cut on a freezing microtome and floated onto gelatin coated slides. After drying overnight, sections were stained with cresyl violet and coverslipped with DPX (Sigma-Aldrich Chemical, St. Louis, MO). Cannula placement was verified, and data were discarded for animals that did not have correct placement.

### 2.6. Drugs

The ( $\pm$ )-MDMA hydrochloride used in this study was synthesized in our laboratory (Nichols et al., 1986) and dissolved in 0.9% saline. (+)-Amphetamine hydrochloride (Sigma, St. Louis) was also dissolved in saline. Prazosin hydrochloride (Tocris, Ellisville, MO) was dissolved in distilled water with brief sonication. MDMA, amphetamine, and the 0.5 mg/kg (1.2  $\mu$ mol/kg) dose of prazosin were administered

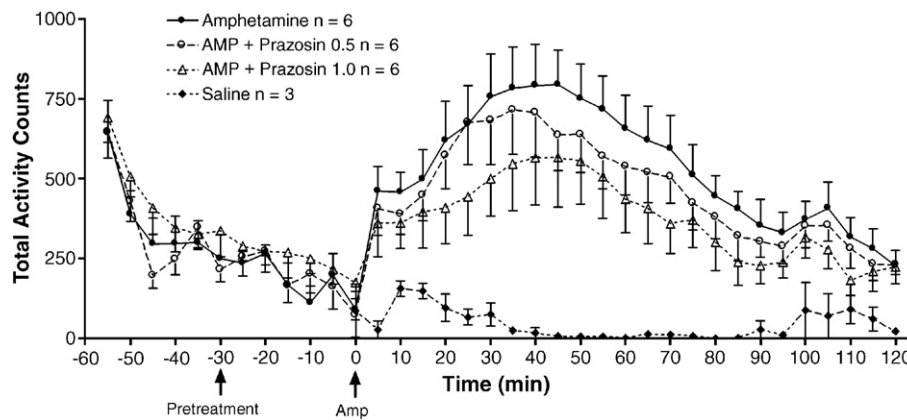


Fig. 1. Illustration of the time course of effect of 2 mg/kg (+)-amphetamine hydrochloride on rat total locomotor activity ( $n=6$ ) and effect of systemic prazosin (0.50 or 1.0 mg/kg;  $n=6$  each) pretreatment. Data were recorded every 5 min and are shown as raw untransformed activity counts.

intraperitoneally (IP) in a volume of 1 mL/kg. The 1 mg/kg (2.4  $\mu\text{mol/kg}$ ) dose of prazosin was administered IP in a volume of 2 mL/kg. The doses of prazosin were selected based on the results of Darracq et al. (1998).

## 2.7. Behavioral procedures

### 2.7.1. Effect of prazosin on amphetamine

Animals were handled and habituated to laboratory conditions for one week, during which they were placed without injection into the Behavioral Pattern Monitor (BPM) for 1 h each day to familiarize them with the testing apparatus. In experiment 1, eighteen animals were divided into the following three test groups, with six animals per group: saline–amphetamine (2 mg/kg); prazosin (0.5 mg/kg)–amphetamine (2 mg/kg); and prazosin (1 mg/kg)–amphetamine (2 mg/kg). On the test day animals were again habituated to the test chamber for 1 h while photo beam breaks were recorded to establish baseline activity. After habituation, animals received an intraperitoneal injection of saline or prazosin (0.5 or 1.0 mg/kg) and locomotor activity was monitored for 30 min. Once the 30 min interval was completed, all animals were injected intraperitoneally IP with 2 mg/kg amphetamine, and motor activity was monitored for another 120 min.

### 2.7.2. Effect of systemic prazosin on MDMA locomotor activity

Animals were handled and habituated as described above. The four test groups for this second experiment included saline–saline ( $n=14$ ), saline–prazosin ( $n=6$ ), saline–MDMA ( $n=6$ ), and prazosin 0.5–MDMA ( $n=6$ ). After the one-hour habituation phase, animals were injected IP with saline or 0.5 mg/kg prazosin. Following the 30-minute pretreatment interval, animals were injected with saline or MDMA (5 mg/kg), returned to the test chamber, and activity counts were recorded for 120 min.

### 2.7.3. Effect of intra-PFC and intra-VTA prazosin on MDMA locomotor activity

In the third experiment, microinjections of prazosin into the prefrontal cortex ( $n=8$  [14–6 discarded after histological

evaluation]) or ventral tegmental area ( $n=6$  [12–6 discarded after histology]) were given to block activation of  $\alpha_1$ -receptors. Following recovery from surgery, during the four days preceding the test day, animals were habituated to the Behavioral Pattern Monitor for 1 h each day. At this time, animals also were exposed to the microinjection procedure by gently restraining them and handling them around the cannula implantation site. On the test day, animals were placed without injection into the BPM chambers for 30 min. Microinjections of saline or prazosin were then administered and animals were returned to the Behavioral Pattern Monitor and locomotor activity was recorded for 30 min to establish baseline activity. After this 30-minute interval, animals were injected intraperitoneally with saline or 5 mg/kg of MDMA and locomotor activity was recorded for 120 min.

### 2.7.4. Topographical analysis of locomotor activity pattern after (+)-amphetamine or MDMA administration

Data collected for the experiments (Sections 2.7.1 and 2.7.2) were used for comparison of topographical components of the locomotor activity pattern. For each group of animals (determined by the treatment regimen) the mean of the number of photo beam crosses along the peripheral zones was compared with the mean of entries into the central zones. One animal with the number of entries closest to the mean for each group (saline–saline, saline–2 mg/kg (+)-amphetamine, saline–5 mg/kg MDMA, and 0.5 mg/kg prazosin–5 mg/kg MDMA) was chosen for graphical representation of the locomotor activity pattern for that treatment in the Behavioral Pattern Monitor.

## 2.8. Data analysis

Because of the variability in the individual 5 min time points, data were averaged for 15 min blocks and then normalized to provide data as percent of baseline photo beam breaks. Baseline is defined as the mean number of photo beam breaks during the two 15-minute intervals just prior to the treatment phase. For overall comparisons, the summed total of normalized activity counts for each rat was used as an approximation to the area under the curve (AUC). Significant differences between groups

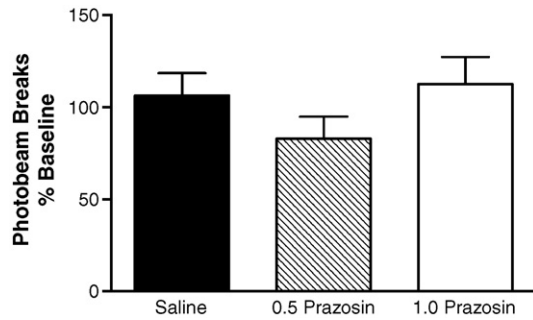


Fig. 2. The effect of systemic prazosin administration on rat spontaneous locomotor activity. Photo beam breaks were recorded during the 30 min following saline or prazosin treatment, and prior to administration of amphetamine or MDMA. Thus, the pretreatment data are pooled, and are expressed as a percent of baseline locomotor activity. Saline  $n=30$ , 0.5 mg/kg prazosin  $n=21$ , 1.0 mg/kg prazosin  $n=6$ . The effect of prazosin alone on locomotor activity was not significant ( $p>0.05$ ).

in experiments 1 and 3 were determined using a one-way analysis of variance followed by a Newman–Keuls post hoc test. A two-way analysis of variance with a Newman–Keuls post hoc test was implemented to detect differences between groups in experiment

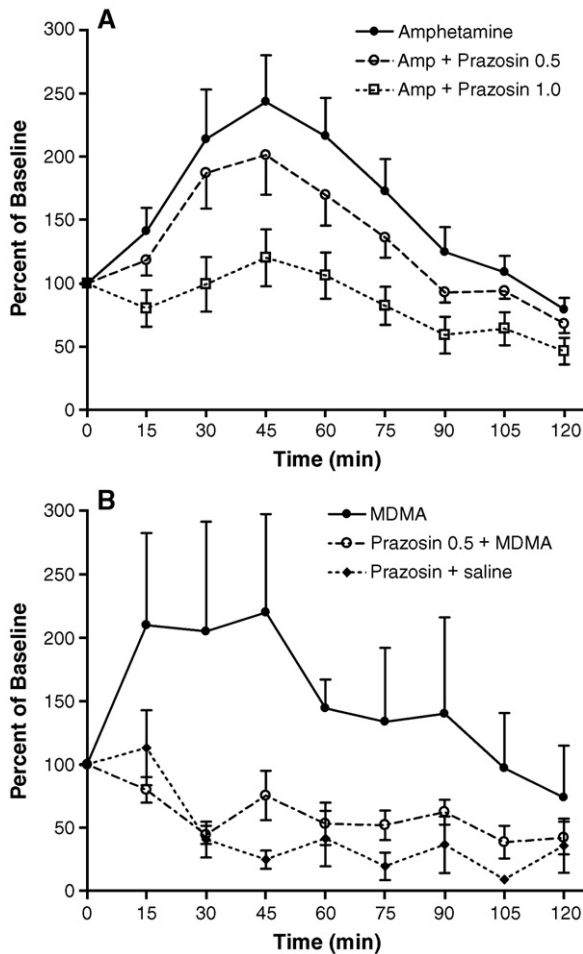


Fig. 3. Effect of prazosin pretreatment (1 mg/kg) on (A) 2 mg/kg (+)-amphetamine- ( $n=6$ ) and, (B) 5 mg/kg MDMA-induced ( $n=6$ ) increased locomotor activity. Photo beam breaks were recorded in 5-minute intervals, averaged over 15 min bins, and normalized to percent of baseline (see Materials and methods).

2. The effect of systemic prazosin administration on spontaneous locomotor activity was analyzed using a one-way ANOVA. The difference between central and peripheral activity was compared using a paired Student’s  $t$  test. The level of significance was set at  $p<0.05$ . Statistical analyses were conducted using Statistica (StatSoft, Tulsa OK) and graphs were generated using Graph-Pad© Prism software (San Diego, CA).

3. Results

3.1. Effect of prazosin on amphetamine

The complete time course for experiment 1 is illustrated in Fig. 1 for the effect of (+)-amphetamine. Increases observed in activity counts at time points –30 and 0 min result from handling and injection. The normalized data from this experiment are shown in Fig. 3A.

Photo beam breaks during the pretreatment phase of each experiment were recorded to determine the effect of prazosin alone on spontaneous motor activity. The data from animals used in experiments 1 and 2 were pooled for this assessment. As shown in Fig. 2, systemic prazosin alone had no significant effect on locomotor behavior. Prazosin injected into the PFC likewise had no significant effect on spontaneous locomotor activity in comparison to intra-PFC saline injections (data not shown). Similarly, intra-VTA injections of prazosin had no statistically significant effect on motor activity (data not shown).

Systemic pretreatment with prazosin attenuated the locomotor response to 2 mg/kg (+)-amphetamine (Figs. 1, 3A, and 4). The results of this experiment are consistent with the findings of Darracq et al. (1998), who had previously reported that prazosin blocked the locomotor stimulant effect of amphetamine. A one-way ANOVA indicated an overall significant effect of prazosin administration on the locomotor response to 2 mg/kg amphetamine ( $p<0.01$ ). All data are expressed as the percentage of baseline, which was generated by the saline–saline group; and thus this plot is not presented in Figs. 3A or 4. The magnitude of

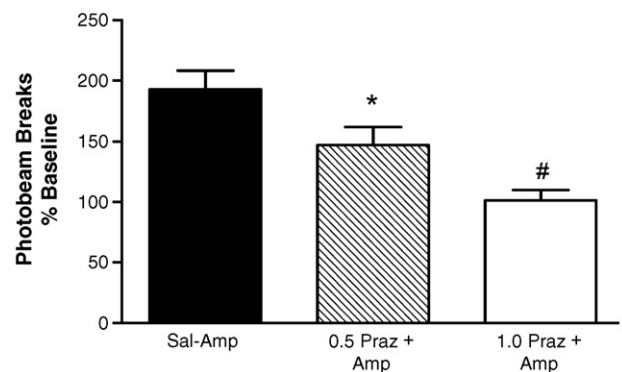


Fig. 4. The effect of systemic prazosin administration on the locomotor response to 2 mg/kg (+)-amphetamine. Pretreatment with 0.5 or 1.0 mg/kg prazosin produced a significant overall effect on the locomotor response to (+)-amphetamine ( $F_{(2,12)}=12.63$ ;  $p<0.01$ ). Significant decreases in (+)-amphetamine-induced hyperactivity were observed in animals pretreated with 0.5 mg/kg ( $*p<0.05$ ) and 1.0 mg/kg ( $#p<0.01$ ) of prazosin. Number of animals used for each treatment  $n=6$ .



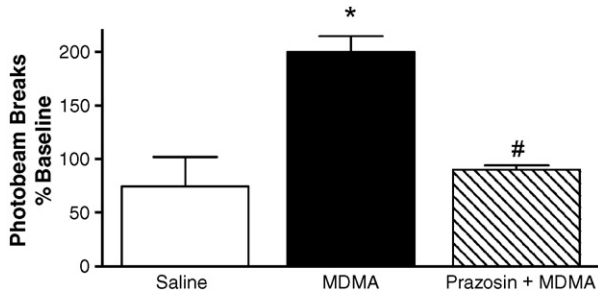


Fig. 5. The effect of systemic prazosin administration on the locomotor response to MDMA. In animals pretreated with 0.5 mg/kg prazosin, the hyperlocomotion generated by 5 mg/kg MDMA ( $*p < 0.01$ ) was significantly reduced, and this effect was not significantly different from prazosin–saline alone; saline–MDMA  $n = 14$ , prazosin–MDMA  $n = 6$ . # $p > 0.05$  versus saline.

the (+)-amphetamine-induced increase of locomotor activity was significantly reduced in animals pretreated with either 0.5 mg/kg ( $p < 0.05$ ) or 1.0 mg/kg prazosin ( $p < 0.01$ ).

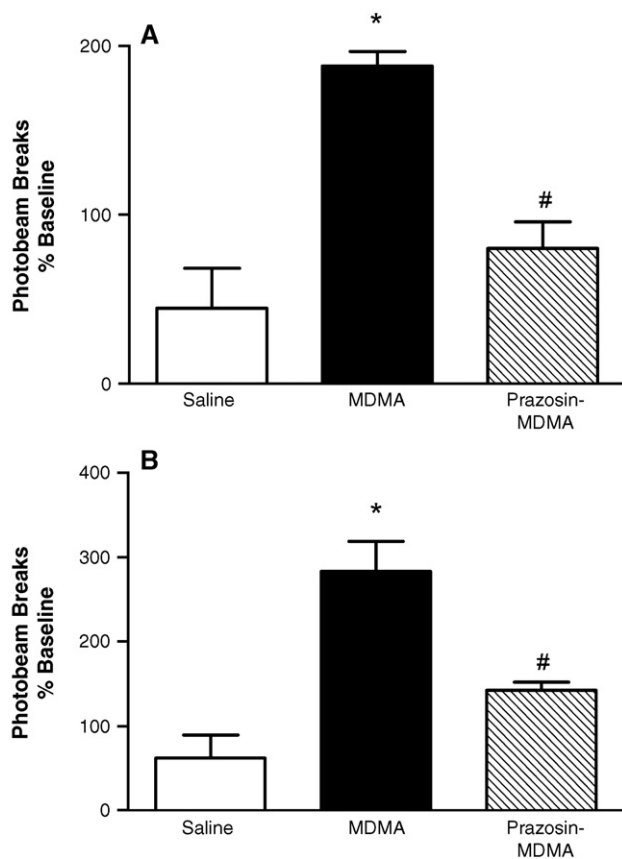


Fig. 6. (A) Locomotor activity was significantly increased by systemic administration of 5 mg/kg MDMA compared to saline ( $*p < 0.001$ ). The response to MDMA was significantly reduced in animals receiving intra-PFC injections of prazosin ( $#p < 0.01$ ). Number of animals used for each treatment:  $n = 11$  for saline–saline,  $n = 6$  for saline–MDMA and prazosin–MDMA. (B) Locomotor activity was significantly increased by 5 mg/kg MDMA compared to saline ( $*p < 0.001$ ). The locomotor response elicited by MDMA was significantly attenuated by intra-VTA injections of prazosin ( $#p < 0.01$ ). Number of animals used for each treatment:  $n = 9$  for saline–saline,  $N = 6$  for saline–MDMA and prazosin–MDMA.

### 3.2. Effect of systemic prazosin on MDMA locomotor activity

As shown in Figs. 3B and 5, prazosin (0.5 mg/kg) significantly ( $p < 0.01$ ) decreased the hyperlocomotion generated by acute administration of 5 mg/kg MDMA. A two-way ANOVA with a post hoc comparison of the saline–MDMA and

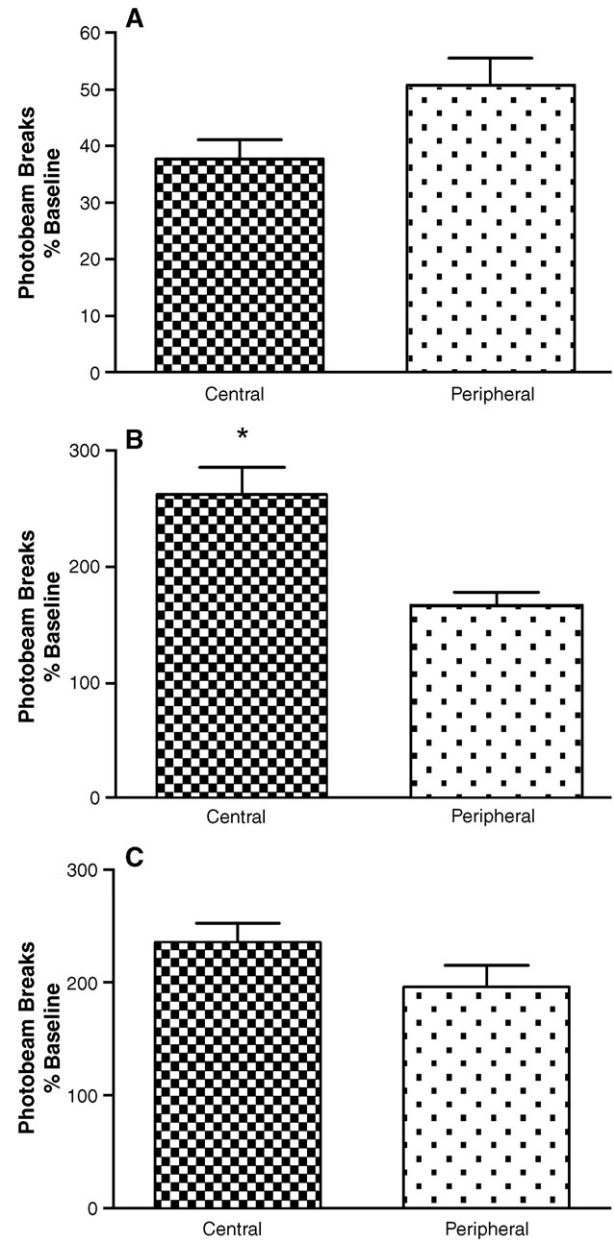


Fig. 7. Comparison of locomotor activity in central versus peripheral areas of the activity chamber (see Materials and methods). (A) The effect of saline on central versus peripheral activity. There was no significant difference between the time spent in the central versus peripheral part of the chamber, although there was a trend to spend more time in the peripheral region of the chamber ( $p = 0.06$ ). (B) The effect of (+)-amphetamine on central versus peripheral activity. Following administration of 2 mg/kg of (+)-amphetamine, animals spent significantly more time in the central area of the test chamber than in the peripheral area ( $*p < 0.01$ ). Number of animals for each treatment  $n = 6$ . (C) The effect of MDMA on central versus peripheral activity. There was no significant difference ( $p > 0.05$ ) between time spent in central versus peripheral areas of the chamber after administration of 5 mg/kg MDMA ( $n = 5$ ).

prazosin–MDMA groups revealed a significant interaction of pretreatment and treatment ( $p < 0.05$ ). Although we also initially pretreated with 1.0 mg/kg prazosin, this dose gave no further reduction in locomotor activity (data not shown).

### 3.3. Effect of intra-PFC and intra-VTA prazosin on MDMA locomotor activity

Histological examination of infusion sites intended for the prefrontal cortex confirmed cannulae traces in the PFC between 2.70 and 4.20 mm anterior to bregma, in the prelimbic, medial optical, and motor cortex regions. Six animals from the

prazosin–MDMA group with cannulae implanted into this region were removed from the study because the internal cannulae could not be completely inserted.

Histological analysis of animals with cannulae implanted into the VTA showed traces from  $-4.25$  to  $-6.04$  mm posterior to bregma. Six animals from the prazosin–MDMA group were dropped from statistical analysis because of incorrect cannula placement.

Intra-PFC injections of prazosin produced a significant overall effect on the locomotor response to MDMA (Fig. 6A). Systemic MDMA administration increased animals' locomotor activity to  $181 \pm 7.6\%$  of baseline ( $p < 0.001$  versus saline), but after intra-

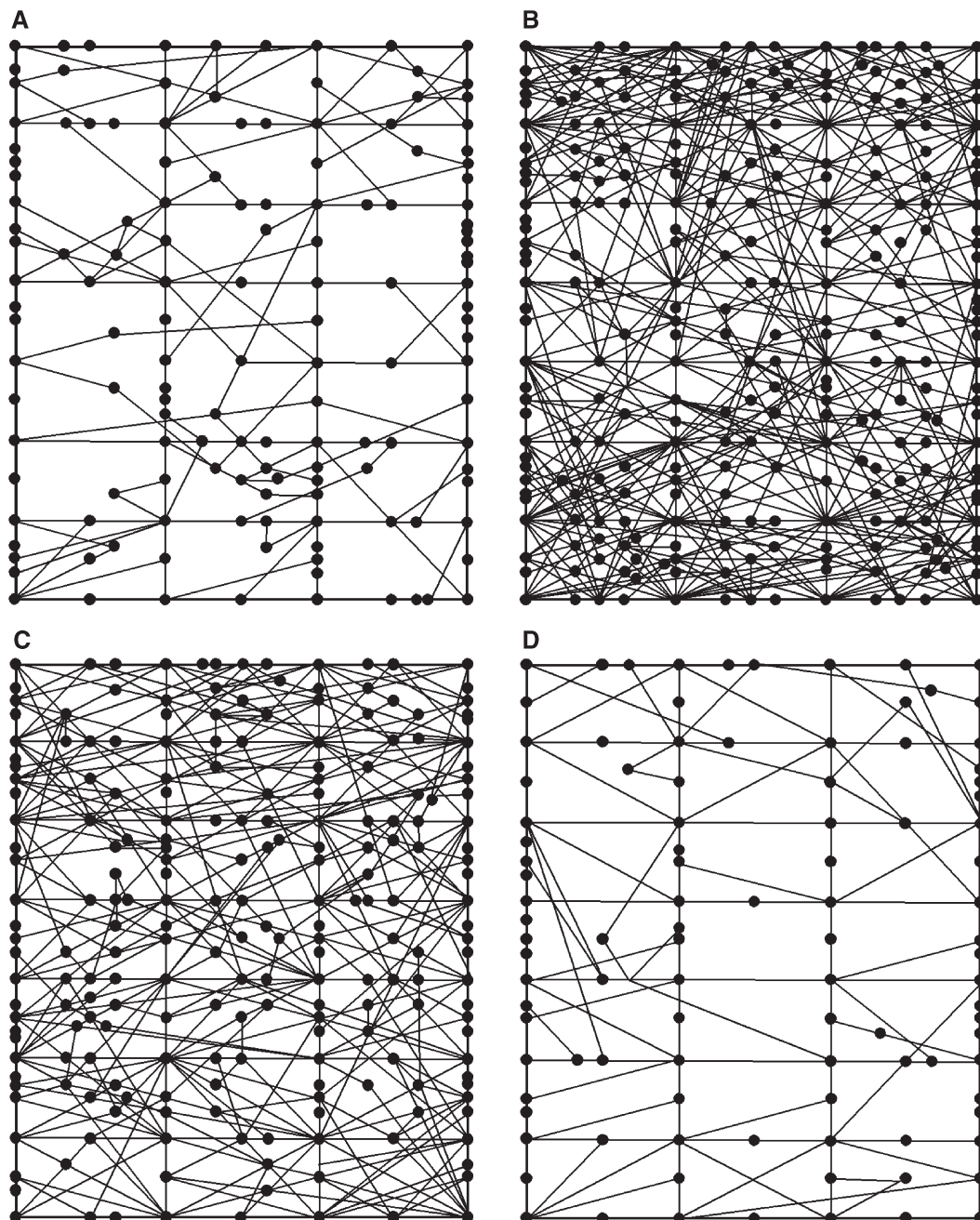


Fig. 8. Representative locomotor activity patterns in the Behavioral Pattern Monitor for (A) saline, (B) 2 mg/kg (+)-amphetamine, (C) 5 mg/kg MDMA, and (D) 5 mg/kg MDMA after pretreatment with 0.5 mg/kg prazosin.

PFC prazosin pretreatment the effect of MDMA was not significantly different from baseline ( $90 \pm 10\%$ ). Thus, blockade of cortical  $\alpha_1$ -adrenergic receptors significantly reduced ( $p < 0.01$ ) the locomotor response to systemic administration of 5 mg/kg MDMA. Pretreatment with intra-VTA injections of prazosin likewise produced an overall significant effect ( $p < 0.01$ ) on MDMA-induced locomotor activity (Fig. 6B).

#### 3.4. Topographical analysis of locomotor activity pattern after (+)-amphetamine or MDMA administration

The Behavioral Pattern Monitor activity data were analyzed to compare the time animals spent in the central versus the peripheral area of the test chamber after administration of saline, 2 mg/kg (+)-amphetamine, and MDMA (5 mg/kg) (Figs. 7 and 8). Systemic (+)-amphetamine administration (Fig. 7B) increased central activity to  $263 \pm 23\%$  of baseline, but gave only a  $166 \pm 12\%$  increase of peripheral activity. Consistent with the findings of Geyer et al. (1987), the present data show that activity in the central part of the chamber was significantly greater ( $*p < 0.01$ ) than peripheral activity after amphetamine administration.

Racemic MDMA increased locomotor activity in both the central ( $240 \pm 17\%$  of baseline) and peripheral ( $200 \pm 19\%$  of baseline) areas of the test chamber (Figs. 7C and 8). No significant difference ( $p < 0.05$ ) was detected, however, between peripheral and central locomotor activity after racemic MDMA treatment. These results are in contrast to the previously reported activity profile of (+)-MDMA (Callaway et al., 1990; Gold et al., 1988).

## 4. Discussion

The present experiments tested the hypothesis that the hyperactivity produced by MDMA in the rat is modulated by activation of  $\alpha_1$ -adrenoceptors in the prefrontal cortex and ventral tegmental area. The main finding is that blockade of  $\alpha_1$ -adrenoceptors by prazosin attenuates the locomotor response to MDMA. Prazosin binds nonselectively to the  $\alpha_{1A}$ -,  $\alpha_{1B}$ -, and  $\alpha_{1D}$ -adrenergic receptors, with 100-fold lower affinity for  $\alpha_{2B}$ - and  $\alpha_{2C}$ -receptors (Bylund et al., 1994). Nevertheless,  $\alpha_{1B}$ -deficient mice demonstrate markedly attenuated locomotor responses to (+)-amphetamine and cocaine (Drouin et al., 2002b), suggesting that this  $\alpha_1$ -adrenergic receptor subtype is the likely target of prazosin in these studies.

The effect of prazosin observed here is similar to the effects reported in other studies examining the role of the  $\alpha_1$ -adrenergic receptor in the locomotor response to amphetamine or cocaine (Arnt 1995; Blanc et al., 1994; Darracq et al., 1998; Dickinson et al., 1988; Drouin et al., 2002a; Drouin et al., 2002b; Snoddy and Tessel, 1985; Wellman et al., 2002). In fact, our data indicate that MDMA-induced motor behavior is more sensitive to  $\alpha_1$ -adrenoceptor blockade than that produced by (+)-amphetamine. Although a higher dose of MDMA (5 mg/kg) was used than (+)-amphetamine (2 mg/kg), at these doses both drugs produced comparable increases in locomotor activity: a 200% increase over baseline following MDMA and a 193% increase after (+)-amphetamine. After pretreatment with 0.5 mg/kg

prazosin, MDMA-induced activity was reduced by 55%, but this dose of prazosin produced only a 21% reduction in (+)-amphetamine-induced behavior. Whereas the larger 1.0 mg/kg dose of prazosin further attenuated the (+)-amphetamine response, the same dose of prazosin produced no further reduction of the locomotor increase by MDMA (data not shown). It seems possible that (+)-amphetamine, which is more potent than MDMA at increasing NE levels in the brain (Steele et al., 1987; Rothman et al., 2000), may produce greater  $\alpha_1$ -adrenoceptor activation and is therefore less responsive to prazosin than MDMA.

Electrophysiology studies in rat PFC slices show that prazosin blocks NE-induced burst firing of cortical neurons, and  $\alpha_1$ -adrenoceptor agonists increase neuronal activity (Bradshaw et al., 1981; Bradshaw et al., 1983; Marek and Aghajanian, 1999), indicating that excitation of cortical  $\alpha_1$ -adrenoceptors can regulate neuronal activity in the cortex. Excitation of PFC neurons modulates mesolimbic transmission through projections to the VTA and nucleus accumbens, with the tegmental projection playing a more dominant role (Karreman et al., 1996; Karreman and Moghaddam, 1996). Thus, noradrenergic–dopaminergic interactions may occur through activation of  $\alpha_1$ -adrenergic receptors in the PFC. Consistent with these *in vitro* data, microinjections of phenylephrine into the PFC of rats increased locomotor activity (Smialowska et al., 1994), whereas microinjections of prazosin into this area abolished the hyperactivity induced by localized or systemic injections of (+)-amphetamine, as well as systemic injections of the opiate morphine (Blanc et al., 1994; Darracq et al., 1998; Drouin et al., 2001).

In light of these results and the role of the PFC in regulating locomotor activity, we examined whether MDMA-induced locomotor activity was sensitive to the blockade of cortical  $\alpha_1$ -adrenoceptors. We observed a significantly decreased locomotor response to MDMA in animals that had received intra-PFC injections of prazosin compared to those injected with saline. Thus, the results of the present microinjection experiments indicate that  $\alpha_1$ -adrenoceptors in the PFC modulate the locomotor response to MDMA.

Locus coeruleus-mediated burst firing of VTA neurons is also sensitive to  $\alpha_1$ -adrenoceptor blockade (Grenhoff et al., 1993), and  $\alpha_1$ -adrenoceptors regulate amphetamine-induced neuronal activity in the VTA and DA release in the nucleus accumbens (Pan et al., 1996). Additionally, the  $\alpha_1$ -adrenergic receptor agonist phenylephrine depolarized VTA neurons in rat brain slices (Grenhoff et al., 1995). In the present experiments prazosin microinjections into the VTA of rats prior to systemic administration of MDMA decreased the locomotor response to MDMA, suggesting that activation of  $\alpha_1$ -adrenoceptors in this region plays a role in the behavioral consequences of MDMA administration. Finally, it is proposed that the intra-VTA prazosin-induced blockade of the locomotor hyperactivity produced by MDMA is linked to the previously demonstrated regulatory role of noradrenergic neurons on cortical DA transmission (e.g. Gresch et al., 1995; Pozzi et al., 1994).

It might be noted that immediately after injection into the PFC or VTA the local concentration of prazosin may approach 1 mM at the injection site. This high concentration would allow some diffusion from the site in both brain areas, as well as



potentially allowing prazosin to interact with nonadrenergic sites where it has much lower affinity. This problem occurs in any microinjection study unless the drug concentration is very low, is given very slowly, or both. As a practical matter, the use of awake and unrestrained rats does not allow prolonged administration times. Nevertheless, our results are consistent with other microinjection studies and we believe the effect observed after local administration is best explained by adrenergic receptor blockade in the specific brain areas targeted.

We also examined the pattern of locomotor activity produced by administration of both (+)-amphetamine and racemic MDMA. Consistent with the previous reports (Geyer et al., 1987), we observed that animals spent more time (>40%) in central than peripheral regions of the chamber following administration of 2 mg/kg (+)-amphetamine. By contrast, several studies from different laboratories have reported observing more peripheral than central activity following (+)-MDMA administration (McCreary et al., 1999; Bankson and Cunningham, 2002; Gold et al., 1988; Callaway et al., 1990). In the present experiments, however, locomotor activity was nearly evenly distributed between the central and peripheral areas of the chamber following administration of 5 mg/kg (±)-MDMA.

In light of these previous studies, our results were somewhat surprising. Nevertheless, there are several important methodological differences between the present and earlier studies, such as habituation and time of activity measurement. Rodents tend to display a natural preference for the perimeters (thigmotaxis) of an environment and avoidance of open areas. In the open field paradigm, avoidance of central areas is used as an indicator of anxiety and is sensitive to anxiolytic drugs (Treit and Fundytus, 1988). In an effort to reduce the effect of a novel environment on locomotor activity, animals used in the present experiments were repeatedly exposed to the test chamber. Most importantly, subjects were allowed 1 h to habituate to the measurement arena prior to drug administration. Thus, the lack of effect of MDMA on central *versus* peripheral activity in the present study could be caused by a reduction in anxiety-like behaviors during the test period.

Perhaps one of the most important differences may be the fact that we used (±)-MDMA, whereas all of the cited previous studies used (+)-MDMA. Racemic MDMA is the only form of the drug found on the illicit market; its (+) enantiomer has never appeared on the street. Although (+)-MDMA is more “potent” in psychostimulant-like behavioral effects in animals than the racemic mixture, the (–) isomer is certainly not inert and probably contributes to the overall pharmacological properties of racemic (±)-MDMA (e.g. Nash et al., 1994; Anderson et al., 1978). We suggest that if the psychopharmacology of MDMA in humans is to be fully understood, the substance that is actually available to human users, racemic (±)-MDMA, is the one that should be studied.

In conclusion, the present experiments have demonstrated that blockade of  $\alpha_1$ -receptors in the prefrontal cortex and ventral tegmental area abolishes the motor stimulant effects of the recreational drug 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) in rats. The motor activating effect of dependence-producing drugs is believed to require an increase

in DA transmission in the mesolimbic projection from the midbrain VTA to the forebrain NAc (Bozarth and Wise, 1981). Thus, the results of the present study, together with the findings of previous work, suggest that  $\alpha_1$ -adrenoceptors are essential for a regulatory role of noradrenergic neurons in cortical dopamine transmission, and that noradrenergic–dopaminergic interactions may underlie the stimulant mechanisms of recreational drugs. We further suggest that additional research is warranted into the pharmacological relevance of noradrenergic pathways to the psychopharmacology of MDMA.

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