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ORIGINAL INVESTIGATION

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Effects of dopamine D_1- or D_2-like receptor antagonists on the hypermotive and discriminative stimulus effects of (+)-MDMA

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Abstract *Rationale:* Both dopamine (DA) and serotonin (5-HT) release are evoked by (+)-MDMA; however, little is known of the contribution of DA D_1- and D_2-like receptors (D_1R and D_2R, respectively) in the behavioral effects of (+)-MDMA. *Objectives:* To test the hypothesis that a D_1R or D_2R antagonist would attenuate the hypermotive or discriminative stimulus effects of (+)-MDMA. *Methods:* Male Sprague-Dawley rats (*n*=164) were pretreated with the D_1R antagonist SCH 23390 (3.125–50 μg/kg, SC) or the D_2R antagonist eticlopride (12.5–50 μg/kg, SC) prior to treatment with (+)-MDMA (3 mg/kg, SC) and locomotor activity was recorded using photobeam monitors. Twelve additional rats trained to discriminate (+)-MDMA (1 mg/kg, IP) from saline in a two-lever water-reinforced FR20 task were administered SCH 23390 (6.25 μg/kg, IP) or eticlopride (12.5 μg/kg, IP) prior to (+)-MDMA (0.375–1.0 mg/kg, IP). Rats were then placed in the drug discrimination chambers and the percent (+)-MDMA appropriate responding and response rate were measured. *Results:* Both SCH 23390 and eticlopride blocked (+)-MDMA-evoked hyperactivity in a dose-related manner; the highest doses of the antagonists also effectively suppressed basal locomotor activity. In rats trained to discriminate (+)-MDMA from saline, SCH 23390 (6.25 μg/kg), but not eticlopride (12.5 μg/kg), blocked the stimulus effects of (+)-MDMA without altering response rate. *Conclusion:* These data indicate that DA released indirectly by (+)-MDMA administration results in stimulation of D_1R and D_2R to enhance locomotor activity. Furthermore, the D_1R appears to play a more prominent role than the D_2R in the discriminative stimulus properties of (+)-MDMA.

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Introduction

Over the past decade, there has been a sharp rise in the use of the substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA), commonly known as "ecstasy." Use of MDMA is especially popular in young adults, among whom the awareness of the possible dangers associated with MDMA has recently begun to rise (Johnston et al. 2003). Likewise, there has been a push to better understand the mechanisms underlying the actions of MDMA in order to better delineate the potential dangers associated with MDMA use.

Two common behavioral measures employed to study the mechanisms of psychostimulants in rodents are measurement of locomotor activity and drug discrimination. In general, the ability of a drug to elicit locomotor hyperactivity correlates with its ability to enhance dopamine (DA) release in the nucleus accumbens (NAc), the terminal region of the DA mesoaccumbens pathway or "reward" pathway (for review, see Erinoff and Brown 1994). Thus, measurement of locomotor activity can be used as an indirect measure of activation of the "reward" circuit (Wise and Bozarth 1987). The NAc has also been implicated in the recognition of the "interoceptive cue" or stimulus effects elicited by psychoactive drugs as demonstrated using the drug discrimination assay, which is thought to model the subjective effects of drugs in humans (for review, see Callahan et al. 1997).

Indeed, (+)- and (±)-MDMA do elicit hyperactivity (Gold et al. 1988; Callaway et al. 1990) and can be readily discriminated from saline in the drug discrimination task (Schechter 1989; present study). Additionally, (±)-MDMA has been shown to enhance extracellular DA release in the NAc (White et al. 1994) at least partially due to reversal of the DA transporter (DAT; Rudnick and Wall 1992). However, unlike its parent compound amphetamine,

which is primarily a DA releaser, (±)-MDMA is actually more potent at releasing serotonin (5-HT; IC_{50}=56.6 ±2.1 nM) via reversal of the 5-HT transporter (SERT; Rudnick and Wall 1992) than DA (IC_{50}=376±16 nM; Rothman et al. 2001). Likewise, many of the behavioral effects of (+)- and (±)-MDMA, including the hypermotive and discriminative stimulus effects, are thought to be mediated by 5-HT neurotransmission (Schechter 1989; Callaway et al. 1990; Baker et al. 1997; Bankson and Cunningham 2001; Frankel and Cunningham 2003). As such, the majority of research has focused on a 5-HTergic contribution to the effects of (+)- and (±)-MDMA. However emerging evidence of an important role for 5-HT-DA interactions in the mechanisms of action of (+)- and (±)-MDMA (for review, see Bankson and Cunningham 2001) has shifted attention to discerning the contribution of the DA system in addition to 5-HT to the effects of MDMA.

Although (±)-MDMA has higher affinity for SERT and is more potent at reversing SERT than DAT, (±)-MDMA evokes a proportionately larger amount of DA release compared to 5-HT release in the NAc (White et al. 1994). Because (+)-MDMA-evoked DA release can be accounted for by both direct actions of (+)-MDMA on DAT as well as via 5-HT stimulated DA release (Koch and Galloway 1997), it is difficult to separately allocate the contribution of 5-HT and DA to these effects. (+)- or (±)-MDMA-induced DA release can be attenuated not only by the DAT inhibitor GBR 12909, but also with the selective 5-HT reuptake inhibitor fluoxetine (Gudelsky and Nash 1996; Koch and Galloway 1997). Additionally, antagonists for the 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} receptors that modulate (+)- and (±)-MDMA-induced locomotor hyperactivity (see Bankson and Cunningham 2001) are also known to modulate basal (Benloucif et al. 1993; Ng et al. 1999; Lucas and Spampinato 2000) and/or stimulated DA release (Parsons et al. 1999; Porras et al. 2002). Thus, since each of these receptors has the capacity to alter DA activity, the observed effects of serotonergic compounds on (+)- and (±)-MDMA-induced locomotor activity may ultimately be related to the ability of these compounds to modulate DA mesoaccumbens pathway activation. Thus it is important to determine the role of DA and the DA receptors in mediating the behavioral effects of (+)- and (±)-MDMA.

Two families of DA receptors, the D_1-like and the D_2-like receptors (D_1R and D_2R, respectively), have been shown to be important in the expression of the hypermotive and discriminative stimulus effects of DA indirect agonists such as cocaine and amphetamine (Callahan et al. 1991; Callahan and Cunningham 1993; Schechter 1997; O'Neill and Shaw 1999). The D_1R family contains the D_1R and D_5R that are primarily post-synaptic and positively coupled to cAMP (for review, see Lachowicz and Sibley 1997). The D_2R family, which includes the D_2R, D_3R, and D_4R, are located both pre- and post-synaptically and are negatively coupled to cAMP, but stimulate PLA2 (Lachowicz and Sibley 1997). Both D_1R and D_2R mRNA and protein are found in high concentrations within the basal forebrain, and are prominently represented in the ventral tegmental area (VTA) and NAc (Dearry et al. 1990; Bouthenet et al. 1991; Landwehrmeyer et al. 1993); D_3R, D_4R, and D_5R mRNA are also present within the brain, but other than high concentrations of D_3R in the NAc shell, these receptors appear to be much less concentrated than the D_1R and D_2R (Bouthenet et al. 1991; Meador-Woodruff et al. 1992; O'Malley et al. 1992; Landwehrmeyer et al. 1993). Blockade of D_1R or D_2R with respective antagonists suppresses basal or stimulated locomotor activation (Agmo and Soria 1999; Chausmer and Katz 2001). Likewise, both D_1R and D_2R have been implicated in control of cocaine- and amphetamine- stimulated locomotor hyperactivity (O'Neill and Shaw 1999) and thus are postulated to be important in (+)- and (±)-MDMA-induced locomotor hyperactivity as well. Indeed, Kehne and colleagues (1996) demonstrated that the D_1R antagonist SCH 23390 and the D_2R antagonist haloperidol blocked (±)-MDMA-induced locomotor hyperactivity. This study used a single dose of the antagonists and a relatively high dose (20 mg/kg) of (±)-MDMA.

The first goal of the present study was to further extend the results of Kehne and colleagues (1996) to test the hypothesis that an antagonist of D_1R or D_2R will attenuate (+)-MDMA-induced locomotor activation in a dose-related manner. In the present experiment, we chose a relatively low dose (3 mg/kg) of (+)-MDMA, which is described to produce robust locomotor hyperactivity (McCreary et al. 1999; Bankson and Cunningham 2002). As the more active isomer, (+)-MDMA is more potent than either (±)-MDMA or (−)-MDMA at eliciting hyperactivity (Callaway et al. 1990). As mentioned above, both D_1R and D_2R antagonists have been shown to effectively suppress basal locomotor activity. Thus, we also aimed to uncover doses of both antagonists that might attenuate (+)-MDMA-induced hyperactivity without altering basal locomotor activation. A second goal of the present study was to determine if administration of a D_1R or D_2R antagonist would alter the discriminative stimulus properties of (+)-MDMA when administered at doses shown to block (+)-MDMA-induced hyperactivity.

Materials and methods

Animals

Adult male Sprague-Dawley rats (n=176; Harlan Sprague-Dawley, Inc., Indianapolis, Ind., USA) weighing 225–350 g at the beginning of the experimental procedures were used. The animals were housed two or four to a cage for drug discrimination or locomotor activity assays, respectively, in a temperature (21–23ºC) and humidity (40–50%) controlled environment and lighting was maintained under a 12-h light-dark cycle (lights on at 7:00 a.m. to 7:00 p.m.). For locomotor activity assays, food and water were available ad libitum (except during habituation and testing procedures). In drug discrimination assays, food was available ad libitum, except during the experimental sessions; however, the amount of water each animal received was restricted to that given during operant training sessions, after test sessions (10–15 min) and on weekends (36 h). All

experimental protocols were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1986) and with the approval by the Institutional Animal Care and Use Committee.

Drugs

The followings drugs were used: (+)-MDMA was obtained from the National Institutes on Drug Abuse (Research Triangle, N.C., USA), R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride [R-(+)-SCH 23390] and S-(−)-3-chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamidehydro-chloride [S-(−)-eticlopride] were obtained from Sigma Aldrich Co. (St Louis, Mo., USA). All drugs were dissolved in sterile saline (0.9% NaCl); doses refer to the weight of the salt. Drug injections were administered subcutaneously (SC) and intraperitoneally (IP) in locomotor activity and drug discrimination experiments, respectively.

Locomotor activity experiments

Apparatus

Locomotor activity was monitored and quantified under low light conditions using a modified open field activity system (San Diego Instruments, San Diego, Calif., USA) housed within sound attenuated chambers. Each of the eight chambers consisted of a clear Plexiglas open field (40×40×40 cm). Two photobeam matrices are used for recording activity: at 4 cm from the floor, a 4×4 matrix counts horizontal activity and at 16 cm from the floor horizontal photobeams count vertical (rearing) activity. The control software (Photobeam Activity Software; San Diego Instruments) was used to count horizontal and vertical activity and data were stored for subsequent statistical evaluation. Video cameras located above the chambers were used to monitor activity continuously without disruption of behavior.

Behavioral procedures

The locomotor activity experiments were conducted in three separate groups of animals according to pretreatment: the first group (n=36) was administered lower doses of D_1R antagonist SCH 23390 (3.125 or 6.25 μg/kg, SC), the second group (n=64) was administered higher doses of SCH 23390 (12.5, 25, or 50 μg/kg, SC), and the third group (n=64) received the D_2R antagonist eticlopride (12.5, 25, or 50 μg/kg, SC). Rats were habituated to the activity monitors for 3 h/day on the 2 days prior to the start of the experiment. On the test day, rats were habituated to the locomotor activity monitors for 30 min prior to receiving pretreatment with either the assigned dose of SCH 23390, eticlopride, or saline, followed 30 min later by treatment with (+)-MDMA (3 mg/kg, SC) or saline (1 ml/kg, SC). Recording of activity in 5-min time epochs for 90 min began immediately following the treatment injection.

Statistical analysis

Total activity counts were summed for each individual animal throughout the 90-min session. Data are presented as mean total activity counts (±SEM) with the dependent measure of total horizontal or vertical activity recorded during the testing session. Since group comparisons were specifically defined prior to the start of the experiment, planned comparisons were conducted in lieu of an overall F-test in a multifactorial anaysis of variance (ANOVA); this statistical analysis has been supported in a number of statistical tests (e.g. Keppel 1973). Significant interactions were followed up with a priori comparisons using Fisher's least significant difference procedure (Keppel 1973). Time course data were broken down into six separate 15-min time bins and a three-way ANOVA was used to detect pretreatment×treatment×time interactions. In the case that a significant interaction was present, differences between treatment groups were determined at each 15-min time point using a one-way ANOVA. All planned comparisons were assessed with Fisher's least significant difference test with the error rate (α) set at 0.05.

Drug discrimination experiments

Apparatus

The procedures were conducted in 12 two-lever operant chambers (Lafayette Instruments Model 80001, Lafayette, Ind., USA or Med Associates Model ENV-001, St Albans, Vt., USA) housed in sound attenuating chambers (Lafayette Instruments Model 80015, or Med Associates Model ENV-015). The operant chambers contained two levers with a water dispenser centered between the levers. Illumination was provided by a 28-V house light; ventilation and masking noise were supplied by a blower. An interface (Med Associates) connected the chambers to a PC computer running Med-PC for Windows software (Med Associates) that controlled and recorded all experimental events.

Design and procedures of drug discrimination analyses

Rats (n=12) were trained to discriminate an injection of (+)-MDMA (1.0 mg/kg, IP) from saline (1 ml/kg, IP) administered 20 min before daily (Monday to Friday) 30-min sessions. In order to maintain a pseudorandomized drug administration schedule, (+)-MDMA and saline were administered irregularly with the restriction that neither training condition prevailed for more than three consecutive sessions. Initially, training began under a schedule of continuous water reinforcement on a fixed ratio (FR) 1 with only the stimulus-appropriate (drug or saline) lever present ("errorless training"); the schedule of reinforcement was incremented until all rats were responding reliably (>300 bar presses on treatment appropriate lever) under an FR 20 schedule for each experimental condition. For half of the rats, responses on the right lever were reinforced following drug administration; for the remaining rats, responses on the left lever were reinforced following drug administration. To control for the possible development of position cues based upon olfactory stimuli, a pseudo-random relationship was maintained between the lever programmed to deliver reinforcement for each consecutive rat run in the same experimental chamber (Extance and Goudie 1981). After responding stabilized on an FR 20 schedule of reinforcement, both levers were presented simultaneously and rats were required to respond on the stimulus-appropriate (correct) lever in order to obtain reinforcement (water). At the start of discrimination training, the session time was shortened from 30 min to 20 min; there were no programmed consequences for responding on the incorrect lever. After responding stabilized, training sessions were shortened from 20 min to 15 min. This phase of training continued until the performance of all rats reached criterion (individual mean accuracies of at least 80% correct prior to the first reinforcer for ten consecutive sessions).

Drug discrimination test procedures

Test sessions were then initiated and were conducted once or twice a week with training (maintenance) sessions intervening on other days. Test sessions included all rats that met the 80% accuracy criterion during the preceding (+)-MDMA and saline maintenance sessions. During test sessions, rats were placed in the chamber as during training sessions and upon completion of 20 responses on either lever or after the session time (15 min) had elapsed, a single

(water) reinforcer was delivered, the house light was turned off and the rat was removed from the chamber. After being returned to the home cages, all rats were allowed 10–15 min of free access to water.

Two pharmacological test manipulations were performed during test sessions in all rats (n=12). In substitution tests, rats were administered (+)-MDMA (0.375, 0.5, 0.75 or 1.0 mg/kg, IP), SCH 23390 (6.25 μg/kg, IP), eticlopride (12.5 μg/kg, IP), or saline (1 ml/kg, IP). In combination tests, rats were tested for lever selection following administration (IP) of SCH 23390 (6.25 μg/kg) or eticlopride (12.5 μg/kg) prior to a dose of (+)-MDMA (0.375, 0.5, 0.75 or 1.0 mg/kg). SCH 23390 or eticlopride were administered 30 min and (+)-MDMA 20 min prior to placement in the operant chambers. The doses of SCH 23390 and eticlopride were chosen based upon the results of the current locomotor experiments, as these doses of the antagonists attenuated (+)-MDMA-induced hyperactivity without significantly altering basal locomotor activity.

Statistical analysis

During training sessions, accuracy was defined as the percentage of correct total responses before the delivery of the first reinforcer; during test sessions, performance was expressed as the percentage of (+)-MDMA-appropriate responses to the total responses prior to the first reinforcer. Response rates (responses per min) were also evaluated during training and test sessions as a measure of behavioral disruption. Response rates were calculated as the total number of responses emitted on either lever before the completion of the first FR 20 on the stimulus appropriate lever (training sessions) or prior to completion of 20 responses on either lever (test sessions), divided by the number of minutes taken to complete the first ratio. During test sessions, data from rats that did not complete the FR 20 within the allotted 15 min period were excluded from analyses. For (+)-MDMA substitution tests, Student's t-test for repeated measures were used to compare the percentage of (+)-MDMA-appropriate lever responding and response rates during test sessions with the corresponding values for the previous (+)-MDMA or saline sessions. Because two comparisons to each test data point were conducted, the experimentwise error rate (α) was adjusted to P<0.025 (0.05÷2 comparisons). For combination tests, a two-way ANOVA for repeated measures was used to assess whether the percentage of (+)-MDMA-appropriate lever responding and response rates observed across four doses of (+)-MDMA (0.375. 0.5, 0.75 or 1.0 mg/kg) differed in the presence versus the absence of a fixed dose of a DA antagonist; a priori comparisons at each dose of (+)-MDMA in the presence and absence of the test drug were conducted using Student's t-test. All comparisons were made with an experimentwise type I error rate (α) set at 0.05 (Keppel 1973), except where noted above where α was set at 0.025.

Results

Locomotor activity experiments

Effects of D_1R antagonist SCH-23390 on spontaneous and (+)-MDMA-evoked activity

Locomotor activity was assessed following pretreatment with SCH 23390 (3.125, 6.25, 12.5, 25, or 50 μg/kg) or saline (1 ml/kg) and treatment with (+)-MDMA (3 mg/kg) or saline (1 ml/kg; Figs. 1 and 2). As stated in Materials and methods, this experiment was conducted in two groups of animals, one of which received lower doses of SCH 23390 (3.125, 6.25 μg/kg) and the other which received higher doses of SCH 23390 (12.5, 25, 50 μg/kg). Statistical analyses comparing the total horizontal or vertical activity counts of the lower dose and higher dose groups determined that there were no significant differences in the levels of horizontal [F(1,13)=0.65, P=0.44] or vertical activity [F(1,13)=0.01, P=0.92] for the Sal/Sal rats in the two groups, nor was there a significant difference in the levels of horizontal [F(1,13)=0.38, P=0.55] or vertical activity [F(1,13)=0.06, P=0.81] for the Sal/(+)-MDMA rats between the two groups (P>0.05). Therefore, data from the two groups were pooled and the mean total horizontal or vertical activity counts for all Sal/Sal (n=14) or Sal/MDMA (n=14) treated rats served as the comparison for tests of all doses of SCH 23390.

There was a main effect of drug treatment observed for both total horizontal [F(11,97)=24.22, P<0.01] and vertical activity [F(11,97)=12.82, P<0.01]. (+)-MDMA significantly increased both mean total horizontal (Fig. 1) and vertical activity (Fig. 2) compared with saline (P<0.05).

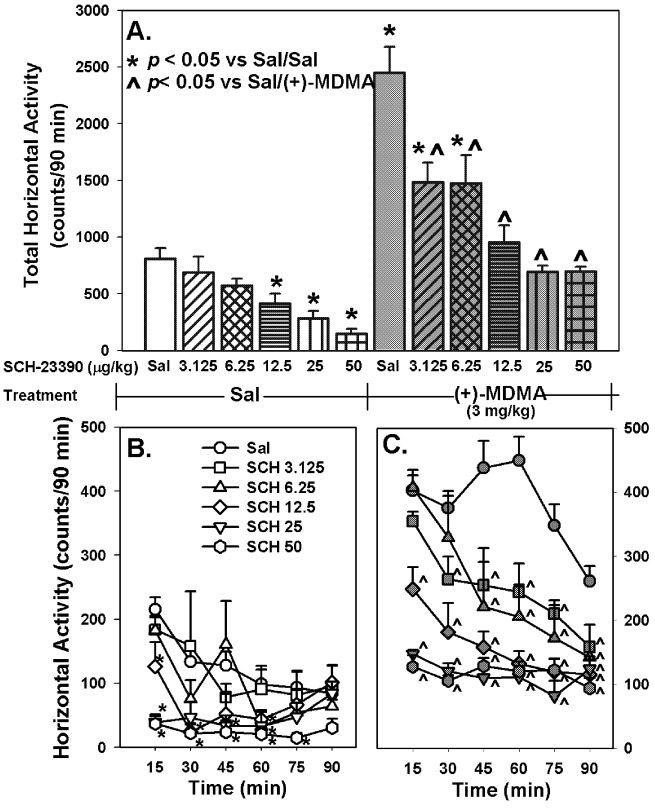

Fig. 1 Effects of the D_1R antagonist SCH 23390 on horizontal hyperactivity induced by (+)-MDMA. **A** Datapoints are represented as mean total horizontal activity (counts/90 min; SEM) in rats pretreated with saline (*Sal*; 1 ml/kg, SC; n=14) or SCH 23390 (*SCH*; 3.125, 6.25, 12.5, 25 or 50 μg/kg, SC; n=6–8) followed by treatment with saline (1 ml/kg, SC) or (+)-MDMA (3 mg/kg, SC). **B**,**C** Datapoints represent the timecourse for horizontal activity in 15-min time bins (SEM) across the 90-min session; pretreatment with saline (1 ml/kg, SC, *circles*) or SCH 23390 [3.125 (*square*), 6.25 (*triangle*), 12.5 (*diamond*), 25 (*inverted triangle*) or 50 μg/kg (*hexagon*), SC] followed by treatment with **B** saline (1 ml/kg, SC; *open symbols*) or **C** (+)-MDMA (3 mg/kg, SC; *closed symbols*). The *asterisk* (*) indicates activity levels significantly different (P<0.05) from Sal/Sal controls. The *caret* (^) indicates activity levels significantly different (P<0.05) from Sal/(+)-MDMA controls

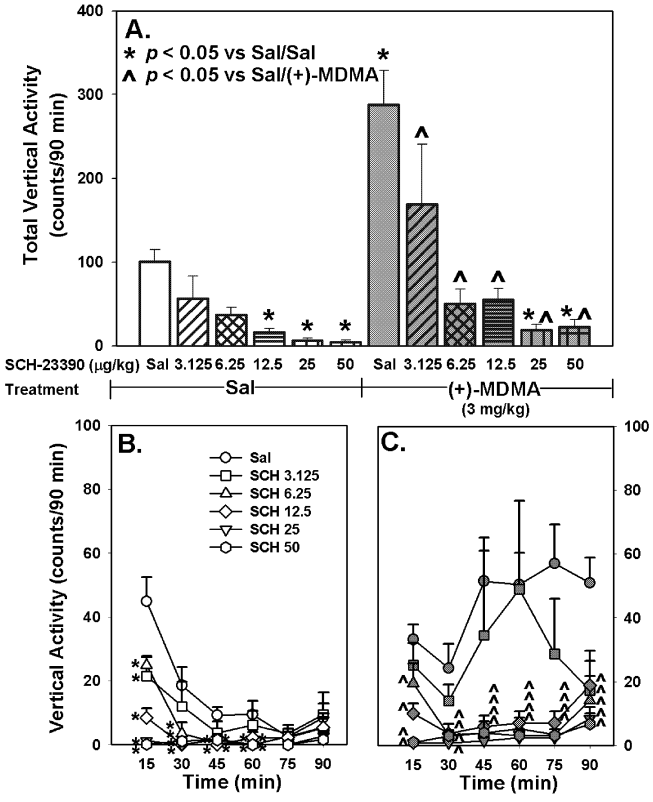

Fig. 2 Effects of the D_1R antagonist SCH 23390 on vertical hyperactivity induced by (+)-MDMA. **A** Datapoints are represented as mean total vertical activity (counts/90 min; SEM) in rats pretreated with saline (*Sal*; 1 ml/kg, SC; *n*=14) or SCH 23390 (*SCH*; 3.125, 6.25, 12.5, 25 or 50 µg/kg, SC; *n*=6–8) followed by treatment with saline (1 ml/kg, SC) or (+)-MDMA (3 mg/kg, SC). **B**, **C** The datapoints represent the timecourse for vertical activity in 15-min time bins (SEM) across the 90-min session; pretreatment with saline (1 ml/kg, SC; *circles*) or SCH 23390 [3.125 (*square*), 6.25 (triangle), 12.5 (*diamond*), 25 (*inverted triangle*) or 50 µg/kg (*hexagon*), SC] followed by treatment with **B** saline (1 ml/kg, SC; *open symbols*) or **C** (+)-MDMA (3 mg/kg, SC; *closed symbols*). The *asterisk* (*) indicates activity levels significantly different (P<0.05) from Sal/Sal controls. The *caret* (^) indicates activity levels significantly different (P<0.05) from Sal/(+)-MDMA controls

Pretreatment with all doses of SCH 23390 significantly decreased (+)-MDMA-induced horizontal (Fig. 1) and vertical hyperactivity (Fig. 2; P<0.05). While the two lower doses of SCH 23390 (3.125 and 6.25 µg/kg) did not alter basal activity, the three higher doses (12.5, 25, 50 µg/kg) significantly suppressed spontaneous horizontal (Fig. 1) and vertical locomotor activity (Fig. 2) in addition to blocking (+)-MDMA-induced hyperactivity (P<0.05). The doses of 25 and 50 µg/kg of SCH 23390 prevented the ability of (+)-MDMA to induce vertical activity; in fact, levels of vertical activity seen were lower than basal levels of vertical activity (Fig. 2; P<0.05).

A significant pretreatment×treatment×time interaction was shown for horizontal [Fig. 1; F(25,599)=1.90, P<0.05] and vertical activity [Fig. 2; F(25,599)=2.47, P<0.05] separated into six different 15-min time bins when comparing all doses of SCH 23390. Examination of the timecourse revealed that the higher doses of SCH 23390 (12.5, 25 and 50 µg/kg) significantly attenuated (+)-MDMA-evoked horizontal hyperactivity at all time points (P<0.05; Fig. 1C), while the lower doses of SCH 23390 (3.125, 6.25 µg/kg) suppressed (+)-MDMA-induced horizontal hyperactivity from 30–90 min and 45–90 min, respectively. Pretreatment with the lower doses of SCH 23390 (3.125 and 6.25 µg/kg) had no effect upon spontaneous horizontal activity, except for a significant suppression of activity with 6.25 µg/kg at 60 min; higher doses of SCH 23390 (12.5, 25 µg/kg) significantly decreased spontaneous horizontal activity during the first 60 min of the session except at the 45 and 30 min time points, respectively, while the 50 µg/kg dose of SCH 23390 suppressed spontaneous horizontal activity throughout the first 75 min of the session (Fig. 1B). All doses of SCH 23390 significantly attenuated (+)-MDMA-evoked vertical activity at all time points (P<0.05; Fig. 2C), except the lowest dose (3.125 µg/kg) at which a significant suppression was observed only at the 90-min time point. Spontaneous vertical activity was suppressed by all doses of SCH 23390 for the first 60 min of the session, except for the lowest dose (3.125 µg/kg), which only suppressed basal vertical activity at the 15 min time point (Fig. 2B).

Effects of D_2R antagonist eticlopride on spontaneous and (+)-MDMA-evoked activity

Locomotor activity was assessed following pretreatment with eticlopride (12.5, 25, 50 µg/kg) or saline (1 ml/kg) and treatment with (+)-MDMA (3 mg/kg) or saline (1 ml/kg; Figs. 3 and 4). There was a significant main effect of treatment observed for both horizontal [F(7,63)=23.90, P<0.01] and vertical activity [F(7,63)=9.80, P<0.01]. (+)-MDMA significantly increased horizontal (Fig. 3) and vertical activity (Fig. 4) when compared to control animals (P<0.05). Pretreatment with all doses of eticlopride significantly decreased (+)-MDMA-induced horizontal (Fig. 3) and vertical hyperactivity (Fig. 4; P<0.05). Eticlopride significantly suppressed spontaneous horizontal activity at the two higher doses (25 and 50 µg/kg), but not at the lowest dose (12.5 µg/kg; Fig. 3); however, all of the doses of eticlopride significantly suppressed spontaneous vertical activity (Fig. 4; P<0.05). As seen with the higher doses of SCH 23390, levels of vertical activity following 25 and 50 µg/kg eticlopride in combination with (+)-MDMA were lower than basal levels of vertical activity (Fig. 4; P<0.05).

A significant pretreatment×treatment×time interaction was shown for horizontal [Fig. 3; F(15,383)=2.36, P<0.01] and vertical activity [Fig. 4; F(15,383)=4.03, P<0.01] separated into six different 15-min time bins. All doses of eticlopride were shown to significantly attenuate (+)-MDMA-evoked horizontal (Fig. 3C) or vertical (Fig. 4C) hyperactivity at all time points except for the lowest dose (12.5 µg/kg), which did not alter (+)-MDMA-evoked vertical activity during the initial 15 min of the session. While the lowest dose (12.5 µg/kg) of eticlopride only suppressed spontaneous horizontal activity at the 60-

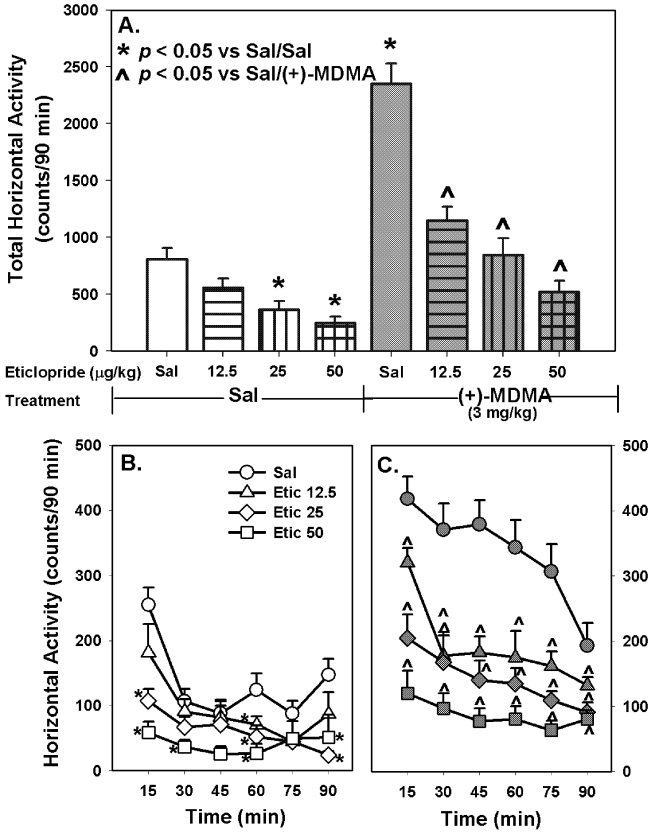

Fig. 3 Effects of the D_2R antagonist eticlopride on horizontal hyperactivity induced by (+)-MDMA. **A** The datapoints represent mean total horizontal activity (counts/90 min; SEM; n= 8) in rats pretreated with saline (*Sal*; 1 ml/kg, SC) or eticlopride (*Etic*; 12.5, 25 or 50 μg/kg, SC) followed by treatment with saline (1 ml/kg, SC) or (+)-MDMA (3 mg/kg, SC). **B**,**C** The datapoints represent timecourse for horizontal activity in 15-min time bins (SEM) across the 90-min session; pretreatment with saline (1 ml/kg, SC, *circles*) or eticlopride [12.5 (*triangle*), 25 (*diamond*) or 50 μg/kg (*square*), SC] followed by treatment with **B** saline (1 ml/kg, SC; *open symbols*) or **C** (+)-MDMA (3 mg/kg, SC; *closed symbols*). The *asterisk* (*) indicates activity levels significantly different (P<0.05) from Sal/Sal controls. The *caret* (^) indicates activity levels significantly different (P<0.05) from Sal/(+)-MDMA controls

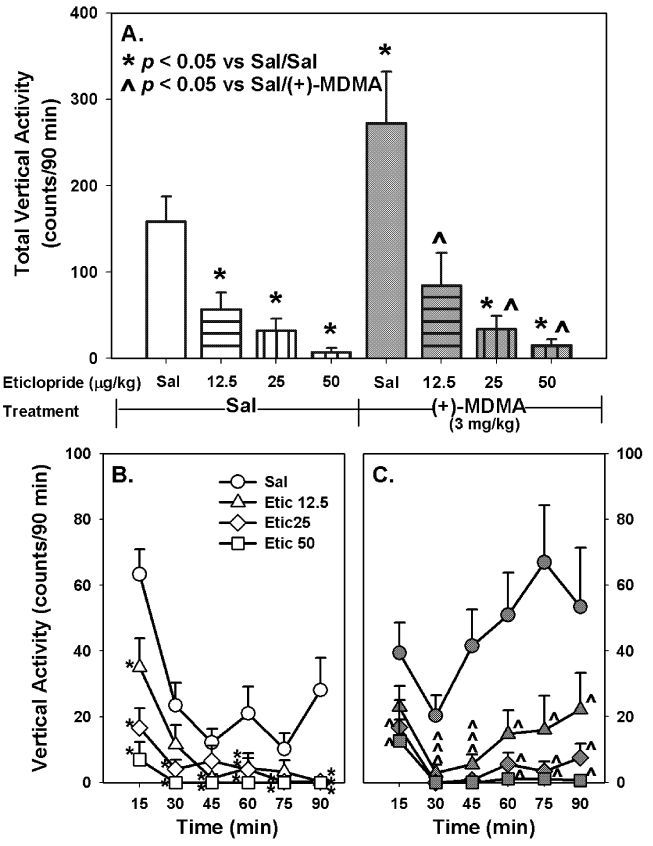

Fig. 4 Effects of the D_2R antagonist eticlopride on vertical hyperactivity induced by (+)-MDMA. **A** The datapoints represent mean total vertical activity (counts/90 min; SEM; n=6–8) in rats pretreated with saline (*Sal*; 1 ml/kg, SC) or eticlopride (*Etic*; 12.5, 25 or 50 μg/kg, SC) followed by treatment with saline (1 ml/kg, SC) or (+)-MDMA (3 mg/kg, SC). **B**,**C** The datapoints represent timecourse for vertical activity in 15-min time bins (SEM) across the 90-min session. Pretreatment with saline (1 ml/kg, SC, *circles*) or eticlopride [12.5 (*triangle*), 25 (*diamond*) or 50 μg/kg (*square*), SC] or followed by treatment with **B** saline (1 ml/kg, SC; *open symbols*) or **C** (+)-MDMA (3 mg/kg, SC; *closed symbols*). The *asterisk* (*) indicates activity levels significantly different (p<0.05) from Sal/Sal controls. The *caret* (^) indicates activity levels significantly different (P<0.05) from Sal/(+)-MDMA controls

min time point, pretreatment with 25 μg/kg and 50 μg/kg eticlopride significantly decreased spontaneous levels of horizontal activity at 15, 60 and 90 min and at 15, 30, 60 and 90 min, respectively (Fig. 3B). Spontaneous vertical activity was significantly suppressed by 50 μg/kg eticlopride at all time points and 25 μg/kg at all but the 45 min time point, while the lowest dose (12.5 μg/kg) of eticlopride suppressed vertical activity at all time points except 30 and 75 min (P<0.05; Fig. 4B).

Drug discrimination experiments

(+)-MDMA dose-response tests

The discrimination between (+)-MDMA (1.0 mg/kg) and saline was acquired in an average of 32 sessions (range: 18–61). Throughout acquisition, response rates (±SEM) during (+)-MDMA sessions (31.12±1.05 responses/min) were not significantly different from saline sessions (35.85 ±1.18 responses/min). (+)-MDMA (0.375–1.0 mg/kg) elicited a dose-related increase in (+)-MDMA-appropriate responding (Fig. 5A, B), whereas saline administration resulted in <10% (+)-MDMA-appropriate responding (Fig. 5A, B). Response rates were stable across all test doses of (+)-MDMA and did not differ from the response rates observed on the previous maintenance days (P<0.025; Fig. 5C, D).

Effects of the D_1R antagonist SCH 23390 and the D_2R antagonist eticlopride on the stimulus effects of (+)-MDMA

Neither SCH 23390 (6.25 μg/kg) nor eticlopride (12.5 μg/kg) mimicked (+)-MDMA, rather each drug produced

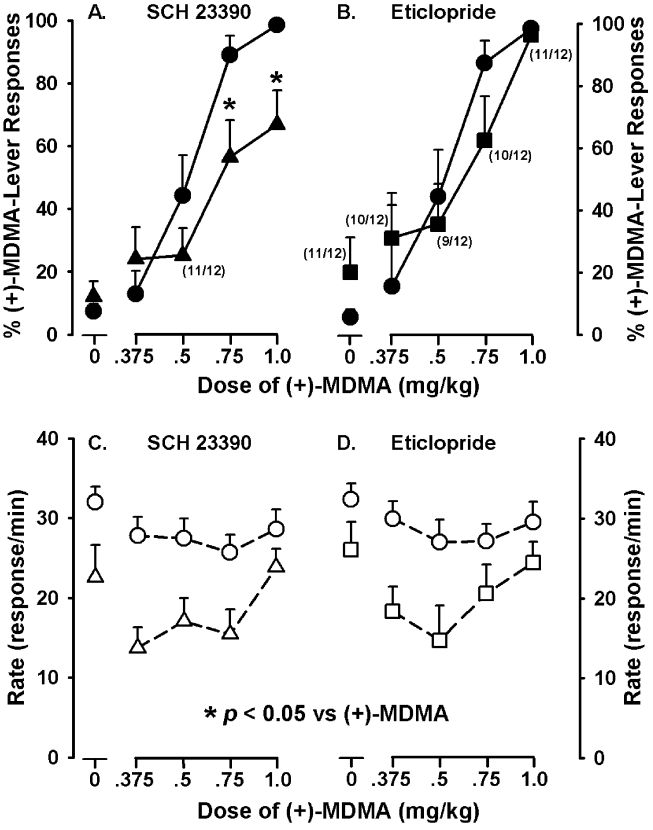

Fig. 5 Effects of the D_1R antagonist SCH 23390 and D_2R antagonist eticlopride in rats trained to discriminate (+)-MDMA. Datapoints represent mean (SEM) **A**,**B** percentage of (+)-MDMA-lever responses (*closed symbols*) and **C**,**D** response rate (*open symbols*) for substitution and combination tests with **A**,**C** SCH 23390 (SCH; 6.25 μg/kg, IP; *triangles*) and **B**,**D** eticlopride (*Etic*; 12.5 μg/kg, IP; *squares*) in rats trained to discriminate (+)-MDMA (1 mg/kg, IP; *circles*) from saline. Data points for substitution tests with saline (*circles*), SCH 23390 (*triangle*) or eticlopride (*square*) are represented by 0 mg/kg dose of (+)-MDMA on the *x*-axis. The number (*n*) of rats completing a FR 20 compared with the number tested (*N*) is 12/12, unless otherwise noted adjacent to individual points. The *asterisk* (*) illustrates datapoints significantly different from performance following the respective dose of (+)-MDMA administration alone ($P<0.05$)

primarily saline-like responding (Fig. 5A, B). Response rates following administration of either SCH 23390 (6.25 μg/kg) or eticlopride (12.5 μg/kg) were not statistically different than the previous (+)-MDMA session (Fig. 5C, D).

A two-way ANOVA revealed a significant pretreatment×treatment interaction for the combination of D_1R antagonist SCH 23390 (6.25 μg/kg) with (+)-MDMA (0.375–1.0 mg/kg) on the percentage of (+)-MDMA-appropriate lever responding [$F(3,32)=3.58$, $P<0.05$; Fig. 5A], but not on response rate [$F(3,32)=1.77$, $P=0.173$; Fig. 5C]. Pretreatment with SCH 23390 was shown to significantly attenuate (+)-MDMA-appropriate lever responding when administered prior to 0.75 and 1.0 mg/kg (+)-MDMA ($P<0.05$). Conversely, there was no significant pretreatment×treatment interaction for the combination of the D_2R antagonist eticlopride (12.5 μg/kg) with (+)-MDMA (0.375–1.0 mg/kg) on the percentage of (+)-MDMA-appropriate responding [$F(3,26)=1.56$, $P=0.223$; Fig. 5B) or the response rate [$F(3,26)=1.44$, $P=0.255$; Fig. 5D].

Although eticlopride (12.5 μg/kg) did not alter the percentage of (+)-MDMA appropriate responding or response rate in animals that completed the FR20, several animals (n=1–3/test session) were excluded from the analyses because they did not complete the FR20 in the allotted 15-min period (Fig. 5B), suggesting that the combination of eticlopride with (+)-MDMA was moderately disruptive to responding. SCH 23390 (6.25 μg/kg), on the other hand, was disruptive to only one subject when combined with (+)-MDMA (0.5 mg/kg; Fig. 5A). Experiments employing higher doses of SCH 23390 (12.5 μg/kg) or eticlopride (25 μg/kg) in combination with the training dose of (+)-MDMA (1 mg/kg) were discontinued upon observation that these doses of the antagonists disrupted responding in greater than 50% of subjects tested (data not shown).

Discussion

The results of the present study support a role for DA receptors in both the hypermotive and discriminative stimulus effects of (+)-MDMA. In support of our hypothesis, administration of the D_1R antagonist SCH 23390 or the D_2R antagonist eticlopride attenuated (+)-MDMA-induced hyperactivity in a dose-related manner. While most doses used in the present study also suppressed spontaneous locomotor activity, lower doses of both SCH 23390 (3.125 and 6.25 μg/kg) and eticlopride (12.5 μg/kg) attenuated (+)-MDMA-induced hyperactivity without significantly altering basal locomotor activity. Thus, the ability of D_1R and D_2R antagonists to attenuate (+)-MDMA-induced hyperactivity as demonstrated in the present study extend the findings of Kehne and colleagues (1996), suggesting that D_1R and D_2R stimulation are integral in the ability of (+)-MDMA to elicit locomotor hyperactivity. Based upon these results, a low dose of SCH 23390 (6.25 μg/kg) and eticlopride (12.5 μg/kg) were chosen to determine the ability of these antagonists to block or shift the dose-response curve for the stimulus properties of (+)-MDMA. SCH 23390 significantly attenuated (+)-MDMA-appropriate responding, resulting in a rightward shift in the (+)-MDMA dose-response curve. Conversely, eticlopride had no effect upon (+)-MDMA-appropriate responding; however, eticlopride moderately disrupted overall lever-responding when combined with any dose of (+)-MDMA tested. The lack of an effect of a D_2R antagonist on the discriminative stimulus effects of (±)-MDMA has previously been reported (Schechter 1989; Goodwin et al. 2003), although, to our knowledge, this is the first report of a significant attenuation of (+)-MDMA-appropriate responding by a D_1R antagonist, suggesting a role for D_1R in the discriminative stimulus properties of (+)-MDMA.

While much of the research investigating the mechanisms of action underlying the effects of MDMA have

focused on the role of 5-HT and the 5-HT receptors (Schechter 1989; Callaway et al. 1990; Baker et al. 1997; Bankson and Cunningham 2001; Frankel and Cunningham 2003), the importance of DA in the behavioral effects of MDMA has become increasingly evident. Early studies demonstrated that 6-hydroxydopamine lesions of DA terminals in the NAc were found to attenuate (±)-MDMA-induced hyperactivity (Gold et al. 1989). As validated here, both D_1R and D_2R antagonists will also attenuate (+)- (present study) or (±)-MDMA-induced hyperactivity (Kehne et al. 1996). These results suggest a fundamental role for the DA system, and in particular DA in the NAc, in the locomotor activating effects of MDMA. Studies discerning the role of DA in the discriminative stimulus effects of MDMA, however, are limited and the results vary depending upon the paradigm used, the isomer chosen, and the time point of administration (Baker et al. 1997; for review, see Cole and Sumnall 2003). A DA component is suggested by the observation that (±)-MDMA mimics the stimulus effects of the DA releaser amphetamine (Glennon et al. 1988) and vice versa (Oberlender and Nichols 1988; Schechter 1989; but see Baker et al. 1997). Additionally, the present results demonstrating that the D_1R antagonist SCH 23390 interferes with the discriminative stimulus effects of (+)-MDMA supplies further evidence for a role for DA in this behavioral effect of (+)-MDMA.

On the other hand, the inability of the D_2R antagonist eticlopride to alter the stimulus effects of (+)-MDMA is in keeping with previous reports (Schechter 1989; Goodwin et al. 2003), all of which cumulatively suggest that the D_2R stimulation may not be an integral component of the discriminative stimulus properties of (+)-MDMA. Interestingly, the role of DA in the stimulus effects of MDMA may be dependent upon the interval of time at which the discrimination is trained relative to the injection of MDMA. Schechter (1989) reported that in rats trained to discriminate (±)-MDMA at a much longer time point (105 min) after injection relative to that typically used (20 min; present results; Schechter 1989; Goodwin et al. 2003), haloperidol did attenuate (±)-MDMA-appropriate responding. Because there is some evidence to suggest that the later time point coincides with the maximal (±)-MDMA-evoked DA release (60–120 min; Yamamoto and Spanos 1988), it is possible that D_1R and D_2R may play differential roles time-locked to variant neurochemical profiles seen after MDMA administration (Yamamoto and Spanos 1988; Schechter 1989). Nonetheless, although further investigation is required to elucidate the potential contribution of D_2R in the discriminative stimulus effects of (+)-MDMA, the present results suggest that D_1R play a prominent role in the discriminative properties of (+)-MDMA.

While the present and previous studies (Gold et al. 1988; Kehne et al. 1996) suggest that DA receptors are involved in the behavioral effects of MDMA, the mechanistic contribution of different receptor subtypes in the control of the hypermotive and discriminative stimulus effects of MDMA has been difficult to decipher. Some of the difficulty may be due to a lack of selectivity of the ligands employed for the D_1R versus D_2R classes and/or for the subtypes of receptors within the two classes. The antagonists employed in the present study, while enabling differentiation of the contribution of D_1R versus D_2R, do not have the ability to distinguish between the individual subtypes of receptors within the respective classes. For example, the D_2R antagonist eticlopride shows moderate selectivity for D_2 (K_i=0.07 nM; Lawler et al. 1999) over D_1 (K_i=113 nM; Hall et al. 1986) receptors but only displays a slight preference for D_2 versus D_3 (K_i=0.36 nM) or D_4 receptors (K_i=64 nM; Lawler et al. 1999). SCH 23390 on the other hand has preferential selectivity for D_1 (K_i=0.37 nM; Lawler et al. 1999) over D_2 (K_i=2367 nM; Levant et al. 1992), but virtually no selectivity over D_5 receptors (K_i=0.47 nM; Lawler et al. 1999). Thus, interpretation of the results of the present study is limited to differentiation between the D_1R and D_2R classes, but not within each class. As such, the availability of more selective compounds for subtypes within the D_1R and D_2R classes is necessary to fully understand the contribution of each of the receptor subtypes to the behavioral effects of (+)-MDMA.

In addition to a lack of selective ligands, another obstacle in the quest for deciphering the individual actions of DA receptor subtypes to control (+)-MDMA-induced behaviors is the apparent synergism between D_1R and D_2R in elicitation of DA-related behaviors (for reviews, see Waddington and O'Boyle 1989; Jackson and Westlind-Danielsson 1994). With regard to locomotor activation, D_1R tone appears to be necessary in order to "enable" D_2R-mediated locomotor stimulation, whereas changes in D_2R tone can alter expression of D_1R agonist-induced hypermotility (see Waddington and O'Boyle 1989). For example, systemic or intra-NAc administration of inactive doses of the D_1R agonist SKF 38393 in combination with suppressive doses of the D_2R agonists RU 24213 or quinpirole, respectively, induced hyperactivity (Starr and Starr 1987; Canales and Iversen 2000). Furthermore, concurrent stimulation of D_1R and D_2R is required to evoke locomotor stimulation in DA-depleted mice (Jackson and Hashizume 1986; but see Arnt 1985), suggesting that simultaneous activation of D_1R and D_2R is important in eliciting locomotor activation. In the present study, (+)-MDMA-induced hyperactivity was blocked by administration of either D_1R or D_2R antagonists suggesting that activation of both receptors are necessary to elicit hyperactivity. Furthermore, (+)-MDMA administered following the higher doses of SCH 23390 or eticlopride, never evoked activity levels greater than baseline levels. Thus, the inability of (+)-MDMA to elicit hyperactivity following the higher doses of SCH 23390 or eticlopride may be due to the lack of synergistic interactions of D_1R and D_2R when one or the other of the receptors is blocked (see Waddington and O'Boyle 1989). These data further speak to the importance of D_1R and D_2R in (+)-MDMA-induced hyperactivity and support the theory that concomitant D_1R and D_2R stimulation may be necessary for elicitation of hyperactivity.

A synergism between D_1R and D_2R can also be inferred from studies of the stimulus effects cocaine, *d*-amphetamine, and (+)- or (±)-MDMA. Coinciding with the inhibitory actions of the D_1R antagonist SCH 23390 on the recognition of the stimulus cue of (+)-MDMA observed in the present study, SCH 23390 completely blocked the discriminative cues for both cocaine and *d*-amphetamine (Nielsen et al. 1989; Smith et al. 1989; Callahan et al. 1991). Conversely, D_1R agonists produce either partial or no substitution for cocaine or *d*-amphetamine (Nielsen et al. 1989; Smith et al. 1989; Callahan et al. 1991). An opposite effect is observed for D_2R ligands, as D_2R antagonists do not consistently block cocaine or (+)- or (±)-MDMA drug discrimination, (present study; Schechter 1989; Callahan et al. 1994; Goodwin et al. 2003), while D_2R agonists reliably substitute for both cocaine and *d*-amphetamine (Smith et al. 1989; Callahan et al. 1991). Thus, these results suggest that D_1R may be necessary, but not sufficient, to mimic the interoceptive cues of cocaine and *d*-amphetamine (Callahan et al. 1994). Furthermore, the inability of D_2R antagonists to fully antagonize the discriminative stimulus cues of psychostimulants may be a result of underlying D_1R stimulation that is retained in these conditions.

Both SCH 23390 and eticlopride suppressed spontaneous locomotor activity in a dose-related manner thereby generating the potential of nonspecific behavioral competition between the suppressive effects of the antagonists vs. the stimulatory effects of (+)-MDMA on locomotor activity. However, examination of the results following administration of the lower doses of the antagonists in the locomotor activity experiments reveals that while 3.125 and 6.25 μg/kg SCH 23390 and 12.5 μg/kg eticlopride produced a modest, non-significant suppression of spontaneous locomotor activity (15%, 30%, and 31%, respectively; see Figs 1A and 5A), these antagonists significantly decreased (+)-MDMA-induced hyperactivity by 39%, 40% and 51% for SCH 23390 at 3.125, 6.25 μg/kg and eticlopride at 12.5 μg/kg, respectively (see Figs 1A and 5A). The greater degree of attenuation produced when the antagonists were administered in combination with (+)-MDMA suggests that the blockade of (+)-MDMA-induced hyperactivity at the lower doses of antagonists was probably not a result of non-specific behavioral suppression, but rather that both D_1R and D_2R play a vital role in the ability of (+)-MDMA to induce hyperactivity. Results from the drug discrimination experiments conducted with the D_1R antagonist SCH 23390 provide further evidence of specific actions of DA receptors in the behavioral effects of (+)-MDMA. The 6.25 μg/kg dose of SCH 23390 was demonstrated to significantly reduce (+)-MDMA-appropriate responding, without altering rates of responding and causing only slight disruption of responding. These results suggest that the effects of SCH 23390 on (+)-MDMA appropriate responding are due to the ability of D_1R antagonism to alter the discriminative stimulus properties of (+)-MDMA, and not interference with responding due to the suppressive effects of the drug.

An unusual finding in the present study was that (+)-MDMA consistently and robustly enhanced vertical (rearing) activity, since (+)-MDMA is most commonly reported to suppress or have no effect on vertical activity (Callaway et al. 1990; McCreary et al. 1999; Bankson and Cunningham 2002). This consistent and robust enhancement of vertical activity by (+)-MDMA was, however, blocked by administration of either the D_1R or D_2R antagonists. Interestingly, the suppressant effects of the higher doses (25 and 50 μg) of SCH 23390 or eticlopride on vertical activity were so intense that (+)-MDMA-evoked vertical activity was significantly lower than that seen in saline treated controls. These data suggest that both D_1R and D_2R activation are necessary to maintain normal rearing activity as well as to induce vertical hyperactivity upon (+)-MDMA administration.

While enhancement of vertical activity by (+)-MDMA is not commonly observed, several studies have reported that suppression of vertical activity occurred only within the first 30 min following (+)-MDMA administration (Callaway et al. 1990; Gold et al. 1988), as was the case in the present study (Figs 4D and 5C). Furthermore, in addition to the enhanced vertical activity observed in the present study, increases in vertical activity have previously been reported following injection of (+)-MDMA directly into the NAc (Callaway and Geyer 1992) and following systemic (+)-MDMA administration in combination with a $5\text{-}HT_{2C}R$ antagonist (Bankson and Cunningham 2002). Unfortunately, the reasons underlying the inconsistencies in the vertical activity response to (+)-MDMA administration are not fully understood.

This phenomenon may be due to behavioral competition between the stimulation of vertical activity and the induction of the 5-HT syndrome by (+)-MDMA. The 5-HT syndrome is characterized, among other effects, by flat body posture and splayed hind limbs (Spanos and Yamamoto 1989). The presence of these components of the 5-HT syndrome may interfere with the ability of the rat to stand on its hind-legs (rear), thereby revealing an inverse relationship between expression of flat body posture and rearing. Indeed, individual differences in response to administration of (+)-MDMA are observed with rearing seen less frequently in animals exhibiting flat body posture (unpublished observations; data not shown). While flat body posture is consistently observed following administration of the current dose (3 mg/kg, SC) of (+)-MDMA, the intensity and duration of the effect appears to vary among individual rats, with some animals displaying flat body posture throughout the duration of the test session and others only for approximately the first 30 min following administration (unpublished observations). Thus, the ability of (+)-MDMA to induce vertical activity may vary depending on the extent of the induction of 5-HT syndrome, and in particular flat body posture. In the present study, seven out of 22 Sal-MDMA treated rats displayed flat body posture; however, only one rat continued to express the behavior after the initial 30-min period (data not shown). Thus the low incidence of flat body posture observed in the present study may have

contributed to the ability of (+)-MDMA to enhance vertical activity.

In conclusion, the present study suggests that DA D_1R and D_2R play critical roles in the expression of basal and (+)-MDMA-induced horizontal and vertical hyperactivity and furthermore that D_1R also contribute to the discriminative stimulus properties of (+)-MDMA. These findings combined with previous reports of the importance of 5-HT in the behavioral effects of (+)-MDMA lends favor to a role for 5-HT-DA interactions in the mechanisms of MDMA action (for review, see Bankson and Cunningham 2001). However, the nature of 5-HT-DA interactions, which has only begun to be characterized, appears to be extremely complex, and may reveal that these two neurotransmitter systems are highly interdependent (see Gudelsky and Nash 1996; Bankson and Cunningham 2001). Future studies investigating the nature of this interaction as well as studies determining the contribution of specific DA receptor subtypes within the D_1R- and D_2R-like classes will further elucidate the role of these systems in the behavioral effects of (+)-MDMA.

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