

Differential Contributions of Dopamine D₁, D₂, and D₃ Receptors to MDMA-Induced Effects on Locomotor Behavior Patterns in Mice

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MDMA or 'ecstasy' (3,4-methylenedioxyamphetamine) is a commonly used psychoactive drug that has unusual and distinctive behavioral effects in both humans and animals. In rodents, MDMA administration produces a unique locomotor activity pattern, with high activity characterized by smooth locomotor paths and perseverative thigmotaxis. Although considerable evidence supports a major role for serotonin release in MDMA-induced locomotor activity, dopamine (DA) receptor antagonists have recently been shown to attenuate these effects. Here, we tested the hypothesis that DA D₁, D₂, and D₃ receptors contribute to MDMA-induced alterations in locomotor activity and motor patterns. DA D₁, D₂, or D₃ receptor knockout (KO) and wild-type (WT) mice received vehicle or (+/–)-MDMA and were tested for 60 min in the behavioral pattern monitor (BPM). D₁ KO mice exhibited significant increases in MDMA-induced hyperactivity in the late testing phase as well as an overall increase in straight path movements. In contrast, D₂ KO mice exhibited reductions in MDMA-induced hyperactivity in the late testing phase, and exhibited significantly less sensitivity to MDMA-induced perseverative thigmotaxis. At baseline, D₂ KO mice also exhibited reduced activity and more circumscribed movements compared to WT mice. Female D₃ KO mice showed a slight reduction in MDMA-induced hyperactivity. These results confirm differential modulatory roles for D₁ and D₂ and perhaps D₃ receptors in MDMA-induced hyperactivity. More specifically, D₁ receptor activation appears to modify the type of activity (linear vs circumscribed), whereas D₂ receptor activation appears to contribute to the repetitive circling behavior produced by MDMA.

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INTRODUCTION

As pioneered by the Segal laboratory and others, animal models of locomotor behavior have been critical tools for understanding the relationships between monoamines and behavior (Geyer and Segal, 1991; Kelly and Iversen, 1976; Lat, 1965; Schiorring, 1979; Segal *et al*, 1975, 1971; Segal and Mandell, 1970; Stinus *et al*, 1980). Such behavioral models have provided a foundation for much of what we know regarding the neural correlates of behavioral effects of drugs of abuse, in particular psychotomimetics such as stimulants and hallucinogens (Adams and Geyer, 1982; Bankson and Cunningham, 2001; Creese,

1983; Eilam *et al*, 1989; Fink and Morgenstern, 1985; Geyer, 1990; Lehmann-Masten and Geyer, 1991; Segal, 1975; Segal *et al*, 1981, 1980; Swerdlow and Koob, 1985). Locomotor behavior paradigms offer a rich profile of behaviors with which to measure the complex interactions between neurochemical systems and the consequent behavioral output (Segal and Geyer, 1985; Segal *et al*, 1981, 1980; Segal and Kuczenski, 1987). The addition of spatial scaling measures to describe locomotor paths in conjunction with locomotor and investigatory behaviors has resulted in further refinement and quantitation of unique behavioral patterns associated with different stimulant classes, leading to a greater understanding of the pharmacological mechanisms of these drugs (Callaway *et al*, 1990; Geyer *et al*, 1986; Paulus and Geyer, 1992). For example, we have found that although amphetamine and its derivative MDMA (3,4-methylenedioxyamphetamine, 'ecstasy') yield similar amounts of hyperactivity, the qualitative behavioral patterns are quite distinctive (Gold *et al*, 1988), an observation that reinforces the hypothesis of distinct pharmacological actions between these two drugs

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(for a review see Martinez-Price *et al*, 2002; Nichols *et al*, 1986).

MDMA acts primarily as a serotonin (5-HT) and dopamine (DA) releaser, by blocking reuptake transporters (Schmidt, 1987). Relative to amphetamine, MDMA is more potent at releasing 5-HT than DA (Crespi *et al*, 1997; Steele *et al*, 1987). Considerable evidence suggests that MDMA-induced hyperactivity is dependent upon its 5-HT-releasing effects (Bengel *et al*, 1998; Callaway *et al*, 1990) and consequent indirect activation of 5-HT_{1B} and 5-HT_{2A} receptors (Bankson and Cunningham, 2002; Callaway *et al*, 1991, 1992; Kehne *et al*, 1996; Scarce-Levie *et al*, 1999). In both rats and mice, MDMA treatment induces a distinctive locomotor exploratory pattern, with increased hyperactivity combined with reduced exploratory behavior and thigmotaxis (Gold *et al*, 1988; Powell *et al*, 2004). We also found that MDMA induces smoother movement patterns with fewer directional changes (Paulus and Geyer, 1992; Powell *et al*, 2004) and that these effects are mimicked by other 5-HT releasers such as MBDB and α -ethyltryptamine (Callaway *et al*, 1991, 1992; Krebs and Geyer, 1993). In mice, 5-HT depletion by parachlorophenylalanine treatment or 5-HT_{1B} gene deletion does not block locomotor activity increases induced by high doses of MDMA, indicating that some of the effects on locomotor activity are via direct agonism at 5-HT receptors or via other neurotransmitter systems (Fantegrossi *et al*, 2005; Scarce-Levie *et al*, 1999).

Although previous findings from this lab and others confirm the predominant involvement of 5-HT in MDMA-induced increases in locomotor activity in both rats and mice, evidence in rats points toward an additional contribution of the DA system (Bankson and Cunningham, 2001). Although MDMA is more potent at releasing 5-HT than DA, the DA efflux after behaviorally active doses of MDMA is equal to or greater than 5-HT efflux (White *et al*, 1994, 1996). Furthermore, evidence suggests that MDMA-induced DA release results secondarily from 5-HT release as well as via blockade of the DA transporter (Koch and Galloway, 1997). Gold *et al* (1989) found that 6-hydroxydopamine lesions of the DA terminals in the nucleus accumbens attenuated MDMA-induced hyperactivity. Hence, DA receptor activation is likely to contribute to MDMA effects on behavior. The five DA receptors currently known are divided into two classes, D₁-like family that stimulate the formation of cyclic adenosine monophosphate (cAMP) and includes the D₁ and D₅ receptors, and the D₂-like family that inhibits cAMP formation and includes the D₂, D₃, and D₄ receptors (for a review see Jaber *et al*, 1996). Recently, Bubar *et al* (2004) suggested that the DA released indirectly by (+)-MDMA administration results in the stimulation of D₁ and D₂ receptors, as both D₁ antagonist and D₂ antagonists partially reversed the hyperactivity produced by MDMA in rats. It appears that at low doses, the D₁ antagonist SCH23390 reduced MDMA-induced activity without having effects on baseline activity. The separation observed with the D₂ antagonist eticlopride, however, was less clear. The authors also pointed out that the antagonists used were not selective enough to also rule out D₃ and D₅ receptor blockade contributions to their effects.

In addition to reducing the locomotor hyperactivity produced by MDMA in rats, SCH23390 has been reported to reverse the striking effects of MDMA-induced persevera-

tion patterns of the locomotor response (Ball *et al*, 2003). This result also parallels our report of D₁ vs D₂ antagonist effects in mice with a DA transporter gene deletion (DAT KO), in which only the D₁ antagonist normalized the perseverative patterns of activity in the DAT KO mice, even though both D₁ and D₂ antagonists reduced overall motor activity in the DAT KOs (Ralph *et al*, 2001). These observations are consistent with other evidence that D₁ receptors contribute critically to stereotyped behavior in mice (Chartoff *et al*, 2001; Daly and Waddington, 1992; Fetsko *et al*, 2003). 5-HT_{1B} receptor activation has been ruled out as a necessary component for MDMA-induced stereotypy in mice (Scarce-Levie *et al*, 1999). Consequently, an additional hypothesis we tested was that the DA D₁ receptor is necessary to observe the MDMA-induced increases in stereotyped locomotor patterns, that is, the increased predictability of movement sequences and/or circling patterns. Hence, the aims of the present study were (1) to determine the effects of MDMA on locomotor and exploratory patterns in mice, and (2) to determine the role of D₁, D₂, and D₃ receptors in MDMA-induced alterations in these behaviors.

MATERIALS AND METHODS

Subjects

DA receptor D₁, D₂, and D₃ wild-type (WT) and knockout (KO) mice (constitutive gene deletion background mice) were used. The D₂ mice (B6.129S2-*Drd2*^{tm1Low}/J) were originally generated at the Oregon Health and Science University (Kelly *et al*, 1998) and backcrossed onto the C57BL/6J background strain for 17 generations. Stocks of D₁ mice (B6.129S4-*Drd1a*^{tm1Lcd}/J; Drago *et al*, 1994) and D₃ mice (B6.129S4-*Drd3*^{tm1Dac}/J; Accili *et al*, 1996) were obtained from the mutant mouse repository at the Jackson Laboratory (Bar Harbor, ME) and were backcrossed onto the C57BL/6J background for 10–12 generations. The study mice were bred using heterozygous pairs and genotyped as described (Ralph-Williams *et al*, 2002), then shipped and housed at the University of California San Diego (UCSD) vivarium, where they were kept in a climate-controlled, reversed light environment (lights on at 2000 hours, off at 0800 hours). D₁ WT mice were used for the MDMA dose-response study. Male and female mice were housed separately ($n = 4/\text{cage}$), and food (Harlan Teklab, Madison, WI) and water were provided *ad libitum*, except during behavioral testing. All behavioral testing started at approximately 5 months of age and took place between 0900 and 1800 hours in an AAALAC-approved animal facility that meets Federal and State requirements for care and treatment of laboratory animals. All experimental protocols were approved by the Institutional Animal Care and Use Committees at both universities.

Drug Treatment

3,4-Methylenedioxymethamphetamine ((+/-)-MDMA) obtained from the National Institute on Drug Abuse was dissolved in 0.9% saline vehicle and administered intraperitoneally (i.p.) at a volume of 5 ml/kg body weight 10 min before behavioral testing.

Apparatus

Locomotor activity was measured in 10 mouse behavioral pattern monitors (BPM; San Diego Instruments, San Diego, CA). The construction and measures for the mouse BPM are based on the rat BPM, as described previously (Geyer *et al*, 1986). By simultaneous monitoring of different responses in sequence and time, the mouse BPM assessed the quantity and aspects of the quality of movement patterns of the mice. Data were collected on the location of the mouse in successive X–Y coordinates, which can be used to produce region locations, locomotor paths, and amount of locomotion (distance traveled). Each Plexiglas chamber contains a 30 by 60 cm holeboard floor enclosed in an outer box that minimizes outside light and noise, with an internal white light houselight. Subject activity was detected by sets of infrared photobeams 1 cm from the floor (2.5 cm apart along the length and the width of the chamber; 24 × 12 X–Y array), recording the location of the mouse every 0.1 s. Another set of 16 photobeams, 1.9 cm apart, located on the Y-axis only, at the height of 6.9 cm from the floor, measured the amount of rearing. Additionally, there are 11 holes (three holes in the floor and eight on the walls; 1.25 cm in diameter, 1.9 cm from the floor). The subject's position was defined across nine unequal regions (four corners, four walls and center; Geyer *et al*, 1986).

Locomotor Pattern Testing

Mice were habituated to the chambers for 1 h at least 1 week before drug testing. A dose response of MDMA was first conducted using 10, 20, and 30 mg/kg ($n=8$) in D₁ WT mice. Vehicle or 20 mg/kg MDMA were used for all subsequent studies. At the start of testing, mice were placed in the bottom left-hand corner of the enclosure, the experimental session was started by an individual button press, and the session lasted 60 min.

The measures obtained were distance traveled (which estimates the total amount of locomotor activity), transitions across regions, center, corner and wall duration and entries, total holepokes and rears, as well as scaling measures. The spatial scaling exponent '*d*' (Paulus and Geyer, 1991) quantifies the geometrical structure of the locomotor path. A value of 1 represents a path in a straight line, 1.5 a meandering path, and 2 a highly circumscribed path. The spatial coefficient of variation (CV) is a measure of the X–Y pattern representing the variation of transitions within the nine-region transition matrix (with 40 permissible transitions). Spatial CV increases when the mouse preferentially repeats certain transitions between the nine regions of the chamber (Geyer *et al*, 1986).

Statistical Analyses

The above measures were analyzed by using two- or three-way analyses of variance (ANOVAs) with sex, genotype, and/or drug treatment as between-subject variables, and time as a within-subject variable. The data were analyzed by first-half and second-halves of the session (min 0–30 and 31–60). Alpha level was set to 0.05. The Biomedical Data Programs (BMDP) statistical software (Statistical Solutions Inc., Saugus, MA) was used for all analyses. For brevity,

only distance traveled and spatial scaling measures specific to the hypothesis will be presented here.

RESULTS

Effect of MDMA on Locomotor Activity and Behavioral Patterns in WT Mice

MDMA administration increased locomotor activity in an inverted U-shaped dose–response function (Distance: $F(3,27) = 10.58$, $p < 0.001$), with slightly greater effects in the second block of testing (Block × MDMA: $F(3,27) = 4.14$, $p < 0.05$). As there were no sex effects or interactions, the data are collapsed across sex. The middle dose, 20 mg/kg, was the most effective, with *post hoc* analysis indicating this group had significantly higher distance traveled compared to vehicle in both time blocks, whereas the 10 mg/kg dose group reached significance only in the second-half of the 60 min test (Figure 1a, $p < 0.05$, 0.01, Dunnett's test). There was a significant effect of MDMA to reduce the spatial *d* scaling measure as well (Figure 1b; MDMA: $F(3,27) = 3.52$, $p < 0.05$). On inspection of the data it appears that there was a U-shaped dose response, with the 20 mg/kg dose group having the lowest spatial *d* in the last block of testing ($p < 0.05$, Dunnett's test; Figure 1b). There was also a nonsignificant trend for MDMA to increase spatial CV (MDMA (3, 27) = 2.39, $p = 0.09$). Based on our findings, in the other measures we conducted an exploratory assessment of the 20 mg/kg dose, revealing that the 20 mg/kg dose group had significantly increased spatial CV compared to vehicle ($\alpha/2$ correction = 0.025; $F(1,14) = 7.92$, $p < 0.025$). We thus chose 20 mg/kg MDMA for our subsequent studies in D₁, D₂, or D₃ receptor WT and KO mice as this was the most effective dose for both the activity and pattern effects of MDMA administration.

Effects of D₁, D₂, and D₃ Receptor Gene Deletion on MDMA-Induced Alterations in Locomotor Patterns

Locomotor activity. D₁: MDMA increased the distance traveled in all groups across the test session (MDMA: $F(1,112) = 441.68$, $p < 0.0001$). In the second block of testing, however, MDMA had greater effects on hyperactivity in D₁ KO compared to WT mice treated with MDMA (Figure 2a and b; $p < 0.05$, Tukey's test) (MDMA × Gene × Block: $F(1,112) = 39.37$, $p < 0.0001$). Although there was a main effect of gene ($F(1,112) = 8.84$, $p < 0.01$), this effect seems to be due largely to the interaction with MDMA, and not to a gene effect alone on activity.

D₂: In contrast to the D₁ KO mice, male D₂ KO mice exhibited significantly less MDMA-induced hyperactivity in the second block of testing; in female mice, this effect was similar but not significant (Figure 2c and d; $p < 0.01$, Tukey's test) (Block × Sex × MDMA × Gene: $F(1,91) = 20.32$, $p < 0.0001$). At baseline (ie vehicle treatment), both male and female D₂ KO mice also appear to exhibit a hypoactive phenotype, especially in the first block of testing (Gene: $F(1,91) = 10.03$, $p < 0.0001$; Block × Gene: $F(1,91) = 13.05$, $p < 0.001$).

D₃: In D₃ KO mice, MDMA-induced hyperactivity was attenuated in female but not male mice across both blocks (Figure 2e and f) (Sex × Gene × Drug: $F(1,111) = 4.27$,

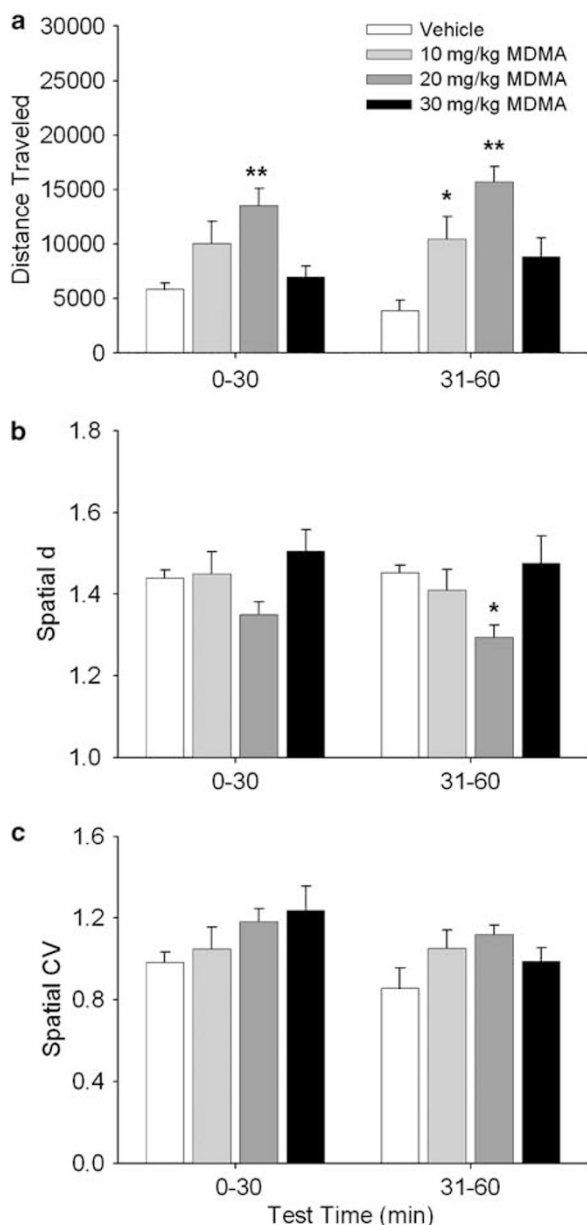


Figure 1 Dose response of MDMA effects on locomotor behavioral patterns. Effect of vehicle, 10, 20, and 30 mg/kg (+/–)MDMA (i.p., 10 min pretest injection) treatment on distance traveled (a), spatial *d* (b), and spatial CV (c). Mice used were WT mice bred in house from heterozygous pairs of the *D₁* line. Data are mean ± SEM; *n* = 7–8/group. **p* < 0.05, ***p* < 0.01, Dunnett's test vs vehicle control.

p < 0.05). Both male and female *D₃* KO mice exhibited similar baseline locomotor activity as WT mice.

Geometrical scaling measures. *D₁*: MDMA significantly increased the linearity of locomotor patterns in both WT and KO mice, as indicated by a reduction in the spatial *d* scaling measure after treatment, with larger effects observed in the second 30 min of testing (Figure 3a, female data not shown) (Block × MDMA: $F(1,112) = 60.8$, $p < 0.0001$). *D₁* KO mice also exhibited significantly lower spatial *d* than controls, indicating that at baseline they have more linear patterns of movements than WT mice (Gene:

$F(1,112) = 24.32$, $p < 0.0001$). MDMA increased spatial CV, in line with the reports of increased thigmotaxis patterns in rodents after MDMA treatment. This effect was slightly more pronounced overall in *D₁* KO mice, indicating that MDMA increased the predictability of spatial locomotor patterns more in *D₁* KO mice (Figure 3b, female data not shown) (Gene × MDMA: $F(1,112) = 4.41$, $p < 0.05$). A representative path (min 41–50) is shown in Figure 3c, which demonstrates the increased linear or smooth path movements in *D₁* KO mice treated with MDMA compared to WT mice.

D₂: MDMA significantly reduced spatial *d* and this effect was larger in *D₂* KO mice (Figure 3a, female data not shown) (MDMA: $F(1,91) = 159.54$, $p < 0.0001$; MDMA × Gene: $F(1,91) = 12.63$, $p < 0.001$). There also was a significant increase in spatial *d* (ie more circumscribed movements) in *D₂* KO mice overall (Figure 3a; Gene: $F(1,91) = 4.63$; $p < 0.05$), although this effect was more pronounced in female mice (data not shown). Most interestingly, the MDMA-induced increases in spatial CV were significantly attenuated in the *D₂* KO mice (Figure 3b) (MDMA × Gene: $F(1,91) = 30.08$, $p < 0.001$). The representative path (Figure 3c, min 41–50) demonstrates the reduction in perseverative thigmotaxis in *D₂* KO mice treated with MDMA compared to WT mice.

D₃: The only interactions of gene with MDMA were observed on spatial CV measures, where MDMA-induced increases in spatial CV were slightly but significantly reduced in *D₃* KO mice across sex (MDMA × Gene: $F(1,111) = 3.99$, $p < 0.05$); however, this interaction seemed largely owing to a slight increase in baseline spatial CV in *D₃* KO compared to WT mice (Figure 3a–c).

DISCUSSION

The goals of the present studies were (1) to characterize the effects of MDMA treatment on locomotor behavior patterns in mice and (2) to determine the respective contributions of DA *D₁*, *D₂*, and *D₃* receptors to these phenomena using constitutive null mutation mice. First, we found that MDMA treatment produced a classic stimulant-like inverted U dose–response curve on locomotor activity, with the 20 mg/kg dose having the greatest effect on activity and locomotor patterns. MDMA increased both the linearity of the mouse's path (reduced spatial *d*) and the repetition of specific locomotor paths (increased spatial CV derived mainly from perseverative thigmotaxis) at the 20 mg/kg dose. Our second finding was that *D₁*, *D₂*, and *D₃* gene deletion had generally small effects on MDMA-induced hyperactivity, supporting the hypothesis that nondopaminergic systems are principally responsible for MDMA effects on locomotor behavior. Nevertheless, *D₁* and *D₂* receptors do contribute to the overall pattern of locomotor behavior seen after MDMA treatment. For example, *D₁*-null mutant mice treated with MDMA exhibited greater increases in distance traveled late in the test session, apparently owing to a larger increase in linear movements over circumscribed movements (ie lower spatial *d* and increased spatial CV). In addition, male *D₂* KO mice exhibited reduced MDMA-induced hyperactivity late in the test session. A similar trend was apparent in the female *D₂* KO mice and both male and female mice were

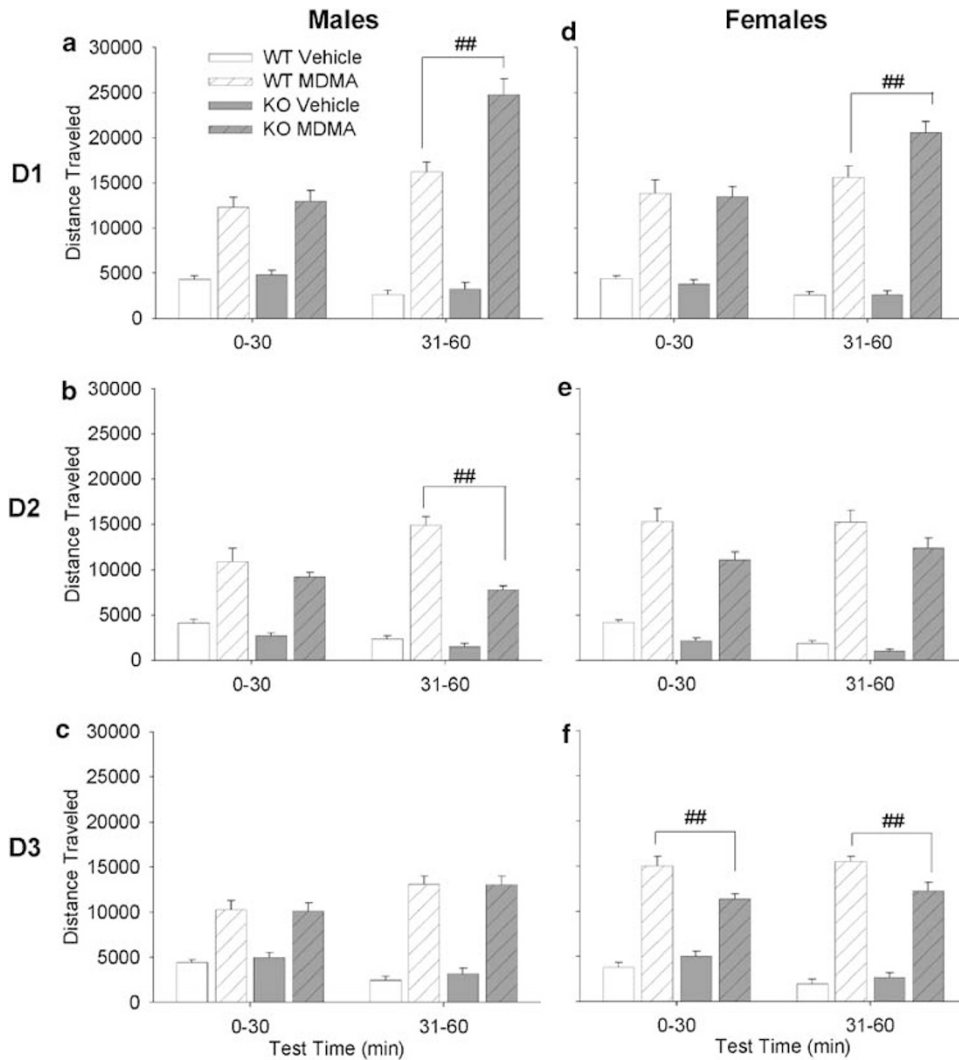


Figure 2 Effect of DA D_1 , D_2 , or D_3 gene deletion on MDMA-induced increases in locomotor activity. Effect of vehicle or 20 mg/kg (+/-)-MDMA (i.p., 10 min pretest injection) treatment on distance traveled in male (a–c) and female (d–f) D_1 , D_2 , and D_3 WT and KO mice. Data are mean \pm SEM; $n = 11$ – 21 . In all three lines, MDMA significantly increased distance traveled (see text for statistics). ## $p < 0.01$ vs WT/MDMA, Tukey's test.

insensitive to the increases in path predictability (ie increased spatial CV) normally produced by MDMA. In the case of D_3 KO mice, female but not male mice exhibited significantly reduced MDMA-induced hyperactivity, but the overall locomotor pattern was not changed. Hence, all three DA receptor subtypes contribute to MDMA-induced hyperactivity, but no one receptor subtype alone is necessary for these effects. Because the three congenic strains exhibited generally similar sensitivity to MDMA effects on locomotor behavior, differences in locomotor activity or patterns of locomotor behavior between D_1 , D_2 , and D_3 KO mice is most likely specific to the gene deletion manipulation.

Previously, the receptor mechanisms underlying MDMA effects on locomotor behavior patterns have been examined predominantly in rats using relatively selective 5-HT and DA receptor antagonists (see Introduction for references). The current studies using mice with specific receptor gene deletions in the recently developed mouse BPM (Geyer *et al*, 1986) complement the previous pharmacological studies of

MDMA effects on locomotor activity and behavioral patterns in rats. For the ascending limb of the MDMA dose–response curve, mice and rats exhibit comparable locomotor response to MDMA, which is characterized by locomotor hyperactivity and straighter, more predictable (ie perseverative) thigmotaxic locomotor paths (Figure 3c) (Gold *et al*, 1988). The inverted U-shaped dose–response curve for the amount and patterns of locomotor behavior presently observed after (+/-)-MDMA in mice has not been reported in rats, although the more potent (+)-MDMA enantiomer has been reported to produce a similar dose–response function on hyperactivity (Bengel *et al*, 1998; Gold *et al*, 1988; Paulus and Geyer, 1992). Using a comparable dose range, Scarce-Levie *et al* (1999) and Powell *et al* (2004) did not observe an inverted U-shaped dose–response curve in locomotor hyperactivity in mice. This disparity may be owing to differences background strain used, which are known to have very different rates of locomotor activity (C57BL/6J in the present studies vs 129/s; Crawley *et al*, 1997). Locomotor effects of 5-HT releasers

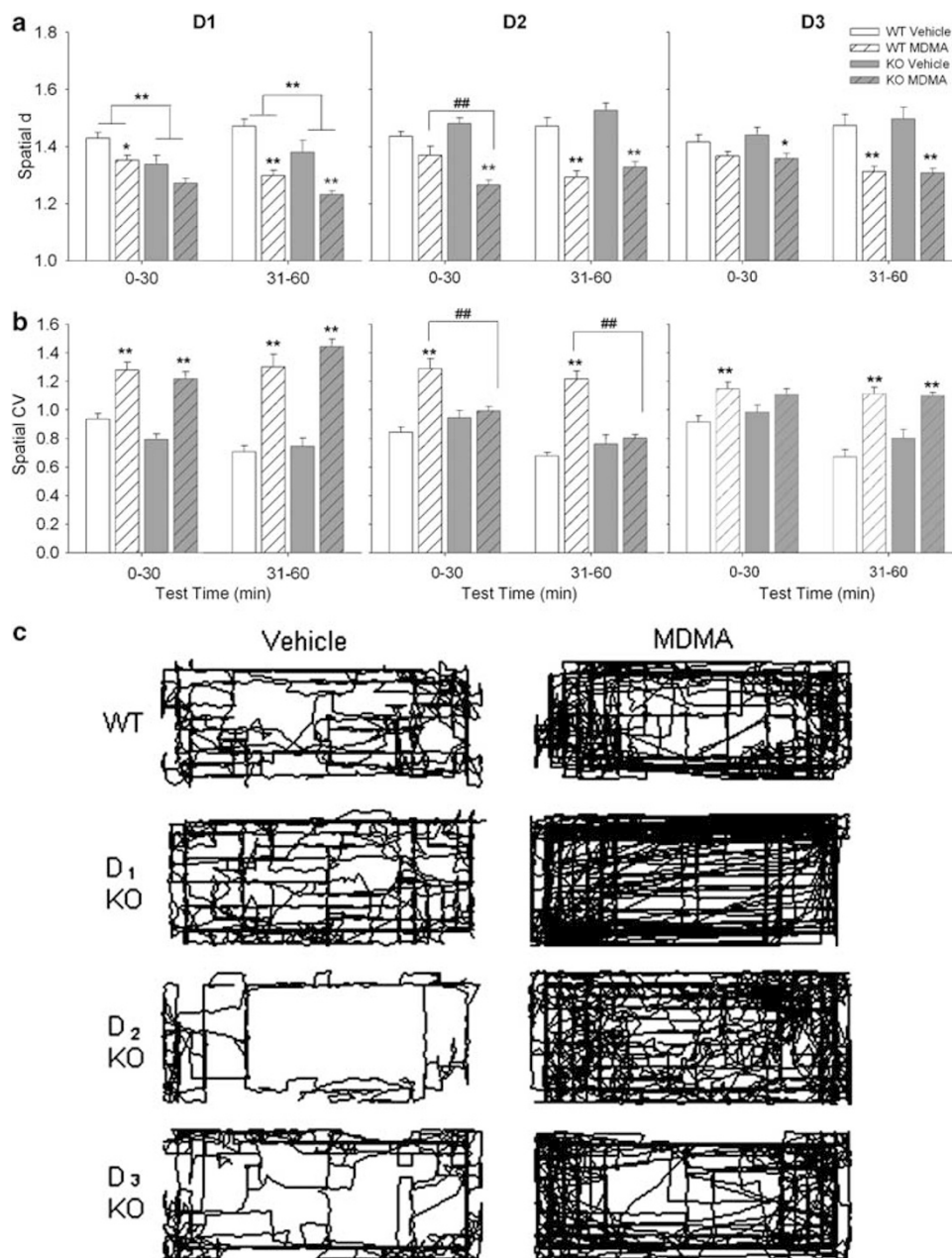


Figure 3 Effect of DA D₁, D₂, or D₃ gene deletion on MDMA-induced increases in linear and predictable spatial locomotor patterns. Panel a–b: Effect of vehicle or 20 mg/kg (+/–)MDMA (i.p., 10 min pretest injection) treatment on locomotor paths as measured by spatial d (a) and spatial CV (b) in male D₁, D₂, and D₃ WT and KO mice. Data are mean ± SEM; $n = 11–21$. * $p < 0.05$, ** $p < 0.01$ vs respective vehicle, ### $p < 0.01$ vs WT/MDMA, Tukey's test. Panel c: Representative locomotor patterns in WT, D₁ KO, D₂ KO, D₃ KO vehicle- and MDMA- (20 mg/kg, i.p.) treated mice (representative WT mouse patterns for vehicle- and MDMA-treated WT mice are from mice of the D₁ cohort). Patterns are from the 41–50 min time point within the 60 min session.

have also been shown to depend somewhat on testing chamber size and conformation (Paulus and Geyer, 1997), which varied across these studies. Despite this minor disparity at the high end of the dose range, the overall effects of MDMA on locomotor activation and organization appears to be fundamentally similar across both rats and mice in the behavioral profile provided by the BPM (present studies, Gold *et al*, 1988; Paulus and Geyer, 1992).

MDMA effects on locomotor activity are mediated primarily by serotonergic activation. In mice, the hyperactivity induced by low doses of MDMA (3–10 mg/kg) is

blocked by deletion of the genes for either the 5-HT_{1B} receptor (Scarce-Levie *et al*, 1999) or the 5-HT transporter (Bengel *et al*, 1998). High doses of MDMA (30 mg/kg), however, induced late-phase increases in hyperactivity in 5-HT_{1B} KO mice, which the authors attributed to a delayed increase in DA release relative to 5-HT release (White *et al*, 1994, 1996; Yamamoto and Spanos, 1988). Our findings in male D₁ and D₂ KO mice after 20 mg/kg MDMA administration support this hypothesis, as both DA receptor KO lines exhibited altered MDMA-induced effects on locomotor activity only in the late phase of testing. In rats, D₁ or D₂

antagonists also appear to be more potent in reducing late-phase MDMA-induced hyperactivity *vs* hyperactivity during initial testing (Bubar *et al*, 2004). On the other hand, female D₃ KO mice were less sensitive to MDMA effects on hyperactivity throughout the testing session. These data indicate that at this dose of MDMA DA release has small, but functional, consequences at earlier phases as well (see also Ball *et al*, 2003).

We found that D₁ KO mice exhibited greater MDMA-induced hyperactivity compared to WT mice. These data contrast with reports in rats that the D₁/D₅ receptor antagonist SCH23390 reduced MDMA-induced hyperactivity (Ball *et al*, 2003; Bubar *et al*, 2004). SCH23390, however, also reduces locomotor activity in D₁ KO mice, indicating that the locomotor-reducing effects of SCH23390 may not be due solely to D₁ receptor blockade (Centonze *et al*, 2003). SCH23390 is an antagonist at both D₁ and D₅ receptors (Lawler *et al*, 1999), as well as 5-HT_{2C} receptors (Millan *et al*, 2001). It is unlikely that the SCH23390-induced blockade of MDMA-induced hyperactivity is via blockade of 5-HT_{2C} receptors, however, as other 5-HT_{2C} antagonists potentiate MDMA-induced hyperactivity (Fletcher *et al*, 2002). As D₅ receptor blockade appears to decrease locomotor activity (Elliot *et al*, 2003), SCH23390-induced attenuation of MDMA-induced hyperactivity may be due, at least in part, to blockade of D₅ receptors. It is important to note that D₁ KO mice exhibit alterations in the neurochemical architecture of the striatum, hence these contrasting findings may also be owing to compensatory changes in the D₁ KO mouse (Ariano *et al*, 1998; Xu *et al*, 1994). More selective D₁ antagonists and/or temporally constrained D₁ receptor-knockdown techniques will be required to clarify these contrasting findings.

The observation that D₁ KO mice exhibit potentiated MDMA-induced hyperactivity is also puzzling when comparing the effects of more specific DA releasers on locomotor activity in D₁ KO mice. D₁ KO mice exhibit reduced sensitivity to the activating effects of cocaine and amphetamine (Xu *et al*, 2000). It is important to consider, however, the overall locomotor activity patterns that were produced in D₁ KO mice, as well as the inverted U-shaped dose-response curve on activity produced by MDMA treatment. First, D₁ KO mice exhibited smoother, less circumscribed locomotor paths during either vehicle or MDMA treatment (lower spatial *d*; Figure 3). Treatment with the 20 mg/kg dose of MDMA significantly increased the linear movements of both groups, most efficaciously during the later phase of testing, resulting in even fewer circumscribed movements. In WT mice, higher doses of MDMA (30 mg/kg) produced less hyperactivity than at lower doses, and the movement path began to become more circumscribed, perhaps reflecting a shift into competing localized stereotyped behaviors at higher doses (Searce-Levie *et al*, 1999). If D₁ activation contributes to the induction of stereotypic behaviors (Chartoff *et al*, 2001; Daly and Waddington, 1992; Fetsko *et al*, 2003), linear locomotor activity may be favored over circumscribed local activity in MDMA-treated D₁ KO mice. Hence, it is possible that the relatively linear movement patterns of the D₁ KO mice resulted in a greater distance traveled compared to WT mice. We have observed a similar pattern of results after the administration of high doses of amphetamine, where D₁ KO

mice exhibit significantly more hyperactivity than WT mice, a pattern that is not reproduced at lower, nonstereotypy-inducing doses of amphetamine (A Kadner, VL Masten, S Caldwell, MA Geyer, unpublished observations). Therefore, at putatively high levels of DA release during the later phase of MDMA effects, D₁ activation may contribute more to the quality of the locomotor movement—linear *vs* circumscribed—as opposed to the quantity *per se*.

D₂ receptor KO mice exhibited very different baseline and MDMA-induced alterations in locomotor activity as compared to D₁ KO mice. We and others have observed that D₂ KO mice exhibit significantly less locomotor activity than WT controls (Figures 2b and e) (Baik *et al*, 1995; Kelly *et al*, 1998; Phillips *et al*, 1998). D₂ KO mice also had a tendency to exhibit more circumscribed movements (increased spatial *d*; Figure 3). With MDMA treatment, D₂ KO mice exhibited significantly less hyperactivity in the later stage of testing compared to WT mice (Figure 2b), similar to results from D₂ antagonist administration in MDMA-treated rats (Ball *et al*, 2003; Bubar *et al*, 2004). Inspection of a representative path of D₂ WT and KO mice treated with MDMA shows that the D₂ KO mice appear to be covering more regions of the chamber and have less dense paths around the walls, corroborating the reduced sensitivity to MDMA effects on spatial CV in these mice (Figure 3c). These data indicate that D₂ receptors may contribute to the perseverative thigmotaxic locomotor pattern after MDMA treatment, and this affect appears to be orthogonal to changes in overall hyperactivity or relative linearity of locomotor path.

D₃ KO mice exhibited no significant differences in locomotor activity compared to WT mice with vehicle treatment, indicating that D₃ receptors do not appear to play a large role in habituated locomotor behaviors. In contrast, previous reports indicate that D₃ KO mice exhibit hyperactivity when exposed to a novel environment (Accili *et al*, 1996). When treated with MDMA in the present studies, female D₃ KO mice exhibited slightly reduced MDMA effects on distance traveled compared to female WTs, although this effect was not replicated in the D₃ KO male mice. The observation that female D₃ KO mice exhibited reduced MDMA locomotor effects is somewhat surprising given previous data, suggesting that D₃ activation reduces locomotor activity. For example, D₃ blockade via antagonists or constitutive gene deletion induces hyperactivity (Accili *et al*, 1996; Waters *et al*, 1993) and D₃ agonists reduce activity (Daly and Waddington, 1993; Svensson *et al*, 1994). Additionally, D₃ KO mice have been reported to exhibit a 'hyperdopaminergic' phenotype, with increased striatal DA tone (Joseph *et al*, 2002; Koeltzow *et al*, 1998) and enhanced D₁/D₂ intracellular signaling (Mizuo *et al*, 2004). Compensatory mechanisms such as reductions in tyrosine hydroxylase and increased DAT expression have been observed in D₃ KO mice (Le Foll *et al*, 2005). This latter point might suggest that D₃ KO mice could have been toward the descending limb of the dose-response curve, similar to the reduced activity observed after higher doses of MDMA. This effect was small and only observed in female mice, however, suggesting a minimal role for D₃ receptors in MDMA-induced locomotor activity. In rats, female rats exhibit significantly more DAT but less D₃ expression in the nucleus accumbens compared to male

rats, although we are unaware of data confirming these findings in mice of the background used in the present studies (Harrod *et al*, 2004). Taken together with the present studies, these data are suggestive that D₃ receptors have differential contribution across male and female mice, but further testing is required to evaluate this possibility.

For these initial studies, we chose the 20 mg/kg dose of MDMA to investigate the contribution of DA receptors to MDMA-induced alterations in locomotor activity. This dose produced the most clear behavioral effects on activity and patterns, which were also most similar to previous reports in rats and mice (see Introduction for references). Our observation of an inverted U-shaped dose-response curve of MDMA effects, in addition to our present findings in D₁ and D₂ KO mice, support the value of future comparative studies of D₁ and D₂ receptor contributions to levels and patterns of locomotor activity over a greater dose range of MDMA. Lower doses would help delineate the specific contributions of these receptors to activity only (path is relatively unaffected at low doses; Figure 1a–c). Higher doses would help determine if these receptors, as hypothesized here, are involved in initiating behaviors that compete with ambulatory movements to create the inverted U-shaped dose-effect pattern observed.

In conclusion, MDMA-induced alterations in locomotor activity remained largely intact in D₁, D₂, and D₃ KO mice, supporting the overall importance of other mechanisms such as 5-HT release for MDMA effects on locomotion in mice. Nevertheless, these results also confirm that these DA receptor subtypes augment MDMA-induced hyperactivity, especially during the later phases of testing in the case of D₁ and D₂ receptors. The present findings indicate that D₁ receptor activation may be important in modifying the type of activity (linear *vs* circumscribed), and that D₂ receptor activation may contribute to the repetitive circling behavior produced by high doses of MDMA. These alterations in locomotor patterns then augment the total activity as measured by distance traversed during testing.

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