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MDMA “ecstasy” alters hyperactive and perseverative behaviors in dopamine transporter knockout mice

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Abstract *Rationale:* Mice lacking the gene for the dopamine transporter (DAT) show a unique behavioral phenotype characterized by locomotor hyperactivity and repetitive circling in a novel environment. The hyperactivity of DAT (–/–) mice can be attenuated by psychostimulants and by serotonin uptake inhibitors, suggesting an important role for serotonin in the attenuation of locomotor hyperactivity in these mice. *Objectives:* These studies characterized the effects of 3,4-methylenedioxymethylamphetamine (MDMA), a serotonin releaser, on the amount and patterns of locomotor activity in DAT (+/+) and (–/–) mice. We compared the locomotor patterns produced by MDMA to those observed in DAT (–/–) mice, and examined whether MDMA altered the hyperactivity and perseverative locomotor patterns in DAT (–/–) mice. *Methods:* The effects of MDMA (10, 30 mg/kg) on locomotor activity in DAT (+/+) mice were measured for 90 min in a video tracker system to determine the optimal dose for subsequent studies in DAT (+/+) and (–/–) mice. The effects of 20 mg/kg MDMA on patterns of locomotor activity in DAT (+/+) and (–/–) mice were measured for 90 min. *Results:* In DAT (+/+) mice, MDMA increased locomotor activity and induced repetitive straight movement patterns. In DAT (–/–) mice, however, MDMA (20 mg/kg) attenuated the characteristic locomotor hyperactivity seen in these mice. In contrast, MDMA potentiated the thigmotaxis and decreased entropy observed in the DAT (–/–) mice. *Conclusions:* The effects of MDMA observed here demonstrate that the

different aspects of the abnormal locomotor behavior exhibited by DAT (–/–) mice can be independently manipulated by pharmacological treatments.

Keywords Dopamine transporter · Hyperactivity · Perseveration · MDMA · Animal model · Mice

Introduction

Dysregulation of the dopamine (DA) system is thought to underlie several psychiatric disorders including schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder (ADHD). Based on the idea that a hyperdopaminergic state underlies some psychiatric illnesses, several studies have focused on the DA transporter (DAT) as a possible candidate gene in linkage studies in patients with schizophrenia, bipolar disorder, and ADHD (Cook et al. 1995; Kelsoe et al. 1996; Crowe and Veland 1998; Fujiwara et al. 1997; Persico and Macciardi 1997; Gill et al. 1997; Greenwood et al. 2001). Recently, DAT mutant mice have been generated that may prove useful in examining the behavioral consequences of dysregulated DA function related to these disease states. DAT (–/–) mice completely lack the gene coding for the DAT and exhibit a chronic hyperdopaminergic tone compared to wildtype DAT (+/+) mice (Giros et al. 1996). DAT (–/–) mice exhibit regulatory changes in neuropeptide gene expression, downregulation of both D₁ and D₂ DA receptors, a 90% decrease in the enzyme tyrosine hydroxylase, and decreased release of DA into the extracellular fluid (Giros et al. 1996; Gainetdinov et al. 1998). Despite these compensatory changes, DAT (–/–) mice are dramatically hyperactive in a novel environment and have impairments in spatial cognitive function (Giros et al. 1996; Gainetdinov et al. 1999a; Spielewoy et al. 2001).

In addition to profound hyperactivity, DAT (–/–) mice display a behavioral profile characterized by perseverative, stereotyped locomotor patterns in the periphery of an open field (Ralph et al. 2001a). A very similar behavioral profile has been observed in rats in response to the

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amphetamine derivative, 3,4-methylenedioxy-N-methylamphetamine (MDMA) (Gold et al. 1988; Callaway et al. 1990, 1991). In mice, MDMA also increases locomotion and decreases exploration of the center (Searce-Levie et al. 1999). As yet, the effects of MDMA on locomotor patterns have not been characterized in mice.

The degree to which MDMA exerts its behavioral effects via the serotonin or dopamine system are unclear. MDMA acts as both a serotonin (5-HT) and DA releaser (Nichols et al. 1982; Schmidt et al. 1987). The locomotor hyperactivity induced by MDMA is blocked by the 5-HT uptake inhibitor fluoxetine in rats (Callaway et al. 1990) and is absent in 5-HT transporter knockout mice (Bengel et al. 1998), suggesting an important role for presynaptic 5-HT release in the behavioral effects of MDMA. The hyperactivity of DAT ($-/-$) mice can be attenuated by psychostimulants such as amphetamine, cocaine, or methylphenidate and by 5-HT uptake inhibitors such as fluoxetine (Gainetdinov et al. 1999b; Spieleswoy et al. 2001). 5-HT agonists such as quipazine and the 5-HT precursors, 5-hydroxytryptophan and L-tryptophan, also attenuate the hyperactivity in DAT ($-/-$) mice. The involvement of serotonergic systems in the hyperactivity seen in DAT ($-/-$) mice appears to be complex, however, as antagonists at 5-HT_{2A} receptors also reduce the motor hyperactivity in these mice (Barr et al. 2003). Since these serotonergic compounds were effective in attenuating the hyperactivity but the noradrenergic manipulations were not, the attenuation of locomotor hyperactivity in DAT ($-/-$) mice with psychostimulants was postulated to be mediated through their effects on 5-HT (Gainetdinov et al. 1999b).

Considering that psychostimulants such as amphetamine, cocaine, and methylphenidate increase locomotor behavior in DAT ($+/+$) mice and attenuate locomotor hyperactivity in DAT ($-/-$) mice (Gainetdinov et al. 1999b; Spieleswoy et al. 2001), we sought to determine whether DAT ($-/-$) mice would show a similar reduction in locomotor activity in response to the 5-HT and DA releaser, MDMA. Thus, the goals of these studies were to: 1) characterize the effects of MDMA on the structure of locomotor patterns as previously done in rats; 2) compare these patterns to those observed in DAT ($-/-$) mice; and 3) examine whether the hyperactivity and perseverative locomotor patterns in DAT ($-/-$) mice would be attenuated by MDMA.

Materials and methods

Animals

The male and female DAT mutant mice [DAT ($+/+$) $n=33$; DAT ($-/-$) $n=21$] used in these experiments were derived from a breeding colony at the Veterans Administration Hospital, San Diego, Calif., USA. DAT ($+/+$) and DAT ($-/-$) mice were generated using parental DAT ($+/-$) mice from Duke University (Giros et al. 1996). All mice were maintained in an AAALAC-approved animal facility at the Veterans Hospital, San Diego. This facility meets all Federal and State requirements for animal care. Mice from each strain were group housed ($n=4/\text{cage}$) in a climate-

controlled animal colony with a reversed day/night cycle (lights on at 8:00 p.m., off at 8:00 a.m.). Mouse pups were weaned at 4 weeks, housed with same-sex littermates, and genotyped by PCR. All behavioral testing started at approximately 5 months of age and occurred between 9:00 a.m. and 6:00 p.m. Food (Harlan Teklab, Madison, Wisc., USA) and water were available throughout the experiments, except during behavioral testing.

Drugs

($\pm$)-MDMA (3,4-methylenedioxymethamphetamine) was obtained from the National Institute on Drug Abuse and was dissolved in 0.9% saline. All injections were given at a volume of 5 ml/kg body weight, intraperitoneally (IP), 10 min prior to behavioral testing.

Apparatus

Locomotor activity was measured using a video-tracker (VT), which tracked mice in 4 adjacent white Plexiglas enclosures (41×41×34 cm). The four adjacent VT enclosures were surrounded by an opaque plastic curtain. Each mouse was tested individually in a separate enclosure and had no contact with the other mice. A video camera, mounted 158 cm above the enclosures, provided the signal for the Polytrack digitizer (San Diego Instruments, San Diego, Calif., USA). The signal was processed to obtain the left-uppermost coordinate for each of the four animals simultaneously. The signal was stored in a PC computer for further off-line processing. For this investigation, the (x,y) position (in pixels) of each animal sampled at a rate of 18.18 Hz was used to generate a (x,y,t) coordinate file consisting of the x-location, the y-location, and the duration of time (t) spent at that location. The spatio-temporal resolution of each event recorded was 0.32 cm, 0.32 cm, 55 ms, which corresponded to a maximum speed of 25 cm/s.

Locomotor pattern testing

Each mouse was placed in the bottom left hand corner of each enclosure at the start of the test session. In experiment 1, female DAT ($+/+$) mice were administered saline ($n=5$), MDMA 10.0 mg/kg ($n=6$) or MDMA 30.0 mg/kg ($n=7$) 10 min prior to placement in the enclosure. In experiment 2, male DAT ($+/+$) mice ($n=15$) and DAT ($-/-$) mice ($n=21$) were administered saline or MDMA 20.0 mg/kg 10 min prior to placement in the enclosure. The mice were then tracked in the enclosure for 90 min, with data being stored in nine 10-min blocks. Four categories of measures were obtained. First, the amount of locomotor activity was assessed as the number of transitions made between pre-defined areas of the open field, i.e. the wall area (4), corner area (4), or center area (1) (see Geyer et al. 1986). Second, the amount of time spent in the center of the open field was assessed to determine a potential avoidance of the center (often interpreted as increased anxiety) and/or thigmotaxis (hugging the walls of the arena). Third, the geometric patterns of locomotor activity were quantified by the spatial scaling exponent, d , as described in detail elsewhere (Paulus et al. 1999). Briefly, the spatial scaling exponent, d , measures the degree to which consecutive movements are along a straight line ($d \approx 1$), are characterized by meandering patterns ($d \approx 1.5$), or include many directional changes or circumscribed movements ($d \approx 2$). Fourth, average entropy was used to quantify the predictability of locomotor activity patterns in DAT ($+/+$) and ($-/-$) mice. Average entropy quantifies the predictability of sequences of transitions across different areas in the VT chamber. For example, a mouse that circles repeatedly along the outer edges of the arena would move through areas 1, 2, 3, 6, 9, 8, 7, 4 and then back to 1. This pattern results in a low entropy measure if this sequence of movements is repeated many times while the animal is in the VT chamber. In comparison, an animal that moves through different areas of the VT chamber via various routes would generate a higher level of entropy. In short, the average entropy quantifies the

diversity of different routes an animal takes while in the VT chamber. Thus, spatial d quantifies the geometric pattern of locomotion and average entropy quantifies the degree of predictability of locomotor patterns. The combination of spatial d and average entropy (e.g. low spatial d , low entropy) can be used to determine the degree of perseverative locomotor activity.

Statistical analyses

Two- or three-way analyses of variance (ANOVAs) were used to compare the amount of locomotor activity (transitions), the time spent in the center, spatial d , and average entropy (experiment 2). Genotype and/or drug treatment were between-subjects variables, and time was a within-subjects variable. For dependent measures in which time was included as a within-subject variable, pairwise ANOVAs were conducted and alpha was decreased by half ($P<0.025$) following the overall ANOVA. For the dependent measures that were collapsed across the 90-min session, post hoc analyses were carried out using Tukey's. Alpha level was set to 0.05. Computations were carried out using Biomedical Data Programs (BMDP) statistical software (Statistical Solutions, Inc., Saugus, Mass., USA).

Results

Experiment 1: MDMA dose response

An MDMA dose-response study was completed to determine the effects of MDMA on locomotor patterns and to select an appropriate dose of drug to test in DAT mice. To ensure that the effects of MDMA on locomotor activity were transferable to DAT mice, the dose response of MDMA was performed in female DAT (+/+) mice ($n=5-7$ /group; Table 1). There was a significant main effect of drug treatment on transitions [$F(2,15)=73.48$, $P<0.001$], but no drug by block interaction. The main effect of drug was due to the significant increase in locomotor activity at the 30 mg/kg dose of MDMA [pairwise ANOVA; $F(1,10)=82.93$, $P<0.001$] with no significant effect at the 10.0 mg/kg dose of MDMA (Table 1). There was a significant main effect of MDMA on spatial d [$F(2,15)=36.11$, $P<0.001$], with the 30 mg/kg dose of MDMA producing straighter patterns of motor behavior as indicated by a decrease in spatial d [pairwise ANOVA; $F(1,10)=53.0$, $P<0.001$; Table 1]. There was also a significant drug by time interaction [$F(8,120)=2.83$, $P<0.01$], with spatial d increasing in the saline and 10.0 mg/kg MDMA group over the 90-min session, but remaining the same across time in the 30.0 mg/kg MDMA group. In addition, there was a significant effect of MDMA on time spent in the center of the test chamber [$F(2,15)=5.73$, $P<0.05$]. While the 10.0 mg/kg dose of MDMA had no effect on center entries, the 30.0 mg/kg

dose produced a trend to increase time spent in the center [pairwise ANOVA; $F(1,10)=4.22$, $P=0.067$]. It was clear that the 30.0 mg/kg dose of MDMA significantly altered both the amount and the pattern of locomotor behavior, but that the 10.0 mg/kg dose of MDMA had minimal effects on both activity and spatial d . Based on these results and previous reports in the literature, a 20.0 mg/kg dose of MDMA was chosen to test in the DAT (+/+) and (-/-) mice (Searce-Levie et al. 1999).

Experiment 2: MDMA in DAT mice

Transitions

As expected, the DAT (-/-) mice were hyperactive compared to the DAT (+/+) mice when treated with vehicle [genotype, $F(1,15)=18.19$, $P<0.001$]. When the DAT (+/+) and (-/-) mice were treated with MDMA (20.0 mg/kg), there was main effect of genotype [$F(1,32)=15.22$, $P<0.001$] but no main effect of drug on transitions. Most importantly, a significant genotype by drug interaction [$F(3,32)=12.64$, $P<0.005$] on transitions followed by subsequent pairwise ANOVAs confirmed the hypothesis that MDMA would increase locomotor activity in DAT (+/+) mice [drug, $F(1,15)=10.26$, $P<0.01$] and attenuate the hyperactivity seen in the DAT (-/-) mice (Fig. 1). Although there was no main effect of drug on transitions in DAT (-/-) mice, there was a significant block by drug interaction [$F(8,168)=4.36$, $P<0.0001$], with MDMA-treated mice showing less locomotion initially which did not decrease over time. In contrast, vehicle-treated DAT (-/-) mice showed locomotor habituation towards the end of the session.

Center duration

There was a main effect of drug on time spent in the center [$F(1,32)=9.24$, $P<0.01$], but no effect of genotype and no genotype by drug interaction. Post-hoc tests (Tukey's), based on the a priori hypothesis, revealed that treatment with MDMA significantly decreased the amount of time spent in the center region of the apparatus in the DAT (-/-) mice ($P<0.05$), but not in the DAT (+/+) mice (Fig. 2A). In fact, MDMA-treated DAT (-/-) mice spent virtually no time in the center.

Table 1 Dose response of ($\pm$)-MDMA (0, 10, 30 mg/kg, IP) in female DAT (+/+) mice. Data represent the mean ($\pm$ SEM) transitions, center duration, and spatial d over the 90-min test session. Data $n=5-7$ /group

($\pm$)-MDMA mg/kg	Transitions	Center duration	Spatial d
0	524.67 (152.2)	2.04 (1.06)	1.74 (0.031)
10	1254.14 (620.7)	0.55 (0.30)	1.71 (0.097)
30	4915.14 (416.0)*	9.78 (3.26)	1.30 (0.053)*

* $P<0.001$ versus vehicle-treated mice

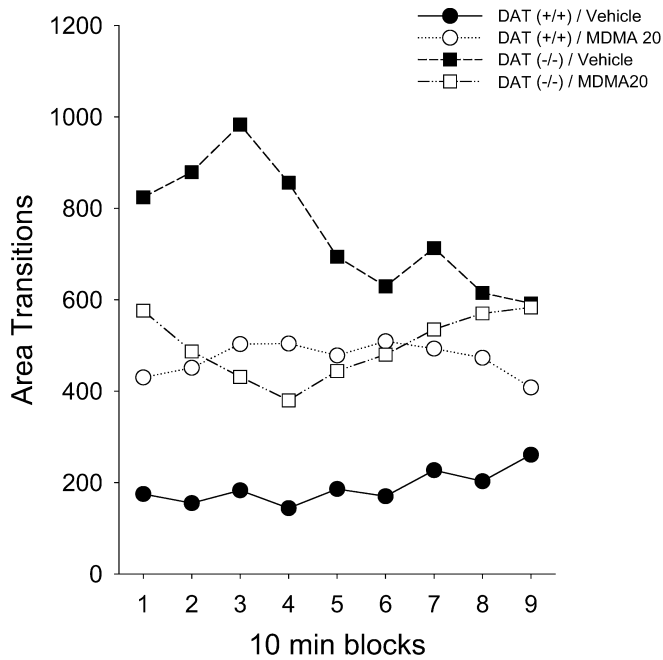


Fig. 1 Effect of ($\pm$) MDMA (20.0 mg/kg, IP) on locomotor behavior (area transitions) in DAT (+/+) and (-/-) mice over a 90-min session. Vehicle-treated DAT (-/-) mice were hyperactive compared to vehicle-treated DAT (+/+) mice. MDMA increased locomotor activity in DAT (+/+) mice and decreased locomotor activity in DAT (-/-) mice. Data are expressed as mean $\pm$ SEM for each 10-min block. $n=7-11$ /group

Spatial scaling exponent, d

Vehicle-treated DAT (+/+) mice exhibited a mixture of straight, meandering, and circumscribed movements, while the DAT (-/-) mice were significantly more active and displayed straighter sequences of locomotor activity (see Fig. 3). These differences in patterns of locomotion were confirmed quantitatively using the spatial scaling exponent, d (Fig. 2B), a measure that quantifies the degree to which sequences of movements are straight ($d \cong 1$) or circumscribed ($d \cong 2$) (Paulus and Geyer 1991; Ralph et al. 2001b). There were main effects of genotype

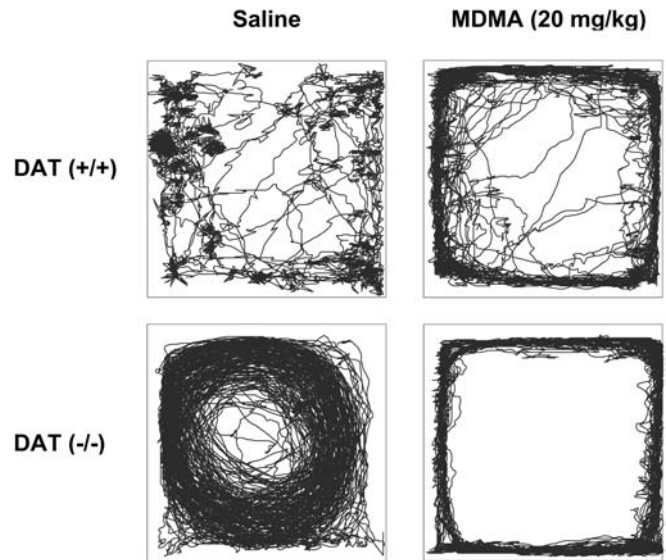


Fig. 3 Representative locomotor patterns in DAT (+/+) and (-/-) mice treated with saline or MDMA (20 mg/kg, IP) during the first 10 min of the 90-min test session. MDMA decreased the amount of locomotor activity and potentiated thigmotaxis and perseverative behavior in DAT (-/-) mice, but had no effect on spatial d in DAT (-/-) mice

[$F(1,32)=15.06$, $P<0.001$] and drug [$F(1,32)=17.31$, $P<0.001$], as well as a genotype by drug interaction [$F(3,32)=4.90$, $P<0.05$] on spatial d . As in earlier studies (Ralph et al. 2001a), vehicle-treated DAT (-/-) mice exhibited more perseverative patterns of motor activity (straighter patterns of locomotor behavior as indicated by lower spatial d values) than the vehicle-treated DAT (+/+) controls ($P<0.05$). Although MDMA increased the straight patterns of locomotion, as evidenced by a decrease in spatial d in the DAT (+/+) mice ($P<0.01$), MDMA did not further reduce spatial d in the DAT (-/-) mice, despite a trend in this direction (Fig. 2B).

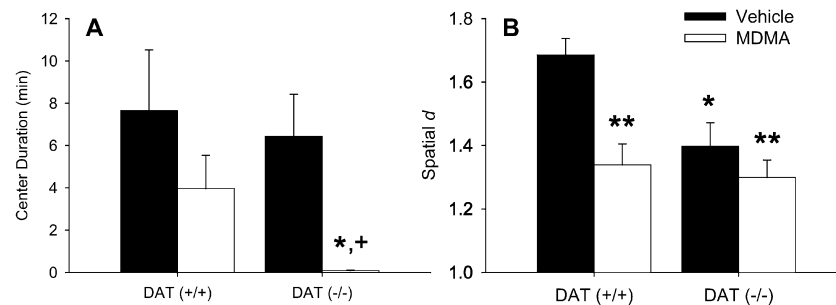
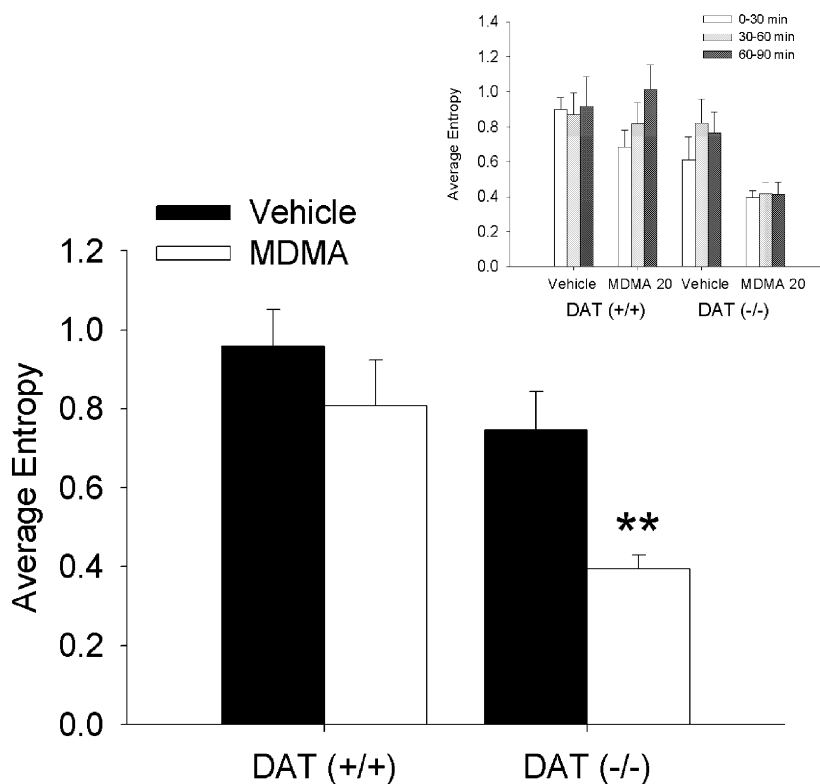


Fig. 2 Effect of ($\pm$) MDMA (20.0 mg/kg, IP) on **A** center duration and **B** spatial d in DAT (+/+) and (-/-) mice over a 90-min session. The spatial scaling exponent, d , measures the degree to which consecutive movements are along a straight line ($d \cong 1$), are characterized by meandering patterns ($d \cong 1.5$), or include many

directional changes or circumscribed movements ($d \cong 2$). There was a main effect of MDMA for both center duration and spatial d , and a main effect of genotype for spatial d . Data are expressed as mean $\pm$ SEM. $n=7-11$ /group. * $P<0.05$, ** $P<0.01$ vs DAT (+/+)/Vehicle group, + $P<0.05$ vs DAT (-/-)/Vehicle group, Tukey's

Fig. 4 Effect of ($\pm$)-MDMA (20.0 mg/kg, IP) on average entropy in DAT (+/+) and (-/-) mice collapsed across 90-min session. Average entropy quantifies the predictability of sequences of transitions across different areas in the VT chamber. *Inset*: average entropy over the 90 min session, in 30-min time blocks. There were main effects of genotype and drug on average entropy, $P < 0.01$. Data are expressed as mean $\pm$ SEM. $n = 7-11$ /group. ** $P < 0.01$ vs all other groups, Tukey's



Average entropy

The movement sequences generated by DAT (-/-) mice relative to DAT (+/+) had lower entropy values overall, i.e. the sequences of movements were more predictable than those of wild-type animals [$F(1,34) = 14.59$, $P < 0.001$], which is consistent with the notion that these mice display perseverative patterns of locomotor activity (Fig. 4). There was a $\text{gen} \times \text{drug} \times \text{block}$ interaction [$F(2,68) = 4.24$, $P < 0.05$] with entropy levels in the DAT (+/+)/MDMA and DAT (-/-)/Vehicle groups increasing over the course of the session, suggesting that these mice take more diverse routes with longer exposure to the VT chamber. Based on the a priori hypothesis that DAT (-/-) mice would be differentially sensitive to the effects of MDMA on entropy, we conducted post-hoc analyses on average entropy values (Tukey's). By itself, MDMA had no significant effect on entropy in DAT (+/+) mice, although the group means were slightly lower in the MDMA-treated mice (Fig. 4). In the DAT (-/-) mice, however, MDMA had a robust effect, such that the DAT (-/-)/MDMA group showed significantly lower entropy than all other groups ($P < 0.001$), confirming that the decreased entropy in DAT (-/-) mice was potentiated by MDMA.

Discussion

The current studies revealed four main findings. First, in wild-type mice, MDMA induced a behavioral profile of

increased locomotor activity and straight, perseverative movements, as indicated by decreased spatial d . Second, in contrast to the increased locomotor activity in wild-type mice produced by MDMA, this compound decreased locomotor activity in DAT (-/-) mice. Third, MDMA reduced center duration in DAT (-/-) mice. Fourth, MDMA potentiated the highly predictable, perseverative pattern of locomotor activity in DAT (-/-) mice as indicated by a decrease in entropy. In combination, these findings support the notion that the hyperactivity in DAT (-/-) mice is attenuated by the 5-HT releaser MDMA, but that MDMA does not reverse all aspects of the abnormal locomotor behavior in these mice. Rather, increased 5-HT release potentiated both the perseverative movement patterns and the avoidance of the center exhibited by DAT (-/-) mice.

As we have reported previously (Ralph et al. 2001a), DAT (-/-) mice are hyperactive and display perseverative locomotor patterns. The pattern of motor activity produced by MDMA in DAT (+/+) mice (i.e. locomotor hyperactivity and straight, perseverative movements) is strikingly similar to the patterns observed in vehicle-treated DAT (-/-) mice (Ralph et al. 2001a) or in rats treated with MDMA or related releasers of presynaptic 5-HT (Gold et al. 1988; Callaway et al. 1990, 1991). The increases in locomotor activity at the 20 and 30 mg/kg doses of MDMA are consistent with those observed by Searce-Levie et al. (1999) in 129/Sv mice. Despite the fact that MDMA increased locomotor activity in DAT (+/+) mice, it attenuated locomotor hyperactivity in DAT (-/-) mice. Although MDMA is a releaser of both 5-HT

and DA (Nichols et al. 1982; Schmidt et al. 1987), the locomotor-stimulating effects of MDMA in rats are thought to be mediated primarily through its effects on 5-HT release (Callaway et al. 1991; Geyer and Callaway 1994). For example, other amphetamine derivatives that act as 5-HT releasers but have little or no direct effect on DA release also increase locomotor activity and induce perseverative locomotor patterns (Callaway et al. 1991). Furthermore, both the 5-HT-releasing and locomotor-stimulating effects of MDMA are blocked by pretreatment with the 5-HT uptake inhibitor fluoxetine (Callaway et al. 1991). Mice lacking the 5-HT transporter (SERT) are insensitive to the locomotor-stimulating effects of MDMA (Bengel et al. 1998). Additionally, fluoxetine (10 mg/kg) attenuates locomotor hyperactivity induced by racemic MDMA in mice (Fantegrossi et al. 2003). Thus, to the extent that MDMA exerts its locomotor-stimulating effects through 5-HT, its attenuation of locomotor hyperactivity in DAT (–/–) mice may also take place through increased release of 5-HT.

As reviewed above, MDMA also acts as a DA releaser (Nichols et al. 1982; Schmidt et al. 1987), which may potentially contribute to its locomotor-stimulating effects (Bankson and Cunningham 2001) and its attenuation of locomotor hyperactivity in DAT (–/–) mice. It is possible that DA release can be increased in certain brain areas in DAT (–/–) mice after MDMA, as has been demonstrated in the nucleus accumbens following amphetamine and cocaine (Carboni et al. 2001; Gainetdinov et al. 2002). Nevertheless, even if MDMA directly or indirectly stimulates DA transmission, it is unlikely that MDMA is able to modulate behavior by further increasing DA levels because DAT (–/–) mice already show profound elevations in synaptic DA concentrations.

Administration of MDMA increases 5-HT release and locomotor activity in mice. Thus, the effect of MDMA on decreasing locomotor activity in DAT (–/–) mice seems paradoxical and similar to that reported with other psychostimulants (Gainetdinov et al. 1999b; Spieleswoy et al. 2001). An inverted U-shaped dose response function for serotonergic effects on locomotor activity in DAT (–/–) mice toward the descending arm of the dose response function would explain this seemingly paradoxical effect. Although such an explanation may be relevant to the effects of fluoxetine (Brocco et al. 2002), no such effect has been reported for MDMA. Moreover, it does not appear that MDMA produces psychostimulant-like stereotypy in mice. Scarce-Levie et al. (1999) described “stereotypy” at high doses of MDMA (30.0 mg/kg), which was characterized by repetitive locomotor movements and did not result in decreased locomotion (as is the case with the classic focal stereotypies induced by amphetamine). Although we did not directly measure stereotypy in the current study, we can infer from the low spatial *d* values at the 30 mg/kg dose (more straight locomotor trajectories) that the decreased locomotor activity in DAT (–/–) mice was not due to a corresponding increase in focused stereotypy. Our measure of spatial *d* has been shown to be sensitive to the induction of focal

stereotypy with high doses of amphetamine in mice (Ralph et al. 2001b). The observation of decreased entropy in DAT (–/–) mice given MDMA suggests that the mice are engaging in more perseverative patterns of locomotor activity, or locomotor stereotypy, which is qualitatively very different from the focal stereotypy induced by amphetamine.

In marked contrast to the MDMA-induced attenuation of the hyperactivity seen in the DAT (–/–) mice, MDMA potentiated other aspects of the behavioral profile characteristic of DAT-deficient mice. Specifically, MDMA enhanced the reduction in entropy and dramatically potentiated the decrease in time spent in the center area of the chamber in DAT (–/–) mice. Both MDMA and the reduction in the DAT gene led to striking increases in straight locomotor patterns as reflected in reductions in the spatial *d* measure. Hence, the absence of an interaction between MDMA and the DAT deletion on spatial *d* is likely due to a floor effect, as spatial *d* was already very low in the vehicle-treated DAT (–/–) mice (Fig. 2B). The findings with the entropy and center duration measures further support the observation that the diversity of different routes in the VT chamber, which is measured by the predictability of locomotor patterns, can be dissociated from changes in the level of locomotor activity. Thus, different behavioral components of the DAT (–/–) phenotype may represent distinct syndromes. Further support for this hypothesis comes from the recent finding that a D₁ but not a D₂ dopamine antagonist reduced the perseverative movement patterns in DAT (–/–) mice, even though both antagonists reduced the amount of locomotor activity (Ralph et al. 2001a). In some cases, however, drug treatments have similar effects on both the amount of hyperactivity and the altered patterns of activity in DAT-deficient mice. For example, the 5-HT_{2A} antagonist M100907 significantly reduces the amount of hyperactivity in the DAT (–/–) mice and also normalizes the abnormal pattern of locomotor activity in these mice, as confirmed by the spatial *d* measure (Barr et al. 2003). Similarly, the antimanic agent valproate normalizes both the hyperactivity and the perseverative patterns of locomotor activity seen in DAT knockdown mice lacking roughly 90% of the DAT protein (Ralph-Williams et al. 2003). Thus, the effects of MDMA observed here, like those of the DA D₂ antagonist raclopride (Ralph et al. 2001a), are important because they confirm that the different aspects of the abnormal locomotor behavior exhibited by DAT (–/–) mice can be independently manipulated by pharmacological treatments.

It is likely that multiple 5-HT receptors are involved in the behavioral effects of MDMA and in its complex effects on the locomotor abnormalities in DAT (–/–) mice. An important role for the 5-HT_{1B} receptor has been implicated in the locomotor-stimulating effects of MDMA because MDMA-induced hyperactivity in rats can be blocked with 5-HT_{1B} antagonists (Callaway et al. 1992) and mimicked by administration of 5-HT_{1B} agonists (e.g. Rempel et al. 1993). Similarly, the locomotor-

stimulating effects of MDMA are attenuated in 5-HT_{1B} receptor knockout mice and can be blocked by the 5-HT_{1B} antagonist GR127935 (Searce-Levie et al. 1999). At the same time, 5-HT_{2A} and 5-HT_{2C} receptors may have facilitatory and inhibitory influences, respectively, on locomotor activity after MDMA treatment (Fletcher et al. 2002). Indeed, the selective 5-HT_{2A} receptor antagonist M100907 markedly reduces both the hyperactivity and perseverative patterns of locomotion in DAT (–/–) mice (Barr et al. 2003). Future studies should further investigate the influences of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} agonists and antagonists on the perseverative locomotor patterns observed in DAT (–/–) mice or in mice treated with MDMA.

In conclusion, the effects of MDMA on DAT (–/–) mice are consistent with two different and apparently opposite mechanisms: first, serotonergic antagonism of hyperactivity; second, an exaggeration of alterations in the patterns of locomotor behavior in DAT (–/–) mice, as reflected in measures of perseveration and time spent in the center. These findings confirm and extend the hypothesis that different behavioral components of the DAT (–/–) phenotype may represent distinct and independently modifiable syndromes. Thus, the phenotype exhibited by the DAT (–/–) mice may in fact be multidimensional in nature, which could explain why different groups have suggested that this animal may represent a model for psychiatric disorders as divergent as ADHD, bipolar disorder, and schizophrenia. Future investigations will need to determine whether one can better dissect this phenotype and relate it more closely to dysfunctions of neurotransmitter systems and the different disorders that the DAT (–/–) mouse may model.

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