

---

# Valproate Attenuates Hyperactive and Perseverative Behaviors in Mutant Mice with a Dysregulated Dopamine System

Rebecca J. Ralph-Williams, Martin P. Paulus, Xiaoxi Zhuang, Rene Hen, and Mark A. Geyer

---

**Background:** Dopamine transporter (DAT) knockdown (KD) mice, with approximately 90% loss of expression of the DAT, allow for the examination of the behavioral consequences of a chronically dysregulated dopamine system. The DAT KD mice have hyperdopaminergic tone, are hyperactive, and show impaired response inhibition in a number of paradigms. We hypothesized that the DAT KD mice would also display deficits in prepulse inhibition (PPI) and would be perseverative in their locomotor behavior.

**Methods:** Basal levels of PPI and patterns of locomotor behavior were measured in two cohorts of DAT KD mice. In addition, measurements of locomotor behavior were recorded after pretreatment with 100 mg/kg valproate in both DAT KD and wildtype mice.

**Results:** The DAT KD mice were hyperactive and displayed perseverative motor behavior but had normal levels of PPI. The clinically effective antimania drug valproate significantly attenuated the hyperactivity and perseverative locomotor behavior in the DAT KD mice and had no effect in control mice.

**Conclusions:** The DAT KD mice appear to provide a model of some aspects of manic behavior. With limited models of bipolar disorder, the DAT KD mice might provide a vehicle to screen for new psychiatric therapies to treat mania and its related symptoms. *Biol Psychiatry* 2003;53:352–359 © 2003 Society of Biological Psychiatry

**Key Words:** Dopamine transporter, prepulse inhibition, hyperactivity, perseveration, valproate

## Introduction

Dysregulation of the dopamine (DA) system is thought to underlie several psychiatric disorders including schizophrenia, bipolar disorder, and attention-deficit/hyperactivity disorder (ADHD). Psychotic behavior, mania, and hyperactivity can be triggered in humans by dopaminergic stimulants such as amphetamine, suggesting that a DA-related mechanism may be involved in these behaviors. 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; Crowe and Vieland 1998; Fujiwara et al 1997; Gill et al 1997; Kelsoe 1999; Kelsoe et al 1996; Persico and Catalano 1998; Persico and Macciardi 1997). Animal model paradigms have been used to examine certain aspects of the neurobiological substrates of these disorders. In recent years, DA transporter (DAT) mutant mouse lines have been generated that may prove useful in behavioral studies related to these disease states.

A DAT “knockout” (KO) mouse has been created that completely lacks the gene coding for the DAT (Giros et al 1996). These mice exhibit a chronic hyperdopaminergic tone because, upon stimulation, DA remains in the extracellular space 300 times longer than it does in the wildtype (WT) control animals. In addition, these animals also have profound physiologic alterations (Gainetdinov et al 1998; Giros et al 1996). The DAT KO mutants are dramatically hyperactive in a novel environment, have impairments in spatial cognitive function, and exhibit locomotor hypoactivity in response to psychostimulants such as amphetamine and methylphenidate (Gainetdinov et al 1999). Recently, a second kind of DAT mutant mouse has been created. Unlike the full DAT KO, these “knockdown” (KD) mice have an approximately 90% loss of DAT (Zhuang et al 2001). The generation of the DAT knockdown has been described previously (Zhuang et al 2001). Briefly, the reduction in DAT expression is due to the

---

From the Alcohol and Drug Abuse Research Center, Harvard Medical School and McLean Hospital (RJR-W), Belmont, Massachusetts; Department of Psychiatry, University of California San Diego (MPP, MAG), La Jolla, California; Department of Neurobiology, Pharmacology, and Physiology, University of Chicago (XZ), Chicago, Illinois; Center for Neurobiology and Behavior, Columbia University (RH), New York, New York.

Address reprint requests to Mark A. Geyer, Ph.D., Department of Psychiatry, University of California, San Diego, 9500 Gilman Drive, La Jolla California 92093-0804.

Received March 27, 2002; revised June 12, 2002; accepted June 20, 2002.

insertion of an extra 4-kb DNA sequence into the second exon in the 5'-untranslated region of DAT, resulting in a reduction in gene expression efficiency while keeping the coding sequence intact. These mice also have a chronic hyperdopaminergic tone (e.g., a 70% increase in extracellular striatal DA), but they do not show gross physical changes. Behaviorally, the DAT KD mice are hyperactive in a novel open field and show impaired response inhibition in a number of paradigms (Zhuang et al 2001). The DAT KO and KD mutant mice have been proposed to model aspects of ADHD (Gainetdinov et al 1999; Zhuang et al 2001), but they may more generally model disease states characterized by a hyperdopaminergic tone. Hence, we set out to characterize further the DAT KD mice, mice that had shown no overt compensatory changes based on the DAT mutation, in both startle and locomotor behavioral paradigms.

Although no animal model can fully account for the complexity of psychiatric disorders, selected behaviors that are observed experimentally in humans have been reproduced in animal models. One such behavior is prepulse inhibition (PPI) of the startle response, a form of sensorimotor gating in which the startle response is reduced when the startling stimulus is preceded by a low intensity prepulse (Graham 1975; Hoffman and Ison 1980). Several psychiatric populations characterized by dysregulated DA systems have deficits in PPI, including schizophrenia, psychotic bipolar affective disorder, and ADHD comorbid with other disorders (Braff et al 1992; Castellanos et al 1996; Ormitz et al 1999; Perry et al 2001). Comparable deficits in PPI are produced in rodents by stimulating the DA system with agonists such as amphetamine and apomorphine (Dulawa and Geyer 1996; Mansbach et al 1988; Ralph et al 1999), suggesting that DA is involved in the modulation of PPI. We have previously described similar deficits in PPI in DAT KO mice, mice that were also hyperactive and highly perseverative in their motor behavior (Ralph et al 2001a). Based on the reported deficits in PPI in disorders such as bipolar disorder and ADHD, the evidence of DA-stimulated disruptions in PPI in mice, and the observed hyperactivity phenotype in the DAT KD mice, we hypothesized that the DAT KD mice would show deficits in PPI.

Locomotor hyperactivity has been used for many years as one aspect of modeling the mania phenotype in animals (for review, see Lyon 1991). Because the DAT KD mutant mice exhibit locomotor hyperactivity and because PPI deficits have been reported in patients with bipolar disorder (Perry et al 2001), we hypothesized that the hyperdopaminergic state of the DAT KD mice is consistent with a manic state in humans with bipolar disorder. One approach to address this hypothesis is to examine whether standard pharmacologic interventions to treat manic episodes in

bipolar disorder patients are also effective in reducing locomotor hyperactivity in these mice. Valproate is a standard treatment for manic and hypomanic episodes in subjects with bipolar disorder. Similar to the effects of lithium, valproate reduces hyperactivity, racing thoughts, and other key symptoms of mania within days of treatment (Motohashi 1999; Tohen and Grundy 1999). It has been suggested that the manic episodes during the course of bipolar disorder are consistent with a temporarily dysregulated DA system (Diehl and Gershon 1992; Emilien et al 1999). If indeed a dysregulated DA system underlies some of the key symptoms of mania, and if the DAT KD mice model a dysregulated DA system, we hypothesized that pharmacological agents that successfully treat manic symptoms would attenuate the hyperactivity displayed by the DAT KD mutant mice. Hence, we conducted a valproate dose-response study and observed that 100 mg/kg valproate had no effect on the overall levels of locomotor activity in control mice. We then treated the DAT KD mice with this dose of valproate in an attempt to attenuate their hyperactivity motor phenotype.

## Methods and Materials

### Animals

The DAT KD cohort (Zhuang et al 2001) used in the initial phenotypic characterization was sent to our laboratory from Columbia University (New York, NY;  $n = 12$  male WT and DAT KD). All subsequent mice were derived from breedings at the vivarium at the University of California, San Diego. The DAT KD mice were generated using embryonic stem cells from the 129SvJ strain and were inserted in C57BL/6J blastocyst cells; one of the chimeras was mated with 129SvJ females to generate heterozygous mutants on a 129SvJ genetic background (for details, see Zhuang et al 2001). Heterozygous breeding pairs were used to generate the mice and the DAT protein levels were quantified by western blot analysis (Zhuang et al 2001; performed by XZ at Columbia University). The DAT KD cohort used in the second baseline characterization consisted of 38 WT (female = 23, male = 15) and 28 DAT KD (female = 12, male = 16), whereas 31 WT (vehicle group = 16 [female = 10, male = 6] and valproate group = 15 [female = 8, male = 7]) and 27 DAT KD (vehicle group = 13 [female = 7, male = 6] and valproate group = 14 [female = 10, male = 4]) mice were used in the test with valproate. We used 40 male C57BL/6J mice to complete the valproate dose-response study ( $n = 10$  per treatment group). All of the behavioral testing procedures were approved by an institutional animal care and use committee (IACUC) before the onset of the experiments. All mice were maintained in an animal facility approved by the American Association for Accreditation of Laboratory Animal Care at the University of California, San Diego. This facility meets all federal and state requirements for animal care. Mice from each strain were group housed in a climate-controlled animal colony with a reversed day-night cycle (lights on at 9:00 PM, off at 9:00

AM). All behavioral testing started at approximately 8–9 weeks of age and occurred between 10:00 AM and 6:00 PM. Food (Harlan Teklab, Madison, WI) and water were available throughout the experiments, except during behavioral testing.

### Drugs

Sodium valproate was obtained from Sigma/RBI (St. Louis, MO) and was dissolved in distilled water. Free-base drug weights were used in all drug calculations. In the dose-response study, 100, 200, 400 mg/kg valproate or vehicle were used, whereas the DAT mutant mice were treated with either 100 mg/kg valproate or water. All injections were given intraperitoneally at a volume of 5 mL/kg body weight 1 hour before behavioral testing.

### Apparatus

Startle reactivity was measured using four startle chambers (SR-LAB, San Diego Instruments, San Diego, CA). Each chamber consisted of a clear nonrestrictive Plexiglas cylinder resting on a platform inside a ventilated box. A high-frequency loudspeaker inside the chamber produced both a continuous background noise of 65 dB and the various acoustic stimuli. Vibrations of the Plexiglas cylinder caused by the whole-body startle response of the animal were transduced into analog signals by a piezoelectric unit attached to the platform. These signals were then digitized and stored by a computer. Sixty-five readings were taken at 1-msec intervals, starting at stimulus onset, and the average amplitude was used to determine the acoustic startle response (ASR). Sound levels in dB(A) sound pressure level were measured as described previously (Dulawa et al 1997). The SR-LAB calibration unit was used routinely to ensure consistent stabilimeter sensitivity between test chambers and over time (Geyer and Swerdlow 1998).

Locomotor activity was measured using a video-tracker (VT), which tracked mice in four 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). Using the video-tracking system, a signal from each of four mice was generated simultaneously by tracking the uppermost left coordinate of each mouse in their respective enclosure. The signal was stored in a personal computer for further offline 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 spatiotemporal resolutions of each event recorded were 0.32 cm, 0.32 cm, and 55 msec, which corresponded to a maximum speed of 25 cm/sec.

### Prepulse Inhibition Session

All PPI test sessions consisted of startle trials (PULSE-ALONE), prepulse trials (PREPULSE + PULSE), and no-stimulus trials (NOSTIM). The PULSE-ALONE trial consisted of a 40-msec

120-dB pulse of broadband noise. Acoustic PPI was measured by PREPULSE + PULSE trials that consisted of a 20-msec noise prepulse, 100 msec delay, then a 40-msec 120-dB startle pulse (120 msec onset to onset interval). The acoustic prepulse intensities were 4, 8, and 16 dB above the 65-dB background noise (i.e., 69, 73, and 81 dB). The NOSTIM trial consisted of background noise only. The acoustic section of the test session began and ended with five presentations of the PULSE-ALONE trial; in between, each acoustic or NOSTIM trial type was presented 10 times in a pseudo-random order. There was an average of 15 sec (range: 12–30 sec) between trials. After the mice were placed in the startle chambers, a 65-dB background noise level was presented for a 5-min acclimation period and continued throughout the test session.

The amount of PPI was calculated as a percentage score for each prepulse trial type: % PPI = 100 - {[startle response for PREPULSE + PULSE] / (startle response for PULSE-ALONE)} × 100}. Acoustic startle magnitude was calculated as the average response to all of the PULSE-ALONE trials, excluding the first and last blocks of five PULSE-ALONE trials presented. For brevity, main effects of prepulse intensity (which were always significant) will not be discussed. Habituation of the startle response was analyzed by grouping acoustic startle trials into four blocks (five trials each, grouped by order of presentation). Data from the NOSTIM trials are not included in the Results section because the values were negligible relative to values on trials containing startle stimuli.

### Locomotor Pattern Testing

Each mouse was placed in the bottom left-hand corner of each enclosure at the start of the test session. The movements of the mice were tracked for 15-min in the DAT KD baseline characterization and data were stored in three 5-min blocks. During testing with valproate, the mice were tracked for 30 min, with data being stored in three 10-min blocks. Two categories of measures were obtained. First, the amount of locomotor activity was assessed as the number of entries made into predefined areas of the open field, that is, the wall area, corner area, or center area (see Geyer et al 1986). Second, the geometric patterns of locomotor activity were quantified by the spatial scaling exponent  $d$ , as described in detail elsewhere (Paulus et al 1999). Briefly,  $d$  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 ( $d \cong 2$ ).

### Statistical Analyses

In PPI experiments, genotype was a between-subjects variable, and prepulse intensity and time were within-subjects variables. In locomotor activity experiments, genotype, drug treatment, or both were between-subjects variables, and time was a within-subjects variable. Analyses of variance (ANOVAs) were used to compare means, and Tukey tests were used for post hoc analysis. Where applicable, gender was included as a between-subjects variable. Because there were neither main effects of gender nor interactions with gender, data from female and male mice were combined (data not shown). The computations were carried out

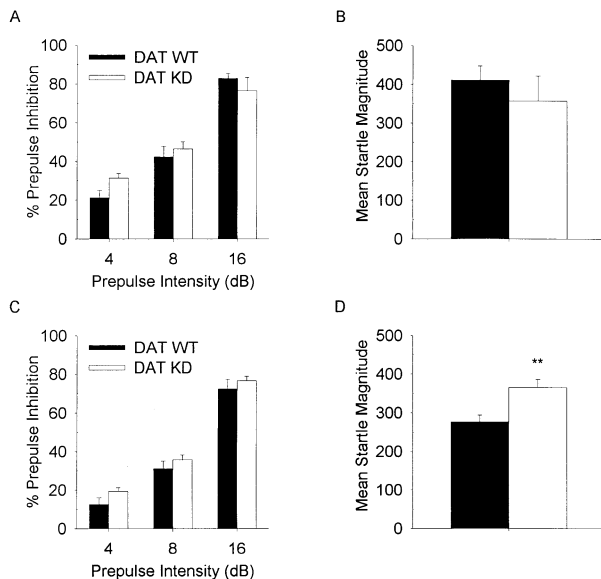


Figure 1. Characterization of prepulse inhibition (PPI) and acoustic startle reactivity in dopamine transporter (DAT) wild-type (WT) and knockdown (KD) mice. There were no significant differences in (A) PPI or (B) in acoustic startle responding between DAT WT and KD mice. Similarly, in a second cohort of DAT KD mice, (C) both WT and KD mice displayed comparable levels of PPI. (D) The DAT KD mice, however, had higher startle response than WT control mice (\*\* $p < .01$ ). Prepulse inhibition values represent mean percent PPI  $\pm$  SEM and startle values (arbitrary units) represent mean startle magnitude  $\pm$  SEM.

using the BMDP statistical software (Statistical Solutions, Saugus, MA).

## Results

### DAT Knockdown Baseline Characterization

The first group of DAT KD mice tested had comparable levels of PPI and acoustic startle reactivity compared with WT control mice (see Figure 1A and 1B). Similarly, testing of a second cohort also revealed no significant difference in PPI between DAT WT and KD mice, indicating that the lack of a PPI phenotype was reliable (see Figure 1C). There was, however, a significant main effect of genotype on acoustic startle responding only in the second cohort [ $F(1,64) = 11.4, p < .01$ ]. Specifically, DAT KD mice had higher acoustic startle reactivity than the WT control mice (see Figure 1D). Despite this difference in startle reactivity, both cohorts of DAT WT and KD mice showed normal habituation to the startle stimuli (data not shown).

Our locomotor studies confirmed the previous finding (Zhuang et al 2001) that DAT KD mice were hyperactive in an open field compared with WT controls. There was a

significant main effect of genotype [ $F(1,62) = 7.0, p < .01$ ] on the number of total entries, confirming that the DAT KD mice were more active than the WT controls. Post hoc comparisons revealed significant group differences in blocks 2 and 3 ( $p < .05$  and  $p < .01$ , respectively; see Figure 2A). In addition, the DAT KD mice exhibited more perseverative patterns of locomotor behavior than did the WT control mice, as evidenced by a significant main effect of genotype on spatial  $d$  [ $F(1,62) = 14.5, p < .001$ ; see Figure 2B]. Specifically, DAT KD mice exhibited significantly straighter sequences of motor patterns (lower spatial  $d$  values) than the WT mice ( $p < .01$ ). There were also significant main effects of block of time on both total entries [ $F(2,124) = 18.5, p < .001$ ] and spatial  $d$  [ $F(2,124) = 9.9, p < .001$ ], but there were no interactions with genotype. Thus, despite baseline differences, both groups of mice appeared to be habituating to the test environment because activity decreased and spatial  $d$  values increased over the test session in both genotypes.

### Valproate Dose Response

A valproate dose-response study was completed to determine an appropriate dose of drug to test in the DAT mice. There was a significant main effect of drug treatment on total entries [ $F(3,41) = 59.4, p < .001$ ], which post hoc comparisons revealed was because 400 mg/kg valproate significantly reduced locomotor activity ( $p < .01$ ; see Figure 3A). Also, 400 mg/kg produced more circumscribed patterns of motor behavior ( $p < .01$ ), as supported by a significant main effect of valproate on spatial  $d$

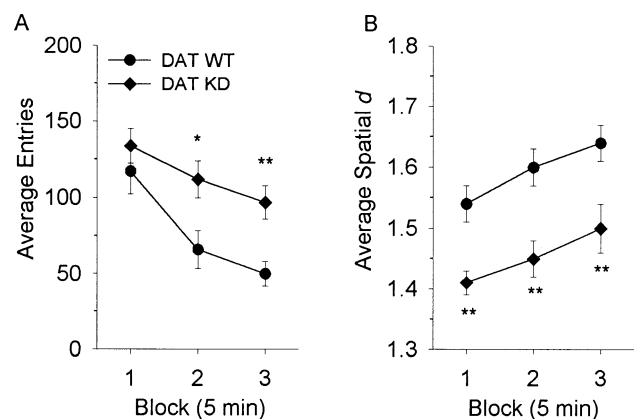


Figure 2. Baseline characterization of locomotor behavior in dopamine transporter knockdown (DAT KD) mice. (A) Similar to previous reports, the DAT KD mice were significantly more active than the DAT wildtype (WT) control mice in blocks 2 and 3. (B) The DAT KD mice displayed more perseverative motor patterns, as evidenced by significantly lower spatial  $d$  values in each block. Values represent means  $\pm$  SEM for three 5-min blocks (\* $p < .05$ , \*\* $p < .01$ ).

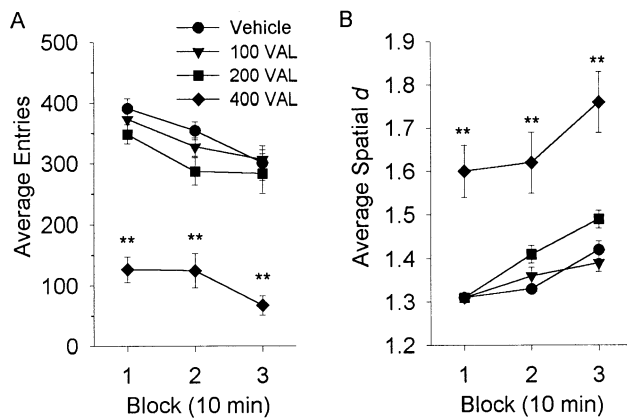


Figure 3. Valproate dose-response study in C57BL/6J mice. 400 mg/kg valproate significantly (A) reduced the amount of activity and (B) increased spatial  $d$  in each block of time (\*\* $p < .01$ ). Values represent means  $\pm$  SEM for three 10-min blocks.

[ $F(3,41) = 32.6, p < .001$ ; see Figure 3B]. It was clear that 400 mg/kg significantly altered both the amount and the patterns of motor behavior; however, at 200 mg/kg, valproate tended to decrease entries and increase spatial  $d$ . Thus, the 100 mg/kg dose of valproate, which had no apparent effects on either activity or spatial  $d$ , was chosen to test in both the DAT KD and KO mice

#### Valproate in DAT Knockdown Mice

When the DAT KD mice were treated with valproate, there were main effects of both genotype [ $F(1,54) = 33.4, p < .001$ ] and drug treatment [ $F(1,54) = 5.1, p < .05$ ] on total entries. Most important, a significant genotype-by-drug interaction [ $F(1,54) = 6.1, p < .05$ ] on total entries confirmed the hypothesis that valproate would block the hyperactivity seen in the DAT KD mice. As expected, the DAT KD mice were hyperactive compared with the WT mice when treated with vehicle ( $p < .01$ ). Treatment with valproate, however, reduced the hyperactivity in the DAT KD mice in blocks 1 and 2 ( $p < .01$  and  $p < .05$ , respectively), while having no effect in the WT control mice (see Figure 4A).

Vehicle-treated DAT WT mice exhibited a mixture of straight, meandering, and circumscribed movements, whereas the DAT KD mice were significantly more active and displayed straighter sequences of locomotor activity (see Figure 5). These differences were confirmed quantitatively using total entries and the spatial scaling exponent,  $d$ , which 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 2001a, 2001b). In measures of spatial  $d$ , there were main effects of genotype [ $F(1,54) = 26.5, p < .001$ ] and drug treatment [ $F(1,54) =$

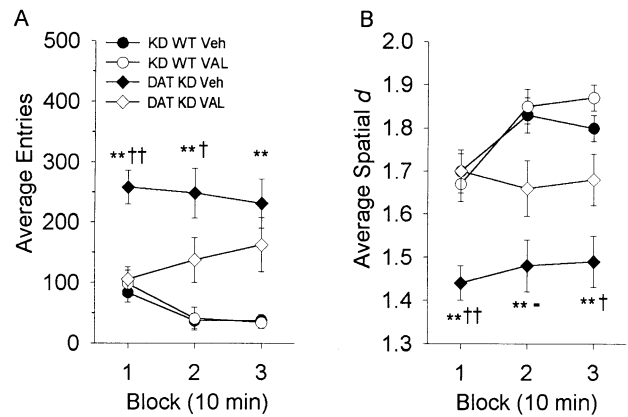


Figure 4. Characterization of locomotor behavior in dopamine transporter (DAT) wildtype (WT) and knockdown (KD) mice with 100 mg/kg valproate. (A) The DAT KD mice were hyperactive compared with the WT control mice. Treatment with valproate attenuated the hyperactive behavior of the DAT KD mice in blocks 1 and 2, while having no effect on the DAT WT mice. (B) DAT KD mice were more perseverative in the motor patterns than the DAT WT control mice. Valproate had no effect on DAT WT mice, but treatment with the drug significantly attenuated the DAT KD patterns of locomotor activity. Values represent means  $\pm$  SEM for three 10-min blocks (\* $p < .10$ , \*\* $p < .01$  compared with WT mice; † $p < .05$ , †† $p < .01$  compared with vehicle-treated mice).

8.3,  $p < .001$ ], as well as the critical genotype by drug interaction [ $F(1,54) = 5.3, p < .001$ ]. As in earlier studies, the DAT KD mice exhibited perseverative patterns of

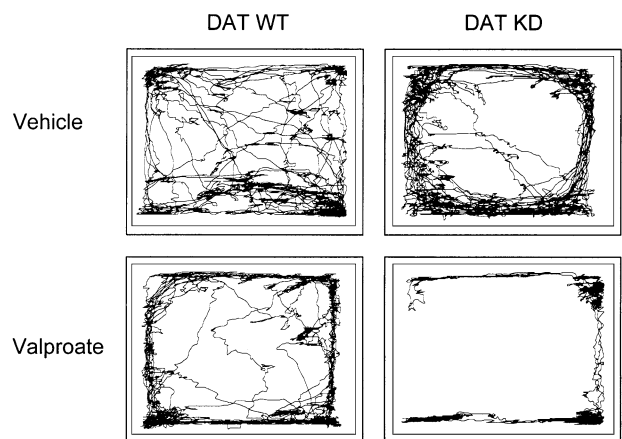


Figure 5. Patterns of motor behavior in individual dopamine transporter (DAT) wildtype (WT) and knockdown (KD) mice after treatment with either vehicle or 100 mg/kg valproate. Patterns were reconstructed using the (x,y,t) coordinates of one mouse per treatment group for the first 10 min of motor behavior sampled. Sample patterns were selected based on data closest to the group means for both the total amount of activity and average spatial  $d$  for each genotype and treatment.

motor activity (straighter patterns of locomotor behavior as indicated by lower spatial  $d$  values) than the KD WT control mice ( $p < .01$ ). Whereas drug treatment had no effect on spatial  $d$  in the WT control mice, valproate attenuated the perseverative patterns of motor behavior (diminished the predominance of straight sequences of locomotor activity as evidenced by increased spatial  $d$ ) seen in the DAT KD mice ( $p < .01$ ,  $p < .10$ , and  $p < .05$  in blocks 1, 2, and 3, respectively; see Figure 4B).

## Discussion

In this study, we examined the behavioral phenotypes of DAT KD mice and their role as possible models for a dysregulated DA system, a state that is thought to contribute to schizophrenia, bipolar disorder, and ADHD. We obtained four main results. Unlike the full DAT KO, DAT KD mice do not show deficits in PPI compared with WT controls. Similar to the DAT KO mice, however, the DAT KD mice are hyperactive and display perseverative motor patterns. Following a dose-response study, the DAT KD mice were treated with 100 mg/kg valproate. The drug significantly attenuated the hyperactivity in the DAT KD mice but had no effect on motor behavior in the WT controls. Finally, valproate also diminished the degree of perseverative straight locomotor patterns in DAT KD mice.

The DAT KD unexpectedly displayed normal levels of PPI compared with WT control mice, despite their hyperdopaminergic state. In contrast, when tested using the same procedures and parameters used here, the full DAT KO mice showed robust and replicable disruptions in PPI (Ralph et al 2001a). Perhaps the differences in the DAT phenotypes relate to the degree to which the DA systems are perturbed in the different DAT mutants. For example, the DAT KD mice might not have a large enough increase in dopaminergic tone to cause deficits in PPI. The full deletion of the DAT causes profound changes in the DA system of the DAT KO mice, with a fivefold increase in DA that persists 300 times longer in the cleft than in control mice (Gainetdinov et al 1998; Giros et al 1996). The DAT knockdown have a 70% increase in extracellular DA, and DA clears at a slower rate than control mice, but they have some transporter to clear the DA from the cleft (Zhuang et al 2001). Consequently, there appears to be enough increased DA tone to produce hyperactivity, but not enough to produce deficits in PPI. It is interesting that a small amount of DAT appears to be sufficient to protect against a PPI deficit. It has been reported that DAT plays a far more important role in the striatum than in the cortex (Tanda et al 1997; Wayment et al 2001; Yamamoto and Novotney 1998). One potential explanation for the phenotypic differences caused by 10% remaining DAT is that

the remaining low levels of DAT are sufficient for maintaining normal cortical but not striatal dopaminergic transmission. Another significant difference between the knockdown and knockout is that the knockout has severe growth retardation (Bosse et al 1997), which might affect general cognitive development. This difference in behavioral sensitivity to alterations in DA function is consistent with reports that higher doses of amphetamine, a drug that increases DA by blocking the DAT, are required to disrupt PPI than are required to produce hyperactivity in mice (Curzon and Decker 1998; Ralph et al 1999; Wenger 1989). Furthermore, DAT heterozygous (+/-) mice, in which the DA system is only partially dysregulated, also exhibit normal PPI (Ralph et al 2001a). The difference in the dysregulation of the DA system of the two DAT mutant strains may also represent different psychiatric disease states. There have been numerous reports of PPI deficits in schizophrenia patients (Braff et al 2001) and, more recently, in currently manic patients with psychotic bipolar affective disorder (Perry et al 2001); however, PPI deficits have not been found in subjects with ADHD except when it was comorbid with other disorders, such as nocturnal enuresis (Ornitz et al 1999) or a tic disorder (Castellanos et al 1996). Thus, the full DAT KO might be more representative of the dysregulated dopaminergic systems seen in schizophrenia, the manic phase of bipolar disorder, and ADHD comorbid with other disorders, whereas the DAT KD might be more analogous to ADHD alone.

As reported previously, the DAT mutant mice were hyperactive compared with their respective WT control mice in measures of the amount of activity (Zhuang et al 2001). In addition, the DAT KD mice also displayed differences in the sequential organization of their behavior. As we have shown previously (Ralph et al 2001a), the full DAT KO had highly perseverative motor patterns. Similarly, the DAT KD mice displayed perseverative motor patterns, moving in significantly straighter sequences than the KD WT mice. In line with these findings, straighter patterns of motor behavior are observed in mice treated with the indirect DA agonist amphetamine, although the effects are strain-dependent (Ralph et al 2001b). Therefore, it appears that increasing DA tone, via removal of the DAT or by related pharmacologic manipulations, leads to a narrowing of the behavioral repertoire of the mouse in which activity is increased in a pattern-specific manner. When the DAT KD mice were treated with the clinically effective antimania drug valproate, however, both their hyperactivity and their perseverative motor behavior were significantly attenuated. Thus, valproate, which reduces hyperactivity, racing thoughts, and other key symptoms of mania in subjects with bipolar disorder during manic episodes (Motohashi 1999; Tohen

and Grundy 1999), also had a normalizing effect in mice with a chronically dysregulated DA system. It has been suggested that the manic episodes during the course of bipolar disorder are consistent with a temporarily dysregulated DA system (Diehl and Gershon 1992; Emilien et al 1999), and there has been a great deal of investigation of the DAT in human gene linkage studies (Cook et al 1995; Crowe and Vieland 1998; Fujiwara et al 1997; Gill et al 1997; Kelsoe 1999; Kelsoe et al 1996; Persico and Catalano 1998; Persico and Macciardi 1997). Taken together with phenotypes reported here, the DAT KD mice may indeed emerge as a useful tool in investigating some disorders characterized with dysregulated DA systems, such as bipolar disorder and ADHD.

Although valproate has been proven to be a highly effective treatment for bipolar disorder, the mechanisms by which it exerts its therapeutic effects are not fully understood. Similarly unclear is the mechanism by which valproate attenuates the behavioral hyperactivity displayed by the DAT KD mice. Some of the reported effects of valproate include enhancing GABAergic inhibition, inhibiting high-frequency firing rates of neurons via voltage-dependent sodium channels, inhibiting the enzyme that converts inositol monophosphates to *myo*-inositol in the phosphoinositol cycle, and increasing glutamate release in the mouse cortex (for review, see Johannessen 2000; O'Donnell et al 2000). Thus, although our studies do not address this issue, one could speculate that the enhanced GABAergic inhibition produced by valproate “dampens” the hyperdopaminergic influences found in the DAT KD mice, resulting in a normalizing of locomotor hyperactivity and perseverative motor patterns; however, it is important to note that any of these processes, or combinations thereof, might be the mechanisms by which valproate exerts its behavioral effects in DAT KD mice.

Valproate clearly attenuates both amount of activity and the pattern of motor behavior in the DAT KD mice, but the effects of the drug on the habituation of the DAT KD mice to the open field remain unclear. In this study, we gave DAT KD mice an acute treatment of valproate with a 1-hour pretreatment time and monitored locomotor activity for 30 min. Although it is possible that valproate treatment reduces habituation in the DAT KD mice, it is equally plausible that the activity-attenuating effects of valproate are wearing off. Thus, further studies are warranted examining the time course of valproate treatment to provide more complete characterization of the effects of the drug in DAT KD mice.

The DAT KD mice provide an opportunity to investigate the behavioral consequences of chronically dysregulated DA systems. The DAT mutation results in hyperactivity and perseverative motor patterns. Valproate, a drug used routinely to treat idiopathic mania, attenuates the

disrupted behavior in the DAT KD mice. The behavioral deficits present in the DAT KD mice are seen in several psychiatric illnesses characterized by hyperdopaminergic states. Although others have suggested that the DAT KD mice provide a model of some aspects of ADHD (Zhuang et al 2001), they might also model some aspects of manic behavior. Thus, the DAT mutant mice could help us better understand the pathophysiology of psychiatric diseases characterized by dysregulated DA systems such as bipolar disorder and could provide a screen for new psychiatric therapies to treat such disorders.

---

These studies were supported by the Veterans Affairs VISN 22 Mental Illness Research, Education, and Clinical Center, and by the National Institute on Drug Abuse (Grant No. DA02925) and the National Institute of Mental Health (Grant No. F31-MH12806, MH61326, MH42228). M.A. Geyer holds an equity interest in San Diego Instruments. The authors thank Virginia Lehmann-Masten for her excellent technical assistance.

---

## References

- Bosse R, Fumagalli F, Jaber M, Giros B, Gainetdinov RR, Wetsel WC, et al (1997): Anterior pituitary hypoplasia and dwarfism in mice lacking the dopamine transporter. *Neuron* 19:127–138.
- Braff DL, Geyer MA, Swerdlow NR (2001): Human studies of prepulse inhibition of startle: Normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* 156:234–258.
- Braff DL, Grillon C, Geyer MA (1992): Gating and habituation of the startle reflex in schizophrenic patients. *Arch Gen Psychiatry* 49:206–215.
- Castellanos FX, Fine EJ, Kaysen D, Marsh WL, Rapoport JL, Hallett M (1996): Sensorimotor gating in boys with Tourette's syndrome and ADHD: Preliminary results. *Biol Psychiatry* 39:33–41.
- Cook EH Jr, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, Leventhal BL (1995): Association of attention-deficit disorder and the dopamine transporter gene. *Am J Hum Genet* 56:993–998.
- Crowe RR, Vieland V (1998): Chromosome 5 workshop. *Psychiatr Genet* 8:73–78.
- Curzon P, Decker MW (1998): Effects of phencyclidine (PCP) and (+)MK-801 on sensorimotor gating in CD-1 mice. *Prog Neuropsychopharmacol Biol Psychiatry* 22:129–146.
- Diehl DJ, Gershon S (1992): The role of dopamine in mood disorders. *Compr Psychiatry* 33:115–120.
- Dulawa SC, Geyer MA (1996): Psychopharmacology of prepulse inhibition in mice. *Chin J Physiol* 39:139–146.
- Dulawa SC, Hen R, Scarce-Lavie K, Geyer MA (1997): Serotonin1B receptor modulation of startle reactivity, habituation, and prepulse inhibition in wild-type and serotonin1B knockout mice. *Psychopharmacology (Berl)* 132:125–134.
- Emilien G, Maloteaux JM, Geurts M, Hoogenberg K, Cragg S (1999): Dopamine receptors—physiological understanding to

- therapeutic intervention potential. *Pharmacol Ther* 84:133–156.
- Fujiwara Y, Yamaguchi K, Tanaka Y, Tomita H, Shiro Y, Kashihara K, et al (1997): Polymorphism of dopamine receptors and transporter genes in neuropsychiatric diseases. *Eur Neurol* 38:6–10.
- Gainetdinov RR, Jones SR, Fumagalli F, Wightman RM, Caron MG (1998): Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis. *Brain Res Brain Res Rev* 26:148–153.
- Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG (1999): Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* 283:397–401.
- Geyer MA, Russo PV, Masten VL (1986): Multivariate assessment of locomotor behavior: Pharmacological and behavioral analyses. *Pharmacol Biochem Behav* 25:277–288.
- Geyer MA, Swerdlow NR (1998): Measurement of the startle response and its use in preclinical measures of prepulse inhibition and habituation. In Crawley JN, Skolnick P, editors. *Current Protocols in Neuroscience*, Vol 2. New York: John Wiley & Sons, 8.7.1–8.7.15.
- Gill M, Daly G, Heron S, Hawi Z, Fitzgerald M (1997): Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. *Mol Psychiatry* 2:311–313.
- Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996): Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 379:606–612.
- Graham FK (1975): Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology* 12:238–248.
- Hoffman HS, Ison JR (1980): Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev* 87:175–189.
- Johannessen CU (2000): Mechanisms of action of valproate: A commentary. *Neurochem Int* 37:103–110.
- Kelsoe JR (1999): Recent progress in the search for genes for bipolar disorder. *Curr Psychiatry Rep* 1:135–140.
- Kelsoe JR, Sadovnick AD, Kristbjarnarson H, Bergesch P, Mroczkowski-Parker Z, Drennan M, et al (1996): Possible locus for bipolar disorder near the dopamine transporter on chromosome 5. *Am J Med Genet* 67:533–540.
- Lyon M (1991): Animal models for the symptoms of mania. In Boulton AA, Baker GB, et al, editors. *Animal Models in Psychiatry*. Clifton, NJ: Humana Press, 197–244.
- Mansbach RS, Geyer MA, Braff DL (1988): Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology* 94:507–514.
- Motohashi N (1999): Algorithms for the pharmacotherapy of bipolar disorder. *Psychiatry Clin Neurosci* 53(suppl):S41–44.
- O'Donnell T, Rotzinger S, Nakashima TT, Hanstock CC, Ulrich M, Silverstone PH (2000): Chronic lithium and sodium valproate both decrease the concentration of myo-inositol and increase the concentration of inositol monophosphates in rat brain. *Brain Res* 880:84–91.
- Ornitz EM, Russell AT, Hanna GL, Gabikian P, Gehricke JG, Song D, Guthrie D (1999): Prepulse inhibition of startle and the neurobiology of primary nocturnal enuresis. *Biol Psychiatry* 45:1455–1466.
- Paulus MP, Dulawa SC, Ralph RJ, Mark AG (1999): Behavioral organization is independent of locomotor activity in 129 and C57 mouse strains. *Brain Res* 835:27–36.
- Paulus MP, Geyer MA (1991): A temporal and spatial scaling hypothesis for the behavioral effects of psychostimulants. *Psychopharmacology* 104:6–16.
- Perry W, Minassian A, Feifel D, Braff D (2001): Sensorimotor gating deficits in bipolar disorder patients with acute psychotic mania. *Biol Psychiatry* 50:418–424.
- Persico AM, Catalano M (1998): Lack of association between dopamine transporter gene polymorphisms and delusional disorder. *Am J Med Genet* 81:163–165.
- Persico AM, Macciardi F (1997): Genotypic association between dopamine transporter gene polymorphisms and schizophrenia. *Am J Med Genet* 74:53–57.
- Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA (2001a): Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: Differential effects of D1 and D2 receptor antagonists. *J Neurosci* 21:305–313.
- Ralph RJ, Paulus MP, Geyer MA (2001b): Strain-specific effects of amphetamine on prepulse inhibition and patterns of locomotor behavior in mice. *J Pharmacol Exp Ther* 298:148–155.
- Ralph RJ, Varty GB, Kelly MA, Wang YM, Caron MG, Rubinstein M, et al (1999): The dopamine D2, but not D3 or D4, receptor subtype is essential for the disruption of prepulse inhibition produced by amphetamine in mice. *J Neurosci* 19:4627–4633.
- Tanda G, Pontieri FE, Frau R, Di Chiara G (1997): Contribution of blockade of the noradrenaline carrier to the increase of extracellular dopamine in the rat prefrontal cortex by amphetamine and cocaine. *Eur J Neurosci* 9:2077–2085.
- Tohen M, Grundy S (1999): Management of acute mania. *J Clin Psychiatry* 60:31–34 discussion:35–36.
- Wayment HK, Schenk JO, Sorg BA (2001): Characterization of extracellular dopamine clearance in the medial prefrontal cortex: Role of monoamine uptake and monoamine oxidase inhibition. *J Neurosci* 21:35–44.
- Wenger GR (1989): The role of control activity levels in the reported strain differences to the behavioral effects of drugs in mice. *Pharmacol Biochem Behav* 32:241–247.
- Yamamoto BK, Novotney S (1998): Regulation of extracellular dopamine by the norepinephrine transporter. *J Neurochem* 71:274–280.
- Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, Hen R (2001): Hyperactivity and impaired response habituation in hyperdopaminergic mice. *Proc Natl Acad Sci U S A* 98:1982–1987.