

# Dopamine is not Required for the Hyperlocomotor Response to NMDA Receptor Antagonists

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N-methyl-D-aspartate (NMDA) receptor antagonists can elicit symptoms in humans that resemble those seen in schizophrenic patients. Rodents manifest locomotor and stereotypic behaviors when treated with NMDA receptor antagonists such as phencyclidine (PCP) or dizocilpine maleate (MK-801); these behaviors are usually associated with an activated dopamine system. However, recent evidence suggests that increased glutamatergic transmission mediates the effects of these NMDA receptor antagonists. The role of dopamine in PCP- and MK-801-induced behavior (eg hyperlocomotion) remains unclear. We used dopamine-deficient (DD) mice in which tyrosine hydroxylase is selectively inactivated in dopaminergic neurons to determine whether dopamine is required for the locomotor and molecular effects of PCP and MK-801. DD mice showed a similar increase in locomotor activity and *c-fos* mRNA induction in the striatum in response to these NMDA receptor antagonists as control mice. Restoration of dopamine signaling in DD mice enhanced their locomotor response to PCP and MK-801. Administration of LY379268, a group II metabotropic glutamate receptor agonist that inhibits glutamate release, blocked PCP- and MK-801-induced hyperlocomotion in both DD and control mice. These results suggest that glutamate, rather than dopamine, is required for the locomotor and molecular effects of NMDA receptor antagonists, but that glutamate and dopamine can act cooperatively.

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## INTRODUCTION

Antagonists of the N-methyl-D-aspartate (NMDA) receptor can produce psychotic symptoms in humans that are indistinguishable from acute episodes of schizophrenia (Snyder, 1976; Javitt and Zukin, 1991; Steinpreis and Salamone, 1993) and exacerbate existing psychoses in schizophrenics (Javitt and Zukin, 1991). As a result, glutamatergic dysfunction has been proposed as an underlying cause of the pathophysiology of schizophrenia (Kim *et al*, 1980; Javitt and Zukin, 1991; Olney and Farber, 1995; Coyle, 1996). Hence, NMDA receptor antagonists such as phencyclidine (PCP), dizocilpine maleate (MK-801), and ketamine have been used extensively to pharmacologically model psychosis in animals. Many of the behaviors produced in rodents treated with NMDA receptor antagonists have relevance to symptoms of schizophrenia in humans. These include disruptions in prepulse inhibition

and deficits in working memory (Bakshi *et al*, 1994; Verma and Moghaddam, 1996). Subanesthetic doses of drugs such as PCP and MK-801 also cause stereotyped motor behaviors and increased forward locomotion (Sturgeon *et al*, 1979; Moghaddam and Adams, 1998; Andine *et al*, 1999). Although positive symptoms of schizophrenia cannot be directly studied in animals, rodents exhibit substantial locomotor activation in response to NMDA receptor antagonists. As the neural circuitry mediating positive symptoms in humans and locomotor activation in rodents may be partly overlapping, we have focused on the locomotor-stimulating effects of NMDA receptor antagonists.

Motor behaviors are often associated with an activated dopamine system (Creese and Iversen, 1973; Kelly *et al*, 1975), and increased dopamine transmission is thought to be a major factor in the psychotomimetic actions of PCP, MK-801, and related drugs. Systemic administration of NMDA receptor antagonists results in an increase in both dopamine and glutamate efflux in limbic brain regions such as the nucleus accumbens (NAc) and prefrontal cortex, presumably through a disinhibitory mechanism (Imperato *et al*, 1990; Miller and Abercrombie, 1996; Adams and Moghaddam, 1998). Systemically administered PCP increases the firing rate of dopaminergic neurons (Freeman and Bunney, 1984; French *et al*, 1993), and dopamine

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receptor antagonists, including antipsychotic drugs such as haloperidol and clozapine, attenuate PCP-induced stereotypies and locomotion (Sturgeon *et al*, 1981; Freed *et al*, 1984). Finally, PCP-induced hyperlocomotion can be reversed by 6-hydroxydopamine (6-OHDA) lesions of the NAc (French and Vantini, 1984; French *et al*, 1985; Steinpreis and Salamone, 1993). Together these findings suggest that dopamine is necessary for the behavioral effects of NMDA receptor antagonists, and are consistent with a hyperdopaminergic hypothesis of schizophrenia (for review, see Davis *et al*, 1991).

However, there is also evidence that dopamine is not required for the actions of NMDA receptor antagonists, but that increases in dopamine levels may be an indirect consequence of activated glutamatergic transmission at non-NMDA receptors (Takahata and Moghaddam, 2003). In support of this, the behavioral effects of PCP correlate more closely with glutamate release in the NAc and prefrontal cortex than with dopamine release (Adams and Moghaddam, 1998). A systemically administered group II metabotropic glutamate receptor (mGlu 2/3) agonist, LY354740, prevents PCP-induced glutamate release in the prefrontal cortex and NAc without affecting dopamine release, and it reduces PCP-induced locomotor activity, stereotypy, and working memory deficits (Moghaddam and Adams, 1998). Some studies have shown that behavioral effects of PCP and MK-801, including locomotor stimulation and potentiation of cocaine reinforcement, can occur without concomitant increases in dopamine release (Druhan *et al*, 1996; Pierce *et al*, 1997; Cornish *et al*, 2001). Rats can be trained to self-administer both PCP and MK-801 in the presence of a dopamine antagonist, suggesting that the rewarding effects of these drugs are dopamine-independent (Carlezon and Wise, 1996). NMDA antagonists have also been shown to produce hyperlocomotion in mice and rats after temporary catecholamine depletion by reserpine (Carlsson and Carlsson, 1989a) or alpha-methyl-para-tyrosine (Lapin and Rogawski, 1995; Swanson and Schoepp, 2002). Taken together, the existing evidence leaves open the question of whether dopamine is required for the psychotomimetic behavioral effects of NMDA receptor antagonists. Most studies that demonstrate effects independent of dopamine are performed in animals with intact dopamine systems, making it difficult to conclude that dopamine is not involved. While lesioning experiments are compelling, they are hampered by the fact that complete lesions result in hypophagia and subsequent death (Ungerstedt, 1971). Incomplete lesions, coupled with resultant hypersensitivity to dopamine (Ungerstedt *et al*, 1974), have made it difficult to definitively rule out a contribution from dopamine.

Here, we use a mouse model in which mice are genetically depleted of dopamine (dopamine-deficient (DD) mice) to test the role of dopamine in the hyperlocomotive response to NMDA receptor antagonists. In DD mice, the gene encoding tyrosine hydroxylase is inactivated specifically in dopaminergic neurons (Zhou and Palmiter, 1995). DD mice are hypoactive and aphagic, and die 3–4 weeks postnatally if not rescued by daily injections of the synthetic dopamine precursor L-3,4-dihydroxyphenylalanine (L-dopa), which temporarily restores dopamine synthesis and regulated release within dopaminergic neurons (Zhou and Palmiter,

1995; Szczypka *et al*, 1999). Dopamine levels in the striatum of DD mice reach approximately 9% of wild-type levels 3 h after L-dopa treatment, but fall to <1% within 24 h (Szczypka *et al*, 1999). DD mice are supersensitive to dopamine, as treatment with the daily regimen of L-dopa causes hyperlocomotion and induces striatal *c-fos* expression—effects not seen in control mice (Kim *et al*, 2000). Using this model, along with pharmacological blockade of glutamate release, we were able to test whether dopaminergic and glutamatergic signaling are required for the locomotor-stimulating effects of PCP and MK-801. In addition, we were able to test the effects of PCP and MK-801 in DD mice after introduction of small amounts of releasable dopamine by pretreatment with a low dose of L-dopa. This allowed us to determine whether dopamine can modulate the locomotor response to NMDA antagonists, something that cannot be easily performed in other models of dopamine depletion.

## MATERIALS AND METHODS

### Generation and Maintenance of DD Mice

DD mice were created as described by inactivating both tyrosine hydroxylase (*Th*) alleles and subsequently targeting a *Th* gene to the dopamine  $\beta$  hydroxylase (*Dbh*) locus (Zhou and Palmiter, 1995). The *Th*<sup>-/-</sup>; *Dbh*<sup>Th/+</sup> pups representing DD mice were identified at 2–3 weeks of age by their runted appearance and behavioral responsiveness to L-dopa. The mice were maintained in a modified specific-pathogen-free facility. DD mice were kept alive by once daily injections of L-dopa (50 mg/kg/day). This dose of L-dopa elicits locomotor activity in DD mice that is similar in magnitude each day (Zhou and Palmiter, 1995; Kim *et al*, 2000). Control mice were littermates of DD mice that had at least one intact *Th* gene and one intact *Dbh* gene; these mice produce nearly normal levels of dopamine and norepinephrine (Thomas *et al*, 1998; Rios *et al*, 1999). L-Dopa treatment of control mice has no effect on locomotor activity, striatal dopamine levels, or Fos immunoreactivity (Szczypka *et al*, 1999; Kim *et al*, 2000), and was therefore not administered to control mice in these studies. Purina chow (5LJ5) and water were available *ad libitum* except during experimental procedures. All procedures were conducted in accordance with guidelines established by the National Institutes of Health and the University of Washington Animal Care Committee.

### Drugs

PCP (phencyclidine hydrochloride) (Sigma, St Louis, MO), MK-801 (dizocilpine hydrogen maleate) (Sigma), SCH 23390 hydrochloride (Sigma), haloperidol (Sigma), and LY 379268 (gift from Dr Darryl Schoepp, Eli Lilly Company) were dissolved in phosphate-buffered saline (PBS; 10 mM phosphate, 150 mM NaCl, pH 7.0). L-Dopa (1.5 mg/ml; Sigma) was dissolved in PBS containing 2.5 mg/ml ascorbic acid. Vehicle control was PBS alone. All treatments were by intraperitoneal (i.p.) injection in a volume of 10  $\mu$ l/g body weight, except L-dopa, which is injected at 33  $\mu$ l/g body weight.

## Behavioral Analysis

Locomotor activity was measured in transparent Plexiglas cages (40 × 20 × 20 cm) set in activity chambers equipped with four infrared beams set 8.8 cm apart (San Diego Instruments Inc., San Diego, CA). The number of consecutive beam breaks is reported as 'ambulations'. All animals were allowed to acclimate to the chambers for at least 12 h prior to beginning a test so that novelty of the testing apparatus was not a factor in the behavioral response to drug administration. Locomotor tests with DD mice were performed 18 h after their last L-dopa (50 mg/kg) injection. Each drug-treatment day was followed by at least a 3-day washout period, during which DD mice were treated only with L-dopa (50 mg/kg) and control mice were left untreated. Because of the limitations of breeding and genetics (2 out of every 11 mice born is DD), both DD mice and their littermate controls were exposed to multiple treatments. Mice were tested in behavioral experiments until one of the following criteria was reached: (1) the mice had received six drug treatments excluding daily L-dopa injections, (2) the age of the mice reached 10 months, or (3) the mice lost > 20% of their body weight or displayed other signs of illness. A total of 40 control and 35 DD mice were used in studies that involved PCP and a total of 37 control and 46 DD mice were used in studies that involved MK-801. On drug testing days, the treatments were randomized, and each mouse was, at some point in the study, treated with either PCP (5 mg/kg) or MK-801 (0.75 mg/kg). Because of our experimental design (mice were continuously added and removed), within-group comparisons using repeated measures were impossible. Therefore, in each figure,

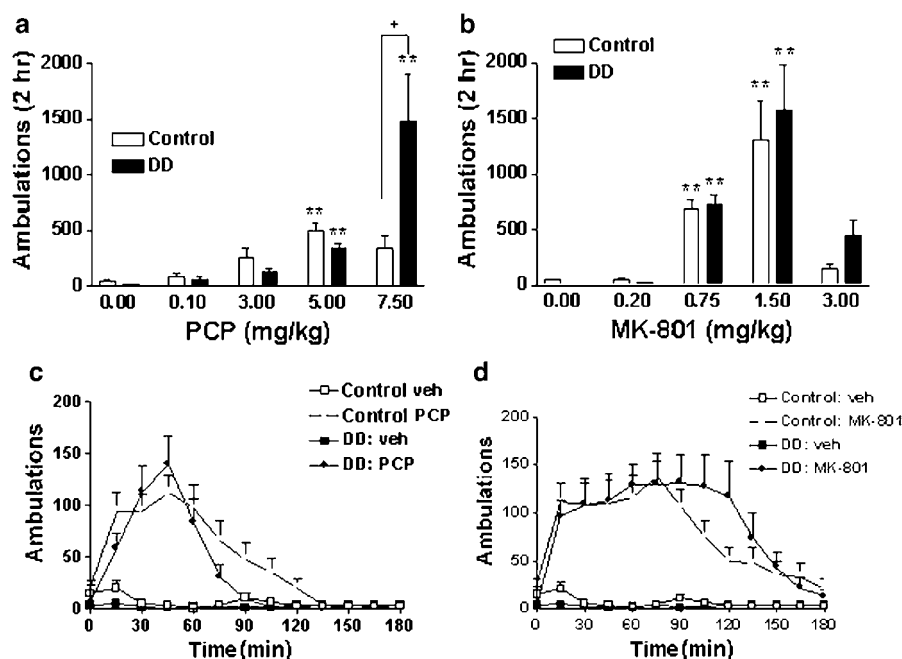
comparisons are made between data from mice treated with a drug of interest and pooled data from all mice treated with either vehicle, PCP (5 mg/kg), or MK-801 (0.75 mg/kg).

## In Situ Hybridization

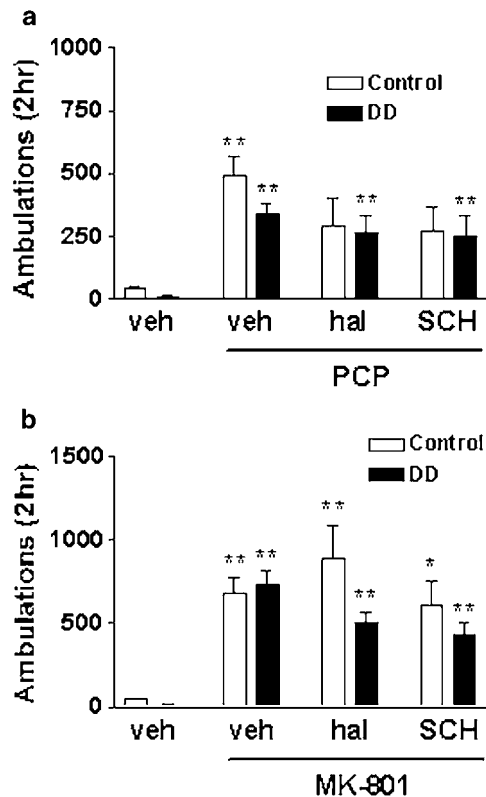
*In situ* hybridization was performed as described (Chartoff *et al*, 1999, 2001) with minor modifications. Briefly, mice were killed 1 h after treatment with vehicle, PCP, or MK-801, and the brains removed and frozen in isopentane on dry ice. Coronal sections (20 μm) were cut with a cryostat, thaw-mounted on Fisherbrand Superfrost microscope slides, and kept at -80°C until used in the hybridization assay. Tissue sections were fixed and subsequently hybridized with an <sup>35</sup>S-UTP-labeled *c-fos* antisense riboprobe transcribed with SP6 RNA polymerase from a linearized rat *c-fos* cDNA template. Tissue sections were hybridized overnight at 58°C and washed at 62°C. Final concentration of probe was 1.5 pmol probe/ml hybridization mix. After washing, slides were dehydrated through a graded alcohol series in which water was substituted by 0.6 M ammonium acetate and air-dried. For visualization of the *c-fos* signal, slides were subsequently dipped in NTB-2 emulsion (Eastman Kodak; diluted 1:1 with 0.6 M ammonium acetate), exposed for 4 weeks, developed, and counterstained with cresyl violet. Sense-strand controls for the *c-fos* riboprobe produced no detectable signal above background (Adams *et al*, 1997).

## Data Analysis

Data assessing pharmacological treatments on locomotor activity in control and DD mice (Figures 1a, b, 2, and 5) were



**Figure 1** PCP and MK-801 cause hyperlocomotion in both control and DD mice. (a and b) Forward locomotion was measured for 2 h in control and DD mice in response to increasing doses of PCP (a) and MK-801 (b). The number of animals in each group is as follows: PCP vehicle (0.0 mg/kg), control  $n = 26$ , DD  $n = 26$ ; PCP 0.1 mg/kg, control  $n = 24$ , DD  $n = 16$ ; PCP 3.0 mg/kg, control  $n = 16$ , DD  $n = 14$ ; PCP 5.0 mg/kg, control  $n = 40$ , DD  $n = 35$ ; PCP 7.5 mg/kg, control  $n = 8$ , DD  $n = 8$ ; MK-801 vehicle, control  $n = 26$ , DD  $n = 26$ ; MK-801 0.20 mg/kg, control  $n = 7$ , DD  $n = 7$ ; MK-801 0.75 mg/kg, control  $n = 37$ , DD  $n = 46$ ; MK-801 1.5 mg/kg, control  $n = 7$ , DD  $n = 7$ ; MK-801 3.0 mg/kg, control  $n = 8$ , DD  $n = 8$ . \*\* $P < 0.001$  compared to the 0.0 mg/kg dose of the corresponding genotype. + $P < 0.05$  comparing DD and control mice treated with PCP (7.5 mg/kg). (c and d) Time course (0–180 min) of locomotor effects of PCP (5.0 mg/kg) (control  $n = 26$ , DD  $n = 21$ ) (c) and MK-801 (0.75 mg/kg) (control  $n = 18$ , DD  $n = 20$ ) (d).



**Figure 2** Effect of dopamine receptor antagonists on NMDA receptor antagonist-induced hyperlocomotion. Haloperidol (hal, 0.5 mg/kg) and SCH 23390 (SCH, 0.2 mg/kg) were administered to control or DD mice 15 min prior to PCP (5 mg/kg) (a) or MK-801 (0.75 mg/kg) (b). The number of animals in each group is as follows: vehicle, control  $n = 26$ , DD  $n = 26$ ; PCP, control  $n = 40$ , DD  $n = 35$ ; hal + PCP, control  $n = 13$ , DD  $n = 18$ ; SCH + PCP, control  $n = 13$ , DD  $n = 17$ ; MK-801, control  $n = 37$ , DD  $n = 46$ ; hal + MK-801, control  $n = 22$ , DD  $n = 21$ ; SCH + MK-801, control  $n = 22$ , DD  $n = 21$ . \* $P < 0.008$ , \*\* $P < 0.0016$  compared to vehicle treatment of the corresponding genotype.

analyzed by two-way ANOVA (genotype  $\times$  treatment). Behavioral data presented in Figure 4 were analyzed by one-way ANOVA. Any significant main effects and interactions revealed in an ANOVA were further examined using the Bonferroni/Dunn test. Since the Bonferroni/Dunn test corrects for multiple comparisons, significance was set at  $P < \alpha/n$  for each experiment, where  $\alpha = 0.05$  and  $n =$  number of comparisons. Data from the *c-fos* *in situ* hybridization study (Table 1) were analyzed by three-way ANOVA (genotype  $\times$  treatment  $\times$  brain region) with repeated measures on brain region, followed by Newman-Keuls *post hoc* tests.

## RESULTS

### PCP and MK-801 Stimulate Locomotion in Both Control and DD Mice

To test whether dopamine is required for the locomotor response to NMDA receptor antagonists, both control and DD animals were injected with PCP (0, 0.1, 3.0, 5.0, and 7.5 mg/kg) or MK-801 (0.0, 0.2, 0.75, 1.5, and 3.0 mg/kg) and locomotor activity was measured for 2 h. The effects of PCP on locomotor activity were genotype- and dose-dependent

(genotype  $\times$  dose interaction:  $F(4,203) = 11.79$ ,  $P < 0.0001$ ) (Figure 1a). Compared to vehicle-treated mice of the corresponding genotype, 5.0 mg/kg PCP significantly increased locomotion in both control and DD mice. Locomotor activity in DD mice treated with PCP (7.5 mg/kg) was significantly greater than that of control mice treated with PCP (7.5 mg/kg). Control mice treated with 7.5 mg/kg PCP engaged primarily in stereotyped behaviors, such as grooming and rearing as has been documented previously (Sturgeon *et al*, 1979), while DD mice exhibited primarily locomotor activity. A higher dose of PCP (10.0 mg/kg) resulted in a stumbling gait and immobility and was therefore not tested further (data not shown). There was no statistically significant difference between control and DD mice at a dose of 3.0 mg/kg PCP. The effects of MK-801 on locomotor activity were dose-dependent (dose:  $F(4,169) = 31.83$ ,  $P < 0.0001$ ): 0.75 and 1.5 mg/kg MK-801 significantly increased locomotion in both control and DD mice compared to vehicle-treated mice of the corresponding genotype (Figure 1b). At 3.0 mg/kg MK-801, locomotor activity appeared uncoordinated and mice had difficulty changing direction in the corners of the chambers as has been noted previously (Carlsson and Carlsson, 1989b). There were no statistical differences between genotypes within each dose of MK-801. The time course of PCP (5 mg/kg)- and MK-801 (0.75 mg/kg)-induced locomotor activity was qualitatively similar between control and DD mice (Figure 1c and d).

### Effect of Dopamine Receptor Antagonists on PCP- and MK-801-Induced Hyperlocomotion

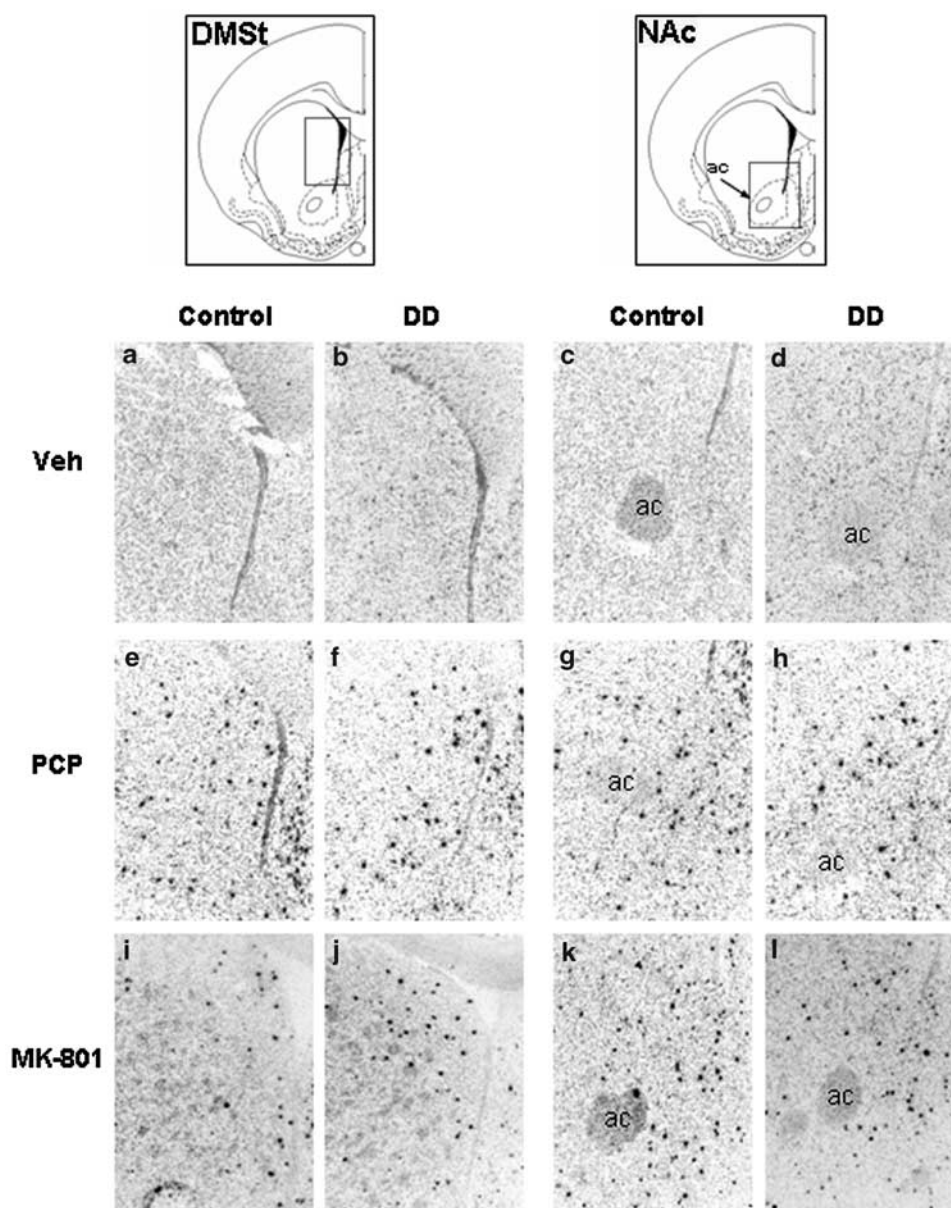
To confirm that the hyperlocomotor response of the DD mice to either PCP or MK-801 was not due to signaling through dopamine receptors, we administered either the dopamine D2 receptor antagonist haloperidol or the dopamine D1 receptor antagonist SCH 23390 to control and DD mice 15 min prior to treatment with NMDA receptor antagonists. The dose of haloperidol chosen (0.5 mg/kg) has been shown to block amphetamine-induced locomotion but not to induce cataleptic behavior in mice (Heusner *et al*, 2003). The dose of SCH 23390 chosen has been shown to block dopamine-induced behaviors and *c-fos* induction in DD mice (Chartoff *et al*, 2001). Locomotor activity in control and DD mice treated with PCP (5.0 mg/kg) was significantly increased compared to vehicle treatment (treatment:  $F(3,180) = 16.92$ ,  $P < 0.0001$ ) (Figure 2a). However, locomotor activity in control mice treated with either haloperidol (0.5 mg/kg) or SCH 23390 (0.2 mg/kg) prior to PCP was not significantly increased compared to vehicle. In contrast, locomotor activity in DD mice treated with haloperidol and PCP, or SCH 23390 and PCP was significantly increased compared to vehicle. Pretreatment of either control or DD mice with the dopamine receptor antagonists did not significantly attenuate PCP-induced locomotion. Locomotor activity in control and DD mice treated with MK-801 (0.75 mg/kg), haloperidol (0.5 mg/kg) and MK-801 (0.75 mg/kg), or SCH 23390 (0.2 mg/kg) and MK-801 was significantly increased compared to vehicle treatment of the corresponding genotype (treatment:  $F(3,213) = 20.02$ ,  $P < 0.0001$ ) (Figure 2b). Pretreatment of either control or DD mice with the dopamine

receptor antagonists did not significantly attenuate MK-801-induced locomotion.

### PCP and MK-801 Induce Striatal *c-fos* Expression

The immediate-early gene *c-fos* is an indicator of transcriptional activity, and PCP has previously been shown to increase striatal expression of *c-fos* in wild-type mice (Svenningsson *et al*, 2003). The striatum receives extensive dopaminergic input and is critical for the regulation of movement (Ungerstedt, 1971; Graybiel *et al*, 1990). *In situ* hybridization was used to assess *c-fos* mRNA in the striatum

of control and DD mice. A 1-h treatment with PCP at 5.0 mg/kg (Figure 3e–g) or MK-801 at 0.75 mg/kg (Figure 3i–l) induced *c-fos* mRNA in scattered cells of the dorsomedial striatum (DMSt) and NAc in both control and DD mice. The *c-fos*-positive cells in these brain regions were distinct and heavily labeled compared to vehicle-treated control sections (Figure 3a–d) that showed faint and sparse background levels of *c-fos* expression. The number of *c-fos*-positive cells was quantified in a subset of sections (see Table 1). Both PCP and MK-801 significantly increased *c-fos* in the two brain regions (treatment  $\times$  region interaction:  $F(2,12) = 4.22$ ,  $P < 0.05$ ). There was no difference



**Figure 3** PCP and MK-801 induce *c-fos* mRNA expression in the striatum and NAc of control and DD mice. Mice were injected with vehicle (veh), PCP (5 mg/kg), or MK-801 (0.75 mg/kg) and killed for *in situ* hybridization 60 min later. (a and c) Control mice, vehicle treatment, DMSt and NAc, respectively. (b and d) DD mice, vehicle treatment, DMSt and NAc. (e and g) Control mice, PCP treatment, DMSt and NAc. (f and h) DD mice, PCP treatment, DMSt and NAc. (i and k) Control mice, MK-801 treatment, DMSt and NAc. (j and l) DD mice, MK-801 treatment, DMSt and NAc. A similar induction of *c-fos*-positive cells (black) is visible for each treatment, regardless of genotype. Representative sections for each treatment and genotype are shown.

**Table 1** Quantification of *c-fos*-Positive Cells

	Control	DD	Control	DD
	Dorsomedial striatum	Dorsomedial striatum	Nucleus accumbens	Nucleus accumbens
Vehicle	15.8 ± 2.0	18.0 ± 2.0	23.2 ± 2.0	18.8 ± 1.2
PCP	68.2 ± 4.0**	81.7 ± 7.4**	70.5 ± 4.2**	80.8 ± 6.5**
MK-801	74.5 ± 3.5**	71.5 ± 4.4**	93.0 ± 8.2**	94.0 ± 2.8**

The number of cells that were positively labeled for *c-fos* was quantified in a subset of mice for the brain regions delineated in Figure 3. Each number represents the mean number of positive cells ± SEM for three mice, two sections per mouse. \*\* $P < 0.01$  compared to vehicle treatment of the corresponding genotype and brain region. No significant differences were found between PCP and MK-801 treatment, or between control and DD mice.

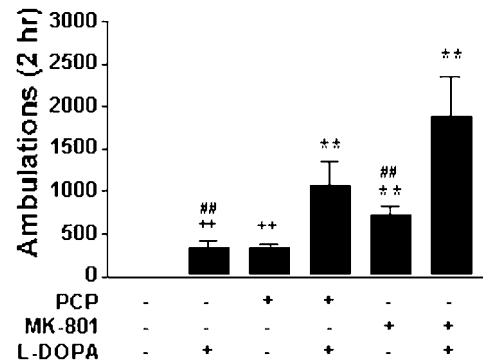
between genotypes within treatments or brain regions: (genotype × treatment:  $F(2,12) = 0.48$ , NS) and (genotype × region:  $F(1,12) = 0.85$ , NS). Induction of *c-fos* by PCP and MK-801 was limited to the DMSt and NAc and was largely absent from the dorsolateral striatum.

### NMDA Receptor Antagonists and L-Dopa Have an Additive Effect on Locomotor Activity in DD Mice

To test whether dopamine can modulate NMDA receptor antagonist-induced locomotor activity, a low dose of L-dopa (20 mg/kg) was administered to DD mice 15 min after either PCP or MK-801 and ambulations were recorded for 2 h. This dose of L-dopa has previously been shown to induce approximately 20% of the hyperlocomotor response observed with the daily dose of L-dopa (50 mg/kg) in DD mice (Szczytko *et al*, 1999). We chose this threshold dose because it does not maximize locomotor activity in DD mice and would allow the detection of potentiating effects of dopamine on the behaviors induced by NMDA receptor antagonists. There was a significant effect of treatment on locomotor activity ( $F(5,140) = 18.98$ ,  $P < 0.0001$ ) (Figure 4). L-Dopa (20 mg/kg) increased locomotor activity to a level qualitatively comparable to that of PCP (5.0 mg/kg), but neither drug caused statistically significant increases in locomotion compared to vehicle using the Bonferroni/Dunn test for multiple comparisons. Administration of L-dopa to PCP-treated mice increased the number of ambulations by a factor of 3. Administration of L-dopa to MK-801-treated (0.75 mg/kg) mice increased the number of ambulations slightly more than two-fold.

### An mGlu 2/3 Agonist Attenuates PCP- and MK-801-Induced Locomotion in Both Control and DD Mice

To determine whether glutamate release is required for NMDA antagonist-induced locomotion in DD mice, we administered LY379268 (10 mg/kg) 15 min prior to either PCP (5.0 mg/kg) or MK-801 (0.75 mg/kg) and recorded locomotor activity for 2 h. Treatment with LY379268 blocked both PCP- (treatment:  $F(1,88) = 15.75$ ,  $P < 0.0001$ ) and MK-801- (treatment:  $F(1,95) = 16.74$ ,  $P < 0.0001$ ) induced locomotion in control and DD mice (Figure 5a and b). LY379268 alone did not significantly affect basal locomotor activity (data not shown).

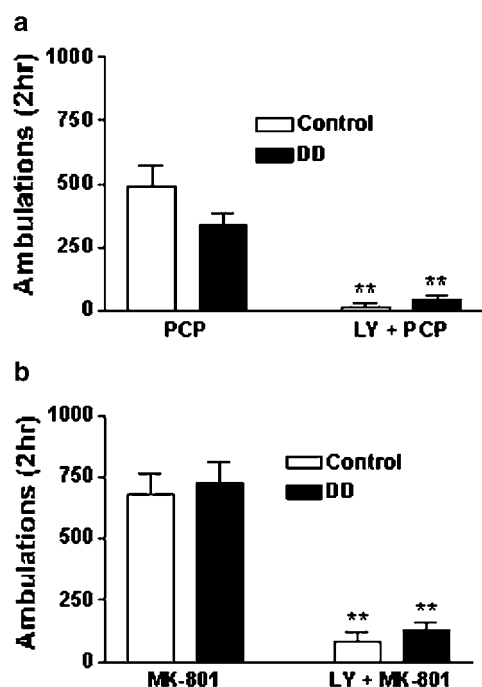


**Figure 4** L-Dopa potentiates NMDA receptor antagonist-induced hyperlocomotion. DD mice were treated with vehicle ( $n = 26$ ), L-dopa (20 mg/kg) ( $n = 20$ ), PCP (5 mg/kg) ( $n = 35$ ), MK-801 (0.75 mg/kg) ( $n = 46$ ), PCP and L-dopa ( $n = 12$ ), or MK-801 and L-dopa ( $n = 7$ ) and locomotor activity was measured for 2 h. PCP and MK-801 were administered 15 min prior to L-dopa. \*\* $P < 0.0006$  compared to vehicle; +  $P < 0.0006$  compared to PCP and L-dopa; ## $P < 0.0006$  compared to MK-801 and L-dopa.

## DISCUSSION

This study uses mice with a genetic deletion of tyrosine hydroxylase specifically in dopaminergic neurons to demonstrate that dopamine is not required for behavioral or transcriptional responses to PCP or MK-801. Furthermore, we provide evidence that glutamatergic neurotransmission is necessary for NMDA receptor antagonist-induced locomotor activity. Specifically, both PCP and MK-801 induced locomotor activity to a similar extent in control and DD mice. Also, neither haloperidol nor SCH 23390 significantly attenuated PCP- or MK-801-induced hyperlocomotion in either control or DD mice. Furthermore, PCP and MK-801 induced similar levels and expression patterns of *c-fos* mRNA in the DMSt and NAc of both control and DD mice. Finally, PCP- and MK-801-induced locomotion was blocked by LY379268, an mGlu 2/3 agonist that acts presynaptically to inhibit glutamate release.

Our data are consistent with studies that suggest that dopamine is not required for the effects of NMDA receptor antagonists. For example, NMDA receptor antagonists have been shown to increase locomotor activity in animals with temporary catecholamine depletion (Carlsson and Carlsson, 1989a; Lapin and Rogawski, 1995; Swanson and Schoepp, 2002). Also, the reinforcing and locomotor stimulatory



**Figure 5** The mGlu 2/3 agonist LY379268 attenuates PCP- and MK-801-induced hyperlocomotion in both control and DD mice. (a) DD and control mice were treated with PCP (5 mg/kg) or LY379268 (LY, 10 mg/kg) plus PCP and locomotor activity was measured for 2 h. (b) DD and control mice were treated with MK-801 (0.75 mg/kg) or LY379268 (LY, 10 mg/kg) plus MK-801 and locomotor activity was measured for 2 h. LY379268 was administered 15 min prior to PCP and MK-801. The number of animals in each group is as follows: PCP, control  $n=40$ , DD  $n=35$ ; LY+PCP, control  $n=8$ , DD  $n=9$ ; MK-801, control  $n=37$ , DD  $n=46$ ; LY+MK-801, control  $n=8$ , DD  $n=8$ . \*\* $P<0.01$  compared to PCP or MK-801 alone of the corresponding genotype.

effects of PCP and MK-801 have been dissociated from dopamine (Carlezon and Wise, 1996; Druhan *et al*, 1996; Pierce *et al*, 1997; Adams and Moghaddam, 1998). However, our findings contrast with some studies that demonstrate a requirement for dopamine in mediating the effects of NMDA receptor antagonists. For example, dopamine receptor antagonists have been shown to reduce the locomotor stimulatory effects of PCP and MK-801 (Sturgeon *et al*, 1981; Freed *et al*, 1984; Lapin and Rogawski, 1995). Also, 6-OHDA lesions can block NMDA receptor antagonist-induced behaviors (French and Vantini, 1984; French *et al*, 1985; Steinpreis and Salamone, 1993).

The discrepancies between the present data and previous studies with antagonists and pharmacological lesions may be explained by several inherent disadvantages of these methods. For example, 6-OHDA lesions are toxic to cells, and the percentage of dopaminergic neurons lesioned is variable and does not usually exceed 95%. Also, pharmacological agents are often nonspecific and affect neurotransmitter systems other than dopamine. In the context of these methodological issues, the variability in results found in many studies is understandable. A potential limitation of using DD mice is that neuroadaptive changes may occur throughout development to compensate for the chronic lack of tyrosine hydroxylase in dopaminergic neurons. Although

DD mice lack dopamine from embryogenesis onward, there are no differences in the levels of dopamine receptors or the dopamine transporter compared to control mice (Kim *et al*, 2000). The basic electrophysiological properties of dopaminergic neurons are also normal (Robinson *et al*, 2004). Likewise, light and electron microscopic analysis of midbrain dopaminergic neurons, their projections, and target neurons in the striatum of DD mice has not revealed any gross differences compared to control mice (Zhou and Palmiter, 1995; Kim *et al*, 2002). In addition, the glutamatergic input to the striatum appears to be normal in that glutamate labeling in synaptic vesicles is normal, extracellular glutamate is normal as measured by microdialysis, and the kinetics of synaptic vesicle release from corticostriatal projections is normal in slice preparations (Bamford *et al*, 2004). The only difference noted thus far is that signaling via either D1 or D2 receptors is greatly enhanced in DD mice compared to controls, such that lower levels of dopamine or dopamine receptor agonists elicit maximal molecular or behavioral effects in DD mice (Kim *et al*, 2000, 2002; Robinson *et al*, 2004). In fact, restoration of dopamine to normal levels in DD mice results in intense stereotypy (Chartoff *et al*, 2001). This hypersensitivity phenomenon is similar to that observed in animals lesioned with 6-OHDA (Zigmond and Stricker, 1984) and can be reversed in DD mice by chronic treatment with L-dopa (Kim *et al*, 2000). While it is impossible to conclude that DD mice are identical to control mice except for the lack of dopamine (and the hypersensitivity to dopamine signaling when dopamine is restored), the existing evidence suggests that DD mice provide a useful model of dopamine depletion that is not encumbered by other neuroadaptations.

In the present study, the locomotor response of DD and control mice to PCP and MK-801 was similar but not identical. Notably, the highest dose of PCP (7.5 mg/kg) elicited significantly more ambulations in DD than in control mice, and there was a similar trend for the highest dose of MK-801 (3.0 mg/kg). There is evidence for a behavioral supersensitivity to NMDA receptor antagonists in rats with neonatal 6-OHDA lesions of the NAC (Moy and Breese, 2002). Likewise, we observed that the control mice were less ambulatory because they were engaged in more stereotypic behaviors. Stereotypy and locomotion are thought to be competing motor activities that involve distinct mechanisms (Segal and Mandell, 1974), and increased striatal dopamine D1 receptor activation may be responsible for the emergence of stereotypies in control mice (Chartoff *et al*, 2001).

While we have used PCP and MK-801 to determine the role of dopamine in the locomotor response to NMDA receptor antagonists, these compounds are not identical. This is revealed in both the dose-response curve, where the response of DD and control mice to PCP is more disparate than the responses to MK-801, and in the fact that PCP-induced locomotion in control mice is more affected by dopamine receptor antagonists than MK-801-induced locomotion in control mice. MK-801 can elicit locomotor activity at doses that do not cause detectable increases in extracellular dopamine (Druhan *et al*, 1996), and PCP, but not MK-801, can act as a dopamine uptake inhibitor (Gerhardt *et al*, 1987). Therefore, it may be that dopamine

has more of a facilitatory role with PCP than with MK-801 in control mice.

The mechanisms responsible for NMDA receptor antagonist-induced locomotion and *c-fos* expression in the absence of dopamine are currently unknown. One possibility is that they lead to an increase in the activation of glutamatergic transmission at non-NMDA receptors, which in turn drives behavior and transcriptional responses. NMDA receptor antagonists have been shown to increase extracellular glutamate levels in both the prefrontal cortex and NAc (Adams and Moghaddam, 1998; Moghaddam and Adams, 1998). It has been proposed that this increase in glutamate may be due to an inhibition of tonic GABA inputs onto cortical pyramidal neurons (Grunze *et al*, 1996; Takahata and Moghaddam, 2003). The prefrontal cortex projects to the DMSt and NAc (Sesack *et al*, 1989), and increased glutamate release into these regions could stimulate non-NMDA receptors, causing cellular activation and locomotion. In such a model for behavioral activation by NMDA receptor antagonists, the release of dopamine is not required for evoking a locomotor response.

To test whether increased glutamate release was responsible for the locomotor activating effects of PCP and MK-801 in DD and control mice, we utilized the mGlu 2/3 agonist LY379268. mGlu 2/3 receptors are localized to corticostriatal axon terminals that synapse in the ventral striatum, and their activation causes long-term depression of excitatory synaptic transmission (Robbe *et al*, 2002). These receptors have also been localized to glial processes, but are absent from postsynaptic dendrites and neuronal cell bodies in the striatum (Jokel *et al*, 2001; Robbe *et al*, 2002). We found that LY379268 blocked PCP- and MK-801-induced locomotion in both control and DD mice. This is consistent with previous work showing that treatment of intact and monoamine-depleted rats with the mGlu 2/3 agonist LY379268 inhibits PCP-induced hyperlocomotion (Moghaddam and Adams, 1998; Swanson and Schoepp, 2002). Antagonists of mGlu 2/3 receptors have been shown to increase locomotor activity in mice (O'Neill *et al*, 2003). The effect of cotreatment of mGlu 2/3 antagonists and PCP is variable: one group found an increase in PCP-induced motor activity (Olszewski *et al*, 2004), yet another group found an incomplete effect on PCP self-administration (Winter *et al*, 2004).

Our results do not explicitly address what neural circuits are involved in the response of control and DD mice to NMDA receptor antagonists. A parsimonious suggestion is that the same corticostriatal circuits that respond to enhanced extracellular dopamine (eg in response to cocaine or amphetamine) can also respond to enhanced extracellular glutamate. However, we cannot rule out the possibility that glutamatergic activation of neural circuits either upstream or downstream of the corticostriatal input is also required for the locomotor response to NMDA receptor antagonists.

Further support for the suggestion that dopamine-responsive corticostriatal circuitry is critical for mediating the effects of NMDA receptor antagonists is our finding that temporary and partial restoration of dopamine synthesis by L-dopa treatment greatly potentiated PCP- and MK-801-induced hyperlocomotion. It has previously been shown by Greenberg and Segal (1985) that repeated daily injections of

amphetamine sensitize rats to the locomotor-stimulating effects of PCP. These authors suggest that the enhanced locomotor activity may be due to an increase in the PCP-stimulated release of mesolimbic dopamine. This is consistent with the idea that dopamine is not required for, but can contribute to, NMDA receptor antagonist-induced hyperlocomotion. It is unlikely that PCP- and MK-801-induced locomotion in DD mice that have not been treated with L-dopa for 18 h is due to a similar increase in dopamine because dopamine levels at this time point are barely detectable at <1% of control.

While glutamate and dopamine may normally act in concert within the striatum, particularly the NAc, to stimulate locomotion, our data suggest that enhanced glutamatergic signaling in corticostriatal circuitry in response to PCP and MK-801 may be sufficient to stimulate behaviors such as locomotion. This is consistent with previous findings (Adams and Moghaddam, 1998; Takahata and Moghaddam, 2003) that showed a temporal dissociation between dopamine release and the locomotor response to PCP and suggests that dopamine release may be an indirect effect of activated glutamate release. As such, the efficacy of dopamine receptor antagonists at blocking NMDA receptor antagonist-induced locomotion can be explained by the hypothesis that dopamine activation is downstream of glutamatergic activation of critical pathways, and blockade of dopamine receptors will impede activity. However, as dopamine receptor antagonists often have undesirable side effects, it appears more effective to target the critical glutamatergic signaling to inhibit behaviors induced by NMDA receptor antagonists. If NMDA receptor antagonist-induced hyperlocomotion is relevant to aspects of psychosis in humans, then our data along with others (Moghaddam and Adams, 1998; Swanson and Schoepp, 2002) underscore the importance of modulating glutamatergic, rather than dopaminergic, signaling when studying schizophrenia and related disorders in humans.

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