Genes, Brain and Behavior (2008) **7:** 568–577

Role of dopamine D1-like receptors in methamphetamine locomotor responses of D2 receptor knockout mice

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Received 20 November 2007, revised 29 January 2008, accepted for publication 13 February 2008

Behavioral sensitization to psychostimulants manifests as an increased locomotor response with repeated administration. Dopamine systems are accepted to play a fundamental role in sensitization, but the role of specific dopamine receptor subtypes has not been completely defined. This study used the combination of dopamine D2 receptor-deficient mice and a D1-like antagonist to examine dopamine D1 and D2 receptor involvement in acute and sensitized locomotor responses to methamphetamine. Absence of the dopamine D2 receptor resulted in attenuation of the acute stimulant effects of methamphetamine. Mutant and wild-type mice exhibited sensitization that lasted longer within the time period of the challenge test in the mutant animals. Pretreatment with the D1-like receptor antagonist SCH 23390 produced more potent reductions in the acute and sensitized locomotor responses to methamphetamine in D2 receptor-deficient mice than in wild-type mice; however, the expression of locomotor sensitization when challenged with methamphetamine alone was equivalently attenuated by previous treatment with SCH 23390. These data suggest that dopamine D2 receptors play a key role in the acute stimulant and sensitizing effects of methamphetamine and act in concert with D1-like receptors to influence the acquisition of methamphetamine-induced behavioral sensitization, traits that may influence continued methamphetamine use.

Keywords: Dopamine receptors, knockout, locomotor activity, neuroadaptation, null mutant, psychostimulant, sensitization

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The physical and psychological effects of methamphetamine are due, in part, to elevation of synaptic monoamine levels, resulting from the disruption of plasma membrane monoamine transporter function and induction of extravesicular release of stored monoamines (Fleckenstein *et al.* 2000; Kilty *et al.* 1991). Sensitivity to methamphetamine likely influences susceptibility to escalating drug use. In fact, the initial stimulant response to amphetamine has been found to predict the likelihood of further drug use (Gabbay 2003; de Wit *et al.* 1986). Although multiple neurochemical factors are known to influence responses to psychomotor stimulants (see recent review by Phillips *et al.* 2008), the complete abrogation of the behavioral activating effects of cocaine in dopamine D1 receptor-deficient mice (Xu *et al.* 1994a, 2000), and the exaggerated excitatory effect of cocaine and methamphetamine in dopamine D4 receptor-deficient mice (Rubinstein *et al.* 1997), show the importance of dopamine receptors (Neve *et al.* 2004) in mediating these responses.

Repeated amphetamine exposure induces neural changes that are detectable through behavioral and biochemical analyses. This 'sensitization' has been most often studied in rodents (Down & Eddy 1932; Pierce & Kalivas 1997), but also documented in humans (Boileau *et al.* 2006; Sax & Strakowski 2001) and may contribute to transitions in drug use from the controlled to compulsive patterns characteristic of addiction (Kalivas *et al.* 2005; Robinson & Berridge 1993; Ron & Jurd 2005). Disruption of sensitization to amphetamine with pharmacological antagonists shows the importance of dopamine receptors in this process and suggests the involvement of both families of receptor subtypes. D1-like receptor antagonists given systemically block both the acquisition and expression of locomotor sensitization to amphetamine (Hamamura *et al.* 1991; Karper *et al.* 2002; Kuczenski and Segal 1999; Ujike *et al.* 1989; Vezina, 1996; Vezina and Stewart, 1989), whereas D2-like receptor antagonists have been found to block the expression (Kuczenski and Segal 1999) and the acquisition in some (Hamamura *et al.* 1991; Ujike *et al.* 1989), but not other (Vezina 1996; Vezina and Stewart 1989) studies.

A complementary approach involves the use of gene deletion to examine the involvement of specific receptor subtypes. Variable methods and differences in genetic background may explain the apparent inconsistencies in results in D1 receptor-deficient mice for studies of the acute and

doi: 10.1111/j.1601-183X.2008.00392.x

sensitized responses to amphetamine (Crawford *et al.* 1997; Karper *et al.* 2002; McDougall *et al.* 2005; Xu *et al.* 2000), and no papers have reported locomotor effects of amphetamine in D2 receptor (D2R)-deficient mice. Glickstein and Schmauss (2004) reported a reduced magnitude of repeated methamphetamine-induced stereotypy in D2R-deficient mice. Therefore, reduced susceptibility to locomotor sensitization in the D2R-deficient mice might be predicted, although reduced susceptibility to stereotypy could lead to increased ability to exhibit locomotor sensitization. In addition, we studied the combination of D2R-deficiency and pharmacological antagonism to explore the role of dopamine D1-like receptors in the complete absence of D2 receptors. We predicted that the D1-like receptor antagonist would attenuate sensitization, but would perhaps have a more profound effect in D2R-deficient mice because of possible compensatory changes resulting in increased dependence upon D1-like receptors in the absence of D2. This approach complements the alternative approach of co-administration of two antagonist drugs and is powerful for identifying specific roles for each of the receptor subtypes.

Materials and methods

Animals

The generation and basic phenotypic analysis of the B6.129S2-*Drd2*$^{tm1/low}$ strain of D2R-deficient mice used in this study have been described previously (Kelly *et al.* 1997; 1998). Mice used here were the offspring of incipient congenic mice that had been backcrossed for five generations to the C57BL/6J (B6) strain. Ten- to 12-week-old littermates of both sexes, born to heterozygous breeder pairs, were used. Mice were group-housed (2–5 per cage) except during testing. Genotypes were determined by Southern blot analysis as described previously (Kelly *et al.* 1997). Studies were approved by the Institutional Animal Care and Use Committees of Oregon Health & Science University and the VA Medical Center and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

A total of 196 mice were studied (95 wild type and 101 D2R deficient), divided equally by sex. The large number of animals, experimental design and available equipment necessitated the consecutive testing of three cohorts of mice (69, 88 and 39 in cohorts 1, 2 and 3, respectively). Genotype, sex and treatment group were equated within each cohort. Results from the first cohort of mice led us to include two higher dose SCH 23390 groups (SCH 0.1 mg/kg and saline, SCH 0.1 mg/kg and methamphetamine) in cohorts 2 and 3.

Locomotor activity testing

Test duration on all days was 60 min, and data were collected in 5-min periods. There were 10 treatment groups per genotype, for a total of 20 groups (n = 9–11/subgroup). The activity apparatus (AccuScan Instruments, Inc., Columbus, OH, USA) and paradigm for sensitization have been described previously (Phillips *et al.* 1994), and the published procedures were followed with only slight modifications. On all test days, mice received two i.p. injections spaced 30-min apart. The injection time interval was chosen to allow a 30-min absorption period on days when the D1-like receptor antagonist SCH 23390 (Sigma/RBI, St Louis, MO, USA; 0.003, 0.01, 0.03 or 0.1 mg/kg prepared in 0.9% saline) was administered; the second injection was administered immediately before testing. Because SCH 23390 is an antagonist of both D1 and D5 dopamine receptors, we have characterized it as a D1-like receptor antagonist throughout this paper. To allow acclimation to the locomotor activity monitors and test procedures, and to obtain baseline activity data, two consecutive days of testing were conducted with saline injections. There were then 4 days spaced 48 h apart (days 3, 5, 7 and 9 of the experiment) on which saline or SCH 23390 was administered prior to saline or 2 mg/kg (+)-methamphetamine (Sigma, St Louis, MO, USA; prepared in 0.9% saline) to study the effects of SCH 23390 on basal activity and on the acquisition and expression of methamphetamine-induced sensitization. Two days after the last SCH 23390 treatment (day 11), all mice were tested after a challenge treatment with 2 mg/kg methamphetamine, that was preceded by a saline injection (no animals received SCH 23390 on the methamphetamine challenge day). The expression of a larger locomotor response to methamphetamine in methamphetamine pre-exposed mice on this test day would indicate that sensitization to methamphetamine had been acquired, and this test allowed us to determine whether prior SCH 23390 treatment affected this expression. On the final test day (day 12), mice were treated with two saline injections to assess the possibility of contextual sensitization. All mice were euthanized following locomotor testing on this day. SCH 23390 doses were chosen from previous studies and from initial responses in the current study that were effective in attenuating drug stimulant effects (Kuribara 1995; Kuribara & Uchihashi 1994; Shen *et al.* 1995). The dose of methamphetamine was chosen to induce acute stimulation and sensitization (Kamens *et al.* 2005; Phillips *et al.* 1994), but was well below doses known to induce stereotypic behaviors in mice (Atkins *et al.* 2001; Glickstein & Schmauss 2004; Karler *et al.* 1998; Yates *et al.* 2007).

Statistics

Data were analyzed initially by multifactor analysis of variance (ANOVA) (with repeated measures when appropriate) using the raw values for total horizontal distance traveled in 60 min (Statview 5.0.1; SAS Institute, Inc., Cary, NC, USA or Stastica; StatSoft, Inc., Tulsa, OK, USA). Complex interactions were further investigated with successive ANOVAs including fewer factors. The sources of two-way interactions were determined using simple main effect analyses. The Tukey highly significant difference (HSD) test was used for mean comparisons. To examine the effects of SCH 23390 alone and in combination with methamphetamine, sigmoidal dose–response curves were fitted for each of the four drug days during the acquisition of sensitization period, using non-linear regression analysis. Half-maximal inhibitory dose (ID_{50}) values ± 95% confidence intervals were then calculated for either SCH 23390 and saline or SCH 23390 and methamphetamine on each day with PRISM 3.0cx (GraphPad Software, Inc., San Diego, CA, USA). To combine data sets across all 4 days, the individual values were normalized relative to the respective groups' mean values for 60-min horizontal distance from saline day 2.

Results

Acute locomotor responses to methamphetamine are diminished in D2R-deficient mice

Only data from mice treated with saline and methamphetamine in the absence of SCH 23390 treatment are represented in Fig. 1. These data were analyzed separately from those for the SCH 23390 treatment groups to determine locomotor responses to acute and repeated methamphetamine. Repeated measures ANOVA identified a significant three-way interaction of genotype, drug and day ($F_{7,252}$ = 4.5, $P < 0.001$). The findings in animals treated with saline were similar to our previously reported results (Kelly *et al.* 1998), wherein D2R-deficient mice exhibited reduced locomotor activity (horizontal distance traveled) in comparison to their wild-type siblings. This conclusion was substantiated by a significant interaction of genotype and day within the repeated saline-treated groups ($F_{7,126} = 5.5$, $P < 0.001$), and simple main effect analyses that detected differences between the wild-type and D2R-null mice of this treatment group on all saline test days (open symbols in Fig. 1a).

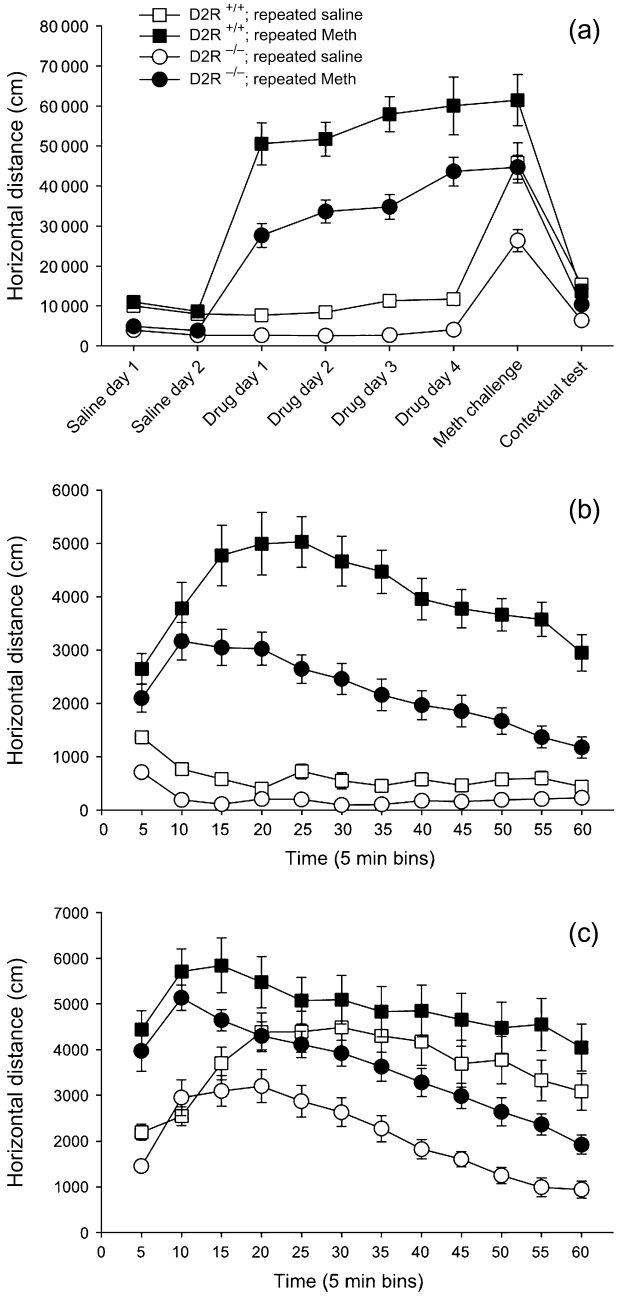

Figure 1: Locomotor activity of D2R-deficient and wild-type mice in response to single or repeated administration of methamphetamine. (a) Summary of the locomotor responses of $D2R^{+/+}$ and $D2R^{-/-}$ mice to repeated administration of saline or 2 mg/kg methamphetamine (Meth) followed by Meth and contextual-cue challenges during the 2-week experiment. The total horizontal distance traveled during each study day's 1-h test session is graphed; mean ± SEM; $n = 10$ per group. Repeated saline group mice received saline injections on all days except the Meth challenge day. Repeated Meth group mice received Meth injections on the drug days and Meth challenge day. See Materials and Methods for a more complete description of the experimental design and subgroups. (b) $D2R^{-/-}$ mice had a reduced excitomotor response when compared to $D2R^{+/+}$ mice after an initial injection of 2 mg/kg methamphetamine on drug day 1. The time–courses of the locomotor responses to saline and methamphetamine are shown in 5-min periods over a total of 1 h; mean ± SEM; $n = 10$ per group. (c) $D2R^{-/-}$ mice exhibited comparable, but more sustained, locomotor sensitization compared to $D2R^{+/+}$ mice on the Meth challenge day. The time–courses of the locomotor responses to methamphetamine are shown in 5 min periods over a total of 1 h; mean ± SEM; $n = 10$ per group.

The stimulant response of D2R-deficient mice upon initial exposure to methamphetamine was less robust and shorter lasting than that of wild-type controls (Fig. 1b). This conclusion was substantiated by the following statistical outcomes. First, there was a significant genotype by drug group interaction for total horizontal distance traveled, when data for drug day 1 (Fig. 1a) were compared for saline vs. methamphetamine groups ($F_{1,36} = 8.3$, $P < 0.01$). Both genotypes exhibited stimulation in response to methamphetamine, but the difference between genotypes was larger after methamphetamine treatment than after saline treatment. A second, time–course analysis more completely characterizes the acute methamphetamine response (Fig. 1b). Repeated measures ANOVA identified a significant genotype × drug × time (5-min segments) interaction ($F_{11,396} = 7.1$, $P < 0.001$) that was associated with an attenuated amplitude and duration of methamphetamine-induced locomotor stimulation in D2R-deficient compared with wild-type mice. Within each genotype, simple main effect analyses identified significant effects of time for the methamphetamine-treated mice (both $P < 0.01$). Locomotor values during the first 10 min of the test were similar for mutant and wild-type mice and showed a similar level of elevation above their respective saline-treated groups. However, in wild-type mice, locomotion continued to increase and remained significantly elevated above their initial 5-min response across minutes 10–50 (Tukey HSD test; all $P < 0.05$), whereas locomotion was elevated above the initial 5-min response for only minutes 10–20 in the D2R-null mice ($P < 0.05$).

D2 receptor-deficient mice express similar levels of sensitization across days, but sensitization to methamphetamine within the time period of the challenge test lasts longer

Both D2R-deficient mice and wild-type mice showed progressive locomotor sensitization to repeated methamphetamine across treatment days (Fig. 1a). There was no significant difference between genotypes in their magnitude of sensitization when data were accumulated across the entire 60-min test session, either when assessed by analysis of covariance with response on the first drug day serving as the measure of acute drug response ($F_{1,17} = 0.05$, $P = 0.82$ for the effect of genotype) or by a two-factor ANOVA ($F_{1,36} = 0.08$, $P = 0.77$ for the genotype by drug group interaction). However, to better characterize expression of the sensitized response, the locomotor time–course on the methamphetamine challenge day was examined (Fig. 1c). There was

a three-way interaction of genotype, drug and time ($F_{11,396} = 2.2$, $P < 0.05$). Independent ANOVAs showed significant drug (saline vs. methamphetamine) × time interactions for both D2R-null ($F_{11,198} = 3.3$, $P < 0.001$) and wild-type mice ($F_{11,198} = 8.2$, $P < 0.001$). Again, as seen in Fig. 1b, activation was sustained for longer in the wild-type mice compared with the D2R-null mice, regardless of whether they were receiving methamphetamine for the first or fifth time. However, when the acute and repeated treatment groups were compared within genotype, sensitization within the 60-min challenge test lasted longer in the D2R-null mice, with significant differences between the acute and repeated methamphetamine treatment groups at all 5-min time periods throughout the 60-min session in these mice and for only the first three 5-min periods for the wild-type mice.

Analysis of time–course data for mice challenged with saline on the contextual test day (not shown), showed no significant interaction of genotype, prior drug treatment and time ($F_{11,396} = 0.95$, $P = 0.49$); however, there was a genotype × treatment interaction for the total locomotion exhibited during the 60-min time period ($F_{1,36} = 4.9$, $P < 0.05$). Simple main effects analyses indicated some contextual conditioning in the D2R-null mice (scores were greater in mice that had received repeated treatments with methamphetamine in this context than in mice that had received mostly saline), but not in the wild-type mice.

D2 receptor-deficient and wild-type mice have comparable responses to D1-like receptor antagonism in the absence of methamphetamine treatment

Figure 2a summarizes the locomotor response data for D2R-deficient and wild-type mice after acute and repeated treatment with SCH 23390 in the absence of methamphetamine on the first (day 1) and final (day 4) drug days during the acquisition phase. Data for days 2 and 3 were intermediate to those for days 1 and 4 and are not shown in Fig. 2a for clarity. A repeated measures ANOVA (SCH 23390 dose × genotype × day) for the 60-min time period on all four drug-treatment days showed significant main effects of genotype ($F_{1,87} = 92.0$, $P < 0.0001$), SCH 23390 dose ($F_{4,87} = 14.8$, $P < 0.0001$) and day ($F_{3,261} = 24.1$, $P < 0.0001$). There was also a significant interaction of genotype and day ($F_{3,261} = 9.9$, $P < 0.0001$) and of genotype and SCH dose ($F_{4,87} = 3.7$, $P < 0.01$). The source of the genotype × day interaction was greater locomotor activity in wild-type compared with D2R-null mice on the final SCH 23390 test day (day 4, Fig. 2a). The source of the genotype × SCH dose interaction was the relatively low activity levels only in the D2R-null group treated with saline (0 mg/kg SCH dose).

Because of the significant baseline differences in locomotor activity between wild-type and D2R-null mice, we normalized the SCH 23390 dose–response data to 100% of activity on saline day 2 for each treatment group, collapsed across all four drug-treatment days. This permitted comparison of the shapes of the dose–response curves (Fig. 2b). The Hill slopes of the sigmoidal dose–response curves were held constant at −1.5. The calculated ID_{50} values and 95% confidence intervals for SCH 23390 from these functions were 0.053 mg/kg (0.032–0.090) for wild-type mice and 0.040 mg/kg (0.024–0.068) for D2R-null mice, which were not significantly different

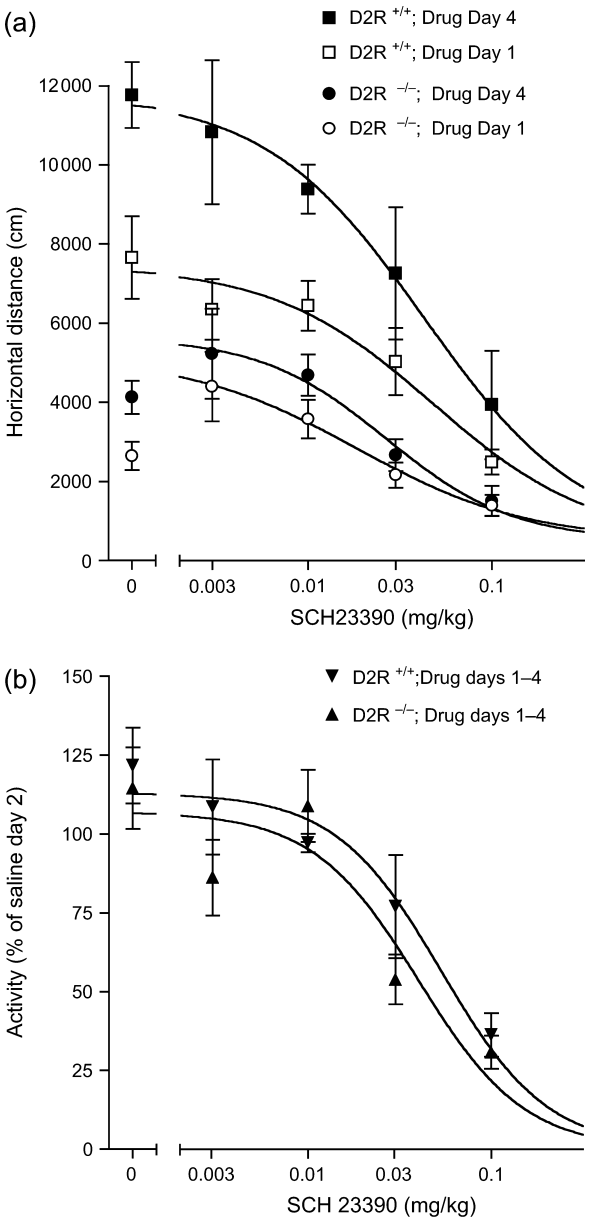

Figure 2: The D1R antagonist SCH 23390 inhibited basal locomotor activity in D2R-deficient and wild-type mice. (a) Dose–response curves for horizontal distance traveled in 60 min from drug days 1 and 4 in groups of mice injected with the indicated doses of SCH 23390 followed by saline; mean ± SEM, $n = 9$ to 11 per group. The non-linear regression curves for the $D2R^{-/-}$ mice only excluded the 0 mg/kg dose to allow convergence of the data. (b) Dose–response curves generated with the combined data from drug days 1 to 4 after normalization of all points to 100% of the mean locomotor activity on saline day 2 for the respective groups; mean ± SEM, $n = 9$ to 11 per group.

from each other ($t_{93} = 0.77$, $P = 0.44$, two-tailed t-test). Similar ID_{50} values were obtained for each genotype from the daily regression curves based on the raw activity data in Fig. 2a, validating our secondary analysis of the data normalized to percentage of saline day 2. Overall, these results show that despite the baseline hypoactivity in D2R-null mice, both genotypes responded with similar sensitivity to acute blockade of the D1-like receptor by SCH 23390 in the absence of methamphetamine.

D2 receptor-deficient mice are more dependent on D1-like receptor activation for their excitomotor responses to methamphetamine compared to wild-type controls

Figure 3a summarizes the locomotor responses of D2R-deficient and wild-type mice after acute and repeated treatment with SCH 23390 in the presence of methamphetamine on the first (day 1) and final (day 4) drug days during the acquisition period. A repeated measures ANOVA of activity across all four drug-treatment days showed significant main effects of genotype ($F_{1,89} = 88.2$, $P < 0.0001$), dose of SCH 23390 ($F_{4,89} = 29.5$, $P < 0.0001$) and day ($F_{3,267} = 44.8$, $P < 0.0001$). There were also significant interactions of genotype and SCH 23390 dose ($F_{4,89} = 3.1$, $P < 0.02$), genotype and day ($F_{3,267} = 2.7$, $P < 0.05$), and SCH 23390 dose and day ($F_{12,267} = 1.8$, $P < 0.05$), but no significant three-way interaction ($F_{12,267} = 1.2$, $P = 0.28$). The absolute levels of activity were lower in D2R-null vs. wild-type mice, consistent with their difference in basal activity level. However, both genotypes exhibited significant induction of sensitization from day 1 to day 4 (follow-up ANOVAs on the individual genotypes: $F_{3,129} = 22.8$, $P < 0.0001$ for wild-type mice; $F_{3,138} = 24.1$ and $P < 0.0001$ for D2R-null mice).

Data were normalized relative to 100% of activity for each respective treatment group on saline day 2 and collapsed across all four drug days to directly compare the shapes of the dose–response curves (Fig. 3b). In contrast to the similar potency of SCH 23390 to decrease basal locomotor activity between genotypes (shown in Fig. 2b), the dose–response curve for SCH 23390 plus methamphetamine was shifted significantly to the left in the D2R-deficient mice, relative to the wild-type mice. ID_{50} values and 95% confidence intervals for SCH 23390 indicated that its potency was increased approximately threefold in the D2R-null mice [0.010 mg/kg (0.006–0.016)] compared with wild-type mice [0.031 mg/kg (0.019–0.049)] ($t_{95} = 3.5$; $P < 0.001$, two-tailed t-test). In other words, SCH 23390 attenuated methamphetamine-induced activation to a greater extent at lower doses in D2R-deficient than in wild-type mice. The same relative shift in dose–response curves between genotypes was observed for all four individual drug days using either the raw locomotor data for horizontal distance traveled or data normalized as a percentage of saline day 2 (not shown).

The curves shown in Fig. 3a were used to calculate the dose of SCH 23390 that reduced locomotor activity on day 4 to the equivalent of the B_{max} acute response to methamphetamine on day 1 for each genotype. This analysis yielded values of approximately 0.008 mg/kg for D2R-deficient mice and 0.03 mg/kg for wild-type mice. The approximately threefold greater dose in wild-type mice closely mirrors the difference in SCH 23390 potency from the calculated ID_{50} values to reduce the excitomotor response to methamphetamine when data were collapsed across the four drug days (Fig. 3b).

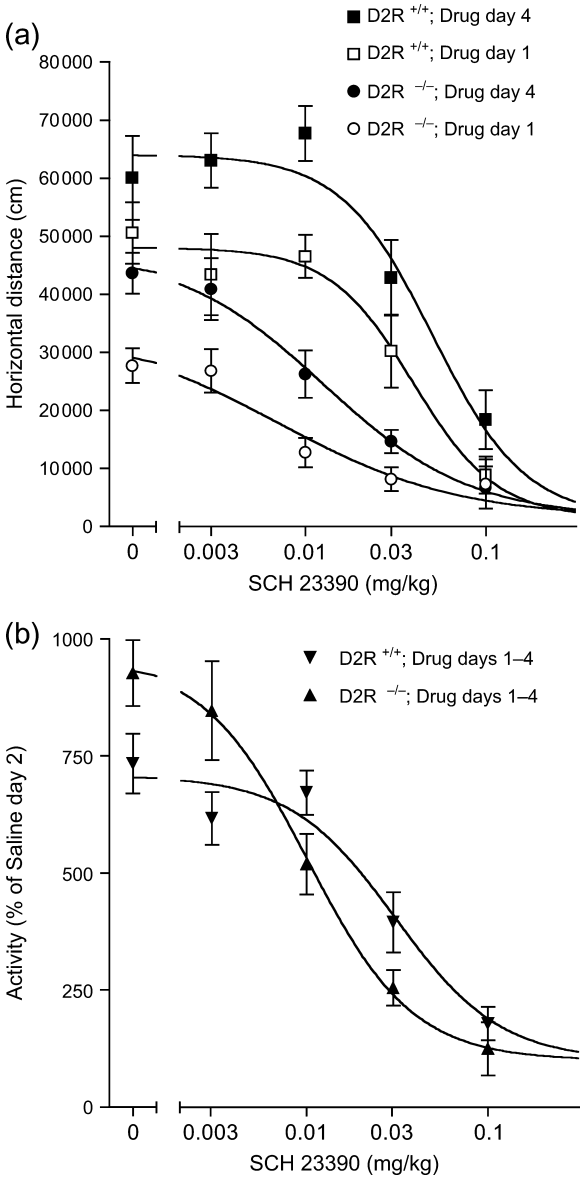

Figure 3: The D1R antagonist SCH 23390 inhibited methamphetamine-stimulated locomotor activity in D2R-deficient and wild-type mice. (a) Dose–response curves for horizontal distance traveled in 60 min on drug days 1 and 4 in groups of mice injected with the indicated doses of SCH23390 followed by 2 mg/kg methamphetamine; mean ± SEM, $n = 8$ to 11 per group. (b) Dose–response curves generated with the combined data from drug days 1 to 4 after normalization of all points to 100% of the mean locomotor activity on saline day 2 for the respective groups; mean ± SEM, $n = 8$ to 11 per group. The curve was shifted significantly to the left in D2R-deficient mice.

D1-like receptor antagonist pretreatment blocks the subsequent expression of methamphetamine sensitization in both D2R-deficient and wild-type mice

Locomotor activity on the methamphetamine challenge day was compared among all treatment groups, with genotype included as a factor. ANOVA showed significant main effects of genotype ($F_{1,175} = 122.9$, $P < 0.0001$) and treatment group ($F_{9,175} = 5.2$, $P < 0.0001$) but no significant interaction between the two factors. Fig. 4a and 4b summarize the data separated by genotype and for clarity, only the highest dose SCH 23390 groups (SCH 23390 0.1 mg/kg alone or SCH 23390 0.1 mg/kg and methamphetamine). Pretreatment with this dose of the D1-like receptor antagonist during repeated methamphetamine administration blocked the expression of methamphetamine sensitization in both wild-type mice and D2R-deficient mice (Tukey HSD; $P < 0.05$, Sal/Meth compared to SCH 0.1/Meth). This effect was specific for sensitization because repeated administration of high dose SCH 23390 without methamphetamine did not prevent a robust acute locomotor response to methamphetamine on the challenge day (Sal/Sal compared to SCH 0.1/Sal; also compare activity levels in Fig. 4 panels a and b with those in panels c and d).

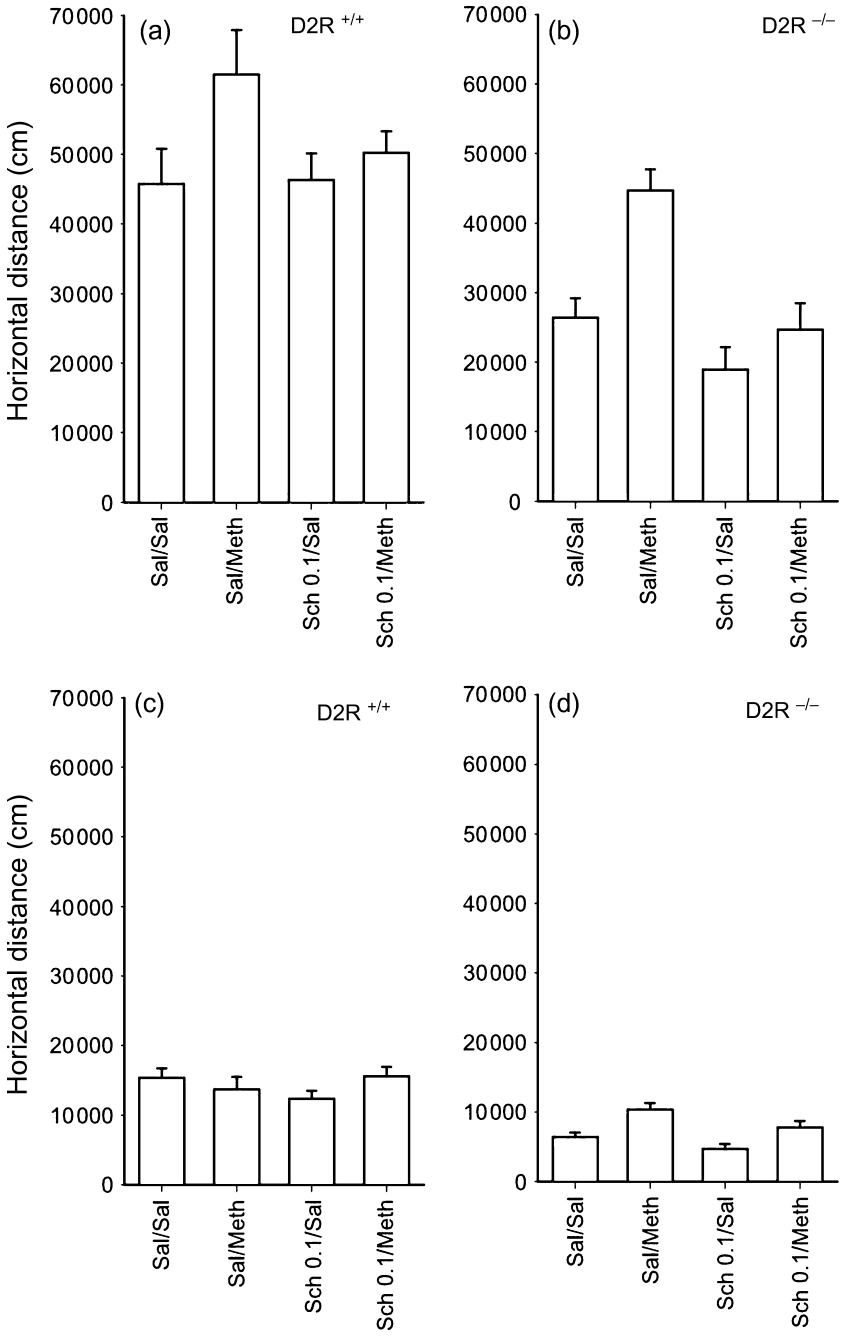

Figure 4: The D1R antagonist SCH 23390 blocked the expression of methamphetamine (Meth) sensitization in D2R-deficient and wild-type mice. Total horizontal distance traveled in 60 min is shown for the Saline (Sal)/Sal Sal/Meth, SCH 0.1/Sal, and SCH 0.1/Meth treatment groups on the Meth challenge day (a, wild-type mice; and b, D2R-deficient mice) and the contextual challenge day (c, wild-type mice; and d, D2R-deficient mice); mean ± SEM, $n =$ 10 per group. Pretreatment of mice with SCH in combination with Meth on the four drug-treatment days blocked the expression of methamphetamine sensitization in both genotypes. However, pretreatment with SCH only did not block the acute locomotor excitatory effect of methamphetamine on the Meth challenge day or cause lower basal locomotor activity on the contextual challenge day in either genotype.

Locomotor activity data from all treatment groups on the contextual day when all animals received only saline injections were also compared, with data grouped on genotype. A two-factor ANOVA showed significant main effects of genotype ($F_{1,175} = 159.7$, $P < 0.0001$) and treatment group ($F_{9,175} = 2.3$, $P < 0.02$) with no significant interaction. Examination of the data presented in Fig. 4c and d suggests that drug-treatment history and context-dependent effects were minimal (no significant mean differences among the four groups shown were detected by pairwise Tukey HSD post hoc comparisons).

Discussion

Sensitivity to some effects of amphetamine has a heritable component (Crabbe *et al.* 1983; Kamens *et al.* 2005; Palmer *et al.* 2005; Phillips *et al.* 2008) that could influence addiction risk. Specific genes have not yet been identified that lead to an increased probability of methamphetamine abuse; however, single gene mutant methods have been increasingly used to study relevant mechanisms. The combination of a dopamine D2 receptor deficiency with a D1-like receptor pharmacological antagonist was used to examine the roles of dopamine D1-like and D2 receptors in acute and sensitized methamphetamine responses. The first novel finding was a significant reduction in the amplitude and duration of the methamphetamine response in D2R-deficient mice, whether they were receiving methamphetamine for the first time or after several prior exposures. However, although the magnitude of sensitization was unaffected by the absence of the D2R when examined across daily sessions, the duration of sensitization within the time period of the final methamphetamine challenge was increased in D2R-deficient compared with wild-type mice, when the response of mice treated for the first time was compared to the response of those receiving their fifth treatment with methamphetamine. The hypothesis that D1-like receptors play an important role in the acute methamphetamine response, as well as the acquisition and expression of sensitization, was supported by data showing that SCH 23390 attenuated each of these responses. However, an increased role of D1-like receptors was shown by examining the effect of the antagonist in the D2R null mutant mice; D2R-deficient mice were more sensitive to the effects of the D1-like receptor antagonist on both the acute and sensitized methamphetamine responses. Changes in D1 receptors and D1-receptor modulated pathways might be predicted to occur in this constitutive knockout. However, the two genotypes were equally sensitive to the effects of SCH 23390 on basal locomotor activity, suggesting that the increased role of D1-like receptors in methamphetamine response in the D2R-deficient mice is not due solely to D1 receptor-related compensatory changes. Taken together, our results support the hypothesis that the dopamine D2 receptor acts in concert with D1-like receptors, in the mechanisms underlying methamphetamine stimulation and sensitization.

Both D1-like and D2 dopamine receptors are necessary for maximum acute methamphetamine response

The importance of dopamine D2 receptors in locomotor activity is well established, and these studies confirm and expand upon previous work that found a *de novo* locomotor phenotype in D2R-deficient mice (Baik *et al.* 1995; Kelly *et al.* 1998). When data were corrected for baseline activity differences, a decrease in the amplitude and duration of the acute locomotor stimulant effects of methamphetamine was apparent in D2R-deficient mice (Fig. 1b). When pretreated with SCH 23390 before methamphetamine treatment, a greater dependency of the D2R-deficient mice on D1-like receptors for their methamphetamine stimulant response was seen (Fig. 3). This result might not have been predicted from a study that showed that c-fos expression in response to methamphetamine was equivalently reduced by pretreatment with SCH 23390 in D2R-deficient and wild-type mice (Schmauss 2000). However, behavior was not measured in that study and this marker of neural activity was examined in the neocortex and not the limbic regions thought to influence locomotor behavior. The importance of D1-like and D2 receptor co-activation in producing a maximal response is not unique to our study (Glickstein and Schmauss 2004; Xu *et al.* 1997).

The increased reliance on D1-like receptors for methamphetamine sensitivity in the mutant mice was not the case for spontaneous locomotor activity; SCH 23390 suppressed locomotor activity equally regardless of D2 receptor status (Fig. 2 b). Because D1 receptor-deficient mice were reported to be impervious to the hypokinetic and cataleptic effects of high doses of SCH 23390 (Xu *et al.* 1994b), it is unlikely that SCH 23390 was acting on other dopamine receptors. This suggests that the compensatory adaptation allowing relatively normal (although somewhat impaired; see Kelly *et al.* 1998) basal locomotor activity in D2R-deficient mice is not mediated solely by signaling through the dopamine D1 receptor.

Methamphetamine sensitization is of similar magnitude, but has a longer time–course in D2R-deficient mice

Despite the reduction in the acute stimulatory effects of methamphetamine in D2R-deficient mice, locomotor sensitization developed over the course of repeated methamphetamine administration in these animals. No significant difference from wild-type siblings was found in the magnitude of sensitization during the acquisition period. However, on the methamphetamine challenge day, behavioral sensitization lasted longer in the D2R-deficient compared with wild-type mice. This longer time–course of sensitization (longer-lasting elevation of activity after methamphetamine treatment in the methamphetamine pre-exposed vs. non-pre-exposed mice) in the D2R-null mice was accompanied by a steeper decline in stimulation after methamphetamine challenge. A difference between the null mutant and wild-type mice in susceptibility to methamphetamine-induced stereotypy (Glickstein & Schmauss 2004), which might compete with the locomotor behavior, cannot be ruled out. However, doses of amphetamine that induce acute and sensitized stereotypic responses have typically been larger than the dose used here, and the administration often more frequent (Atkins *et al.* 2001; Battisti *et al.* 1999; 2000; Glickstein & Schmauss 2004; Karler *et al.* 1998; Yates *et al.* 2007). One study simultaneously measured stereotypic and

locomotor behaviors following treatment with several doses of D-amphetamine in C57BL/6J mice, the background strain of the mice used in our studies (Yates *et al.* 2007). During the 60-min test period corresponding to our study, a 6 mg/kg dose of amphetamine induced robust stimulation that was interrupted by stereotypy. However, stimulation with no stereotypy was seen after a 2 mg/kg dose. Blunted neuronal activation in the striatum indexed by measuring c-fos expression in response to acutely and repeatedly administered methamphetamine corresponded with resistance of the D2R-deficient mice to methamphetamine-induced stereotypy (Glickstein and Schmauss 2004). We have previously found that mice extremely sensitive to the acute stimulant effects of methamphetamine are less likely to self-administer methamphetamine (Kamens *et al.* 2005). The blunted stimulant response of the D2R-null mice in combination with reduced sensitivity to stereotypy and elongated duration of sensitization might predict greater susceptibility to methamphetamine self-administration and relapse. To our knowledge, D2R-null mutant mice have not been tested for methamphetamine reward-related traits.

Overall, our results indicate that the D2 receptor is not essential for methamphetamine-induced sensitization acquisition or expression, but may be important for duration. However, our findings differ from previous pharmacological studies in mice that utilized YM-09151-2 (a D2-like antagonist), or SCH 23390, to effectively block sensitization to methamphetamine (Kuribara & Uchihashi 1993, 1994). There are several possible explanations for these disparate findings. First, the pharmacological studies utilized a different mouse strain (the dd strain) that was apparently much more sensitive to the locomotor depressant effects of SCH 23390 than C57BL/6J mice, the background strain used here. Second, those studies administered drugs by s.c. rather than i.p. injection, which may have produced differences in the pharmacokinetics of drug action. Third, the methamphetamine was administered at longer intervals in the published work; there were 3–4 days between treatments compared to 48 h in our study. However, previous studies have shown our sensitization paradigm to be effective in C57BL/6J mice (Phillips *et al.* 1994). Fourth, D2-like antagonists may also affect D3 receptor signaling pathways that have been postulated to play a role in behavioral sensitization (Jones *et al.* 2007; Chiang *et al.* 2003). Finally, another possible explanation for the different conclusions from the purely pharmacological and genetic studies is that developmental compensations in the relevant circuitry of D2R-deficient mice have changed other aspects of the response system. Perhaps the difference in within-treatment duration reflects the increased dependence on D1-like receptor signaling by the D2R-null mice. A conditional receptor gene inactivation strategy is necessary to fully resolve this issue. In addition to bypassing critical developmental time points when neural adaptations may readily occur, a conditional mutant could also be used to discriminate spatially between ventral tegmental and ventral striatal substrates that are postulated to underlie different aspects of behavioral sensitization (Cador *et al.* 1995; Pierce & Kalivas 1997; Tanabe *et al.* 2004).

D1-like receptors are essential for the acquisition and expression of sensitization in wild-type and D2 receptor-deficient mice

Cador *et al.* (1995) have argued that acquisition and expression of psychostimulant sensitization are separable processes with distinct neural substrates. However, previous pharmacological studies in wild-type mice have shown that SCH 23390 dose-dependently attenuated both the acquisition and expression of methamphetamine sensitization (Kuribara & Uchihashi 1993, 1994). Our results in wild-type mice are concordant with these findings. They are also similar in that expression of sensitization on the methamphetamine challenge day was only prevented by SCH 23390 pretreatment (in combination with methamphetamine) at doses that nearly completely inhibited the locomotor stimulant response on the drug test days during acquisition. The consequences of SCH 23390 pretreatment during acquisition for the expression of methamphetamine sensitization on the methamphetamine challenge day were qualitatively the same in D2R-deficient and wild-type mice.

The amplitude and duration of the initial locomotor response to methamphetamine, but not the induction of behavioral sensitization, was significantly impaired by constitutive absence of the dopamine D2 receptor. During the challenge test, sensitization was sustained for a longer period of time in D2R-deficient mice. Our data are consistent with some other findings suggesting that the D1 receptor serves a more critical function than the D2 receptor in the acquisition of methamphetamine-induced behavioral sensitization. The literature is silent with regard to studies utilizing D1 and D2 receptor antagonist co-administration to study psychostimulant sensitization. However, the finding that D1-like receptors played a more significant role in the sensitized response to methamphetamine in D2R-deficient animals than in their wild-type siblings supports the involvement of D2 receptors as well. Taken together, these studies indicate that both receptors are needed for maximal stimulant response to methamphetamine, and at least one of the two major dopamine receptor subtypes must be functional for the acquisition and expression of sensitization.

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Acknowledgments

Funding for this work was provided by T32 DA076262 (M.A.K.), R01 MH061326 (M.J.L.), R01 DA010913 (T.J.P.), P50 DA018165 (T.J.P.) and the Department of Veterans Affairs (T.J.P.).