Contributions of Dopamine D1, D2, and D3 Receptor Subtypes to the Disruptive Effects of Cocaine on Prepulse Inhibition in Mice

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Deficits in prepulse inhibition (PPI) of startle, an operational measure of sensorimotor gating, are characteristics of schizophrenia and related neuropsychiatric disorders. Previous studies in mice demonstrate a contribution of dopamine (DA) D_1-family receptors in modulating PPI and DA D_2 receptors (D2R) in mediating the PPI-disruptive effects of amphetamine. To examine further the contributions of DA receptor subtypes in PPI, we used a combined pharmacological and genetic approach. In congenic C57BL/6 J wild-type mice, we tested whether the D1R antagonist SCH23390 or the D2/3R antagonist raclopride would attenuate the effects of the indirect DA agonist cocaine (40 mg/kg). Both the D1R and D2/3R antagonists attenuated the cocaine-induced PPI deficit. We also tested the effect of cocaine on PPI in wild-type and DA D1R, D2R, or D3R knockout mice. The cocaine-induced PPI deficit was influenced differently by the three DA receptor subtypes, being absent in D1R knockout mice, partially attenuated in D2R knockout mice, and exaggerated in D3R knockout mice. Thus, the D1R is necessary for the PPI-disruptive effects of cocaine, while the D2R partially contributes to these effects. Conversely, the D3R appears to inhibit the PPI-disruptive effects of cocaine. Uncovering neural mechanisms involved in PPI will further our understanding of substrates of sensorimotor gating and could lead to better therapeutics to treat complex cognitive disorders such as schizophrenia.

INTRODUCTION

Prepulse inhibition (PPI) is a cross-species measure of sensorimotor gating in which startle magnitude is reduced when the startling stimulus is preceded by a low-intensity prepulse (Hoffman and Ison, 1980). PPI deficits are observed in psychotic disorders, such as schizophrenia, and are associated with dopamine (DA) dysregulation (Braff et al, 2001; Swerdlow et al, 2001). PPI deficits are modeled in mice and rats following treatment with psychotomimetic drugs, such as d-amphetamine, cocaine, or phencyclidine (Geyer et al, 2001).

Similar to the rat, the DA D_2 receptor (D2R) subtype is necessary for amphetamine-induced PPI disruptions in mice, while the DA D_1 (D1R), D_3 (D3R), and D_4 (D4R)

receptor subtypes are not (Ralph et al, 1999; Ralph-Williams et al, 2002). DA D_2-like agonists quinpirole and quinelorane failed to disrupt PPI in several strains of mice (Ralph-Williams et al, 2003; Ralph and Caine, 2005), although quinelorane can disrupt PPI in some mouse strains (Ralph and Caine, 2007). Relative to the rat, the D1R plays a more prominent role regulating PPI in mice (Geyer et al, 2002; Ralph-Williams et al, 2003; Ralph and Caine, 2005). The D1R antagonist SCH23390 blocks the PPI-disruptive effect of the direct DA agonist apomorphine in mice, and D_1-family agonists such as SKF82958, SKF81297, and dihydrexidine reduce PPI in mice, supporting the idea that D1R activation is sufficient to produce PPI disruptions in mice (Holmes et al, 2001; Ralph-Williams et al, 2002, 2003). Thus, the roles of DA receptor subtypes in the modulation of PPI in mice appear to differ from those observed in rats.

Cocaine is an indirect DA agonist, which inhibits the reuptake of DA in the synapse, producing behavioral stimulation and precipitating psychosis after sustained use in humans (Bolla et al, 1998). Acute cocaine administration disrupts PPI in rats (Martinez et al, 1999; Byrnes and

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Hammer, 2000) and mice (our unpublished data; Yamashita et al, 2006) at high doses. Other studies, however, have failed to show an acute effect of cocaine on PPI in rats (Varty and Higgins, 1998). The behavioral effects of cocaine appear to be mediated by both D_1- and D_2-family receptors. D2Rs regulate the increase in extracellular DA concentrations elicited by cocaine (Rouge-Pont et al, 2002). The D2/3R antagonist raclopride blocked the locomotor-stimulating effects of cocaine in mice (Chausmer et al, 2002). Xu and colleagues (Xu et al, 1994, 2000; Xu, 1998) found that D1R knockout (KO) mice exhibit an attenuated locomotor response after acute or repeated cocaine and proposed that D3Rs modulate responses to psychostimulants by inhibiting the cooperative effects of postsynaptic D_1- and D_2-family receptors. Carta et al (2000) reported that D3R KO mice show enhanced locomotor activity following cocaine administration. The D1R activates and the D3R inhibits gene expression of extracellular kinases and c-fos induction after acute and chronic cocaine treatment (Zhang et al, 2004). Taken together, these reports suggest that all three DA receptor subtypes regulate the behavioral effects of cocaine and that they may exert different influences on behavior. To examine contributions of the DA D1R, D2R, and D3R subtypes to cocaine-induced deficits in acoustic startle response (ASR) and PPI, we pretreated WT mice with the D1R antagonist SCH23390 or the D2/3R antagonist raclopride, in addition to testing D1R, D2R, and D3R KO mice.

MATERIALS AND METHODS

Animals

Incipient congenic D1R, D2R, and D3R mutant mice were bred and genotyped at Oregon Health & Science University (OHSU) and shipped to the University of California, San Diego (UCSD) for testing. Before testing, mice had a minimum 2-week resting period to acclimate to our facilities to minimize any influences of travel. The wild-type (WT) mice used for the pharmacological experiment were taken from a cohort of D2R mice. WT mice from D1R, D2R, or D3R cohorts display no difference in PPI or ASR (Ralph et al, 1999).

A colony of D1R mutant mice (Drago et al, 1994) was originally established at OHSU from breeding stock of B6.129S4-Drd1a[tm1Jcd] male mice obtained from The Jackson Laboratory (Bar Harbor, ME). The heterozygous (HET) mice had been backcrossed for a minimum of five generations onto the inbred C57BL/6J line before importation and were then backcrossed for two additional generations in Portland (OHSU, Vollum Institute) to produce incipient congenic (N7) mice. N7 C57BL/6J D1R HET breeding pairs were used to generate the mice for behavioral studies. Because the D1R KO mice were growth-retarded (Drago et al, 1994), pups were not weaned from their mothers until 4 weeks of age and were then provided with moistened chow until approximately 8 weeks of age to maximize growth. The D1R mice were genotyped by PCR using a protocol and primer sequences provided by The Jackson Laboratory.

The D2R mutant mice (Kelly et al, 1997, 1998), official strain designation B6.129S2-Drd2[tm1Low], were originally generated at OHSU and backcrossed for 14 generations (N14) onto the inbred C57BL/6J line. N14 C57BL/6J D2R HET breeding pairs were used to generate fully congenic mice used in behavioral studies. Both sexes of D2R KO mice were smaller than their WT siblings at the time of weaning, but they did not require special attention or moistened chow. The D2R KO mice later achieved weight close to that of the WT mice. D2R mice were genotyped by PCR according to the protocol previously described (Díaz-Torga et al, 2002).

D3R mutant mice (Accili et al, 1996), original strain B6.129S4-Drd3[tm1Dac], were also imported from The Jackson Laboratory. The mice were incipient congenic (N5 C57BL/6J D3R), having been backcrossed five times with the inbred strain C57BL/6J at the time of importation. The colony was established at OHSU and backcrossed for two more generations with the C57BL/6J to generate N7 D3R mice. N7 C57BL/6J D3R HET breeding pairs were used to generate mice for behavioral studies. All the mice were viable and fertile and no altered growth phenotype was present in these mutant mice. D3R mice were genotyped by PCR using a protocol and primer sequences provided by the Jackson Laboratory.

Testing

At UCSD, mice were housed in a climate-controlled animal colony with a reversed day/night cycle (lights on at 2000 hours, off at 0800 hours). All behavioral testing was performed on mice between 7 and 33 weeks of age and occurred between 0900 hours and 1700 hours daily. Food (Harlan Teklab, Madison, WI) and water were available ad libitum, except during behavioral testing. All behavioral testing procedures were approved by the UCSD institutional animal care and use committee prior to the onset of the experiments. Mice were maintained in American Association for Accreditation of Laboratory Animal Care approved animal facilities at UCSD. This facility meets all Federal and State requirements for animal care.

Drugs

SCH23390 HCl was dissolved in sterile water at a concentration of 0.2 mg/ml and a dose of 1.0 mg/kg was given subcutaneously 10 min before cocaine administration. Raclopride tartrate was dissolved in 0.9% saline at a concentration of 0.6 mg/ml and a dose of 3.0 mg/kg was given intraperitoneally 10 min before cocaine administration. Cocaine HCl was dissolved in 0.9% saline at a concentration of 8 mg/ml and a dose of 40.0 mg/kg was given intraperitoneally 5 min before behavioral testing. All three drugs were obtained from Sigma/RBI (St Louis, MO) and injected at a volume of 5 ml/kg body weight. The doses of SCH23390, raclopride, and cocaine were chosen based on pilot studies in C57BL/6J mice from our lab, as well as Yamashita et al (2006), for cocaine.

Apparatus

Startle reactivity was measured using eight startle chambers (SR-LAB, San Diego Instruments, San Diego, CA). Each chamber consisted of a clear nonrestrictive Plexiglas

cylinder (inner diameter = 38 cm) 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 ms intervals, starting at stimulus onset, and the average amplitude was used to determine the ASR. The SR-LAB calibration unit was used routinely to ensure consistent stabilimeter sensitivity between test chambers and over time, and sound levels in dB SPL (A scale) were measured as described previously (Geyer and Dulawa, 2003).

PPI 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-ms 120-dB pulse of broad-band noise. PREPULSE + PULSE trials consisted of a 20-ms noise prepulse, 80 ms delay, then a 40-ms 120-dB startle pulse (100 ms onset to onset). The acoustic prepulse intensities were 69, 73, and 81 dB (ie 4, 8, and 16 dB above the 65-dB background noise). The NOSTIM trial consisted of background noise only. 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 s (range: 12–30 s) between trials. A background noise level of 65 dB was presented for a 10-min acclimation period and continued throughout the test session.

Data Analyses

For receptor comparison purposes, ASR and PPI were analyzed during the initial half of the session, where a significant cocaine effect occurred in all WT mice. Unless otherwise indicated, data were collapsed across sex when there were no interactions of cocaine treatment with sex. The amount of PPI was calculated as a percentage score for each acoustic prepulse trial type: % PPI = 100−{[(startle response for PREPULSE + PULSE)/(startle response for PULSE-ALONE)] × 100}. For brevity, data are presented as the average % PPI across the three prepulse intensities tested because of a lack of a treatment × intensity effect during analysis. The magnitude of the ASR was calculated as the average response of the PULSE-ALONE trials, excluding the first block of five PULSE-ALONE trials presented. 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.

Pharmacological Blockade of Cocaine Effects on PPI

The WT cohort consisted of 16 male and 15 female mice. The mice were first characterized in a session to establish their baseline startle magnitude and PPI levels. They were then assigned to their drug treatment groups (40.0 mg/kg cocaine or vehicle), balanced for baseline startle magnitude,

PPI, and startle chamber assignment. The mice were tested in a three-way crossover design with 1 week between tests. Each animal always received either vehicle or cocaine as their treatment over the three tests. For the pretreatment, each animal received vehicle, 1.0 mg/kg SCH23390, or 3.0 mg/kg raclopride in a semi-randomized, counterbalanced order, to complete a within-subject design. Thus, treatment was the between-subject variable and pretreatment was a within-subject variable. A two-way analysis of variance (ANOVA) was used to compare means, and the α-level was adjusted to $P < 0.025$ to accommodate the removal of treatment as a factor in the *post hoc* ANOVAs. The computations were carried out using the BMDP statistical software (Statistical Solutions Inc., Saugus, MA).

Effects of Cocaine in D1R, D2R, and D3R WT and KO Mice

The D1R cohort consisted of 15 WT and 7 KO male mice, and 10 WT and 7 KO female mice. The D2R cohort consisted of 28 WT and 18 KO male mice, and 16 WT and 30 KO female mice. The D3R cohort consisted of 9 WT and 9 KO male mice, and 10 WT and 10 KO female mice. The cohorts were characterized initially in a session to establish their baseline startle magnitude and PPI levels. They were then assigned to their drug treatment groups (40.0 mg/kg cocaine or vehicle), balanced for baseline startle magnitude, PPI, and startle chamber assignment. The cohorts were tested in a semi-randomized, crossover design. After the first treatment, the mice were tested 1–2 weeks later to counterbalance for treatment, to complete a within-subject design. In the statistical analyses, genotype was the between-subject variable and drug was a within-subject variable. A two-way ANOVA was used to compare means and the α-level was adjusted to $P < 0.025$ to accommodate the removal of genotype as a factor in the *post hoc* ANOVAs.

RESULTS

D1 or D2/3 Antagonist Interactions with Cocaine

Cocaine (40 mg/kg) produced PPI deficits in WT mice compared to vehicle pretreated controls ($F_{(1, 29)} = 10.0$; $P < 0.01$) (Figure 1). Pretreatment with SCH23390 (1.0 mg/kg), a D1R antagonist, attenuated the PPI deficit produced by cocaine as evidenced by a main effect of pretreatment ($F_{(1, 29)} = 22.6$; $P < 0.01$) and a pretreatment × treatment interaction ($F_{(1, 29)} = 12.7$; $P < 0.01$) (Figure 1). Pretreatment with raclopride (3.0 mg/kg), a D2/3R antagonist, also attenuated the PPI deficit produced by cocaine as evidenced by a main effect of pretreatment ($F_{(1, 29)} = 15.2$; $P < 0.01$) and a pretreatment × treatment interaction ($F_{(1, 29)} = 8.2$; $P < 0.01$) (Figure 1). There were no effects of cocaine on ASR in WT animals, regardless of pretreatment with vehicle, SCH23390, or raclopride (Table 1). There was a main effect of sex on ASR with male mice exhibiting higher startle than female counterparts in all experiments, regardless of genotype ($P < 0.05$).

D$_1$ Receptor Mice

There was a significant effect of cocaine ($F_{(1, 37)} = 11.6$; $P < 0.01$) and a genotype × cocaine interaction ($F_{(1, 37)} = 4.6$; $P < 0.05$) on PPI in D1R mice. *Post hoc* ANOVAs revealed that cocaine (40 mg/kg) significantly reduced PPI in D1R WT mice ($F_{(1, 24)} = 15.1$; $P < 0.01$), but was ineffective in D1R KO mice (Figure 2a). Regardless of sex or drug treatment, D1R KO mice showed higher startle responses compared to WT control mice ($F_{(1, 37)} = 7.6$; $P < 0.01$) (Table 1).

D$_2$ Receptor Mice

Cocaine (40 mg/kg) produced PPI deficits in both D2R WT and KO mice (main effect of cocaine, $F_{(1, 90)} = 73.9$; $P < 0.01$) (Figure 2b). Nevertheless, there was a genotype × cocaine interaction ($F_{(1, 90)} = 5.2$; $P < 0.05$), indicating that there was a significant difference in the magnitude of the PPI-disruptive effects of cocaine between the two genotypes. Specifically, the PPI-disruptive effects of cocaine were attenuated in D2R KO mice compared to WT mice. Cocaine significantly reduced ASR in male D2 WT mice only (drug × sex × gene interaction $F_{(1, 88)} = 4.8$; $P < 0.05$). Fe-

Figure 1 Prepulse inhibition of the startle response in WT mice after pretreatment with vehicle (VEH), 1.0 mg/kg SCH23390 (SCH) or 3.0 mg/kg raclopride (RAC) and treatment with vehicle or 40.0 mg/kg cocaine (COC). Cocaine decreased PPI in WT mice and both SCH23390 and raclopride attenuated the cocaine-induced PPI deficit. *$P < 0.01$ compared to vehicle control. Data are presented as mean ± SEM.

male D2R KO mice exhibited reduced startle compared to WT littermates, regardless of drug treatment (sex × gene interaction $F_{(1, 88)} = 4.4$; $P < 0.05$) (Table 1).

D$_3$ Receptor Mice

Cocaine (40 mg/kg) produced PPI deficits in both D3R WT and KO mice (main effect of cocaine, $F_{(1, 36)} = 53.8$; $P < 0.01$) (Figure 2c). In addition, there was a genotype × cocaine interaction ($F_{(1, 36)} = 6.1$; $P < 0.01$), indicative of a significant difference in the magnitude of the cocaine-elicited PPI deficit between the two genotypes. In contrast to the pattern observed with D2R mice, the PPI-disruptive effects of cocaine were exaggerated in D3R KO mice compared to WT animals. Cocaine had no effect on ASR in either gender, although there were significant main effects of sex ($F_{(1, 34)} = 9.8$; $P < 0.01$) and genotype ($F_{(1, 34)} = 11.0$; $P < 0.01$). Female mice exhibited lower ASR than their male counterparts and D3R KO mice had a lower ASR compared to WT mice (Table 1).

DISCUSSION

In support of our hypotheses, the results indicated that all three DA receptor subtypes contributed to the PPI-disrupting effects of cocaine in mice, although differentially. First, both the D1R antagonist SCH23390 and the D2/3R antagonist raclopride completely attenuated the PPI disruption produced by cocaine in WT mice. Second, mice lacking the D1R exhibited no cocaine-induced PPI deficits, suggesting that the D1R subtype is necessary for the PPI disruption caused by cocaine. Third, mice lacking the D2R displayed attenuated cocaine-induced PPI deficits, suggesting that activation of the D2R subtype contributes to the PPI disruption caused by cocaine. Fourth, mice lacking the D3R displayed exaggerated cocaine-induced PPI deficits, suggesting that activation of the D3 receptor subtype serves to inhibit the PPI disruption caused by cocaine. Each DA receptor subtype appears to be differentially modulating cocaine-induced PPI disruptions as seen by the lack of response in D1R KO mice, a weak response in D2R KO mice, and an exaggerated response in D3R KO mice.

Mice lacking the DA D1R or those administered the D1R antagonist SCH23390 failed to show a cocaine-induced disruption in PPI. These observations corroborate and
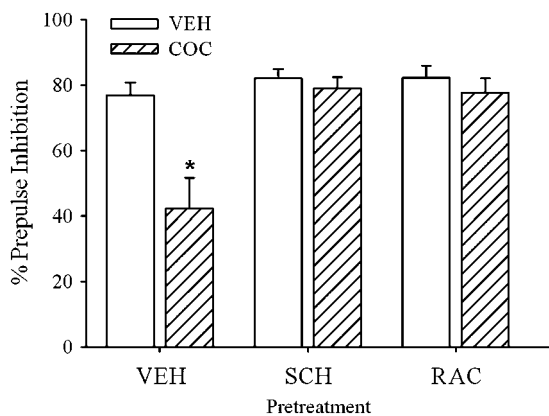
Table 1 Acoustic Startle Response after Treatment with Vehicle or Cocaine (40.0 mg/kg)

ASR	WT			D1R		D2R		D3R	
	VEH	**SCH**	**RAC**	**WT**	**KO**	**WT**	**KO**	**WT**	**KO**
VEH	76.3 ± 13.3	66.4 ± 15.4	62.7 ± 10.5	59.2 ± 10.6	84.4 ± 13.5	111.3 ± 11.3	75.2 ± 7.7	81.9 ± 9.0	56.5 ± 9.6
COC	55.7 ± 10.4	55.5 ± 10.5	67.7 ± 15.5	43.1 ± 6.7	83.8 ± 12.5	75.8 ± 7.7	66.8 ± 7.2	84.1 ± 14.8	43.4 ± 6.2

Similar to PPI, acoustic startle response (ASR) has been collapsed across sex, given that the only interaction of sex with cocaine treatment occurred in male D2 WT mice (see the Results section). Female mice exhibited less startle reactivity than male mice in all genotypes. Cocaine (COC) had no effect on ASR in the WT cohort pretreated with vehicle (VEH), 1.0 mg/kg SCH23390 (SCH), or 1.0 mg/kg raclopride (RAC). Cocaine had no effect on ASR in D1R and D3R mice. In D2R mice, cocaine decreased startle magnitude in male WT animals compared to vehicle controls. Additionally, D1R KO and D3R KO mice exhibited more and less startle than their WT littermates, respectively. D2R KO females also exhibited less startle than female WT mice, but this effect was not replicated in the males (for statistics see the Results section). Data are presented as mean ± SEM startle magnitudes in arbitrary units.
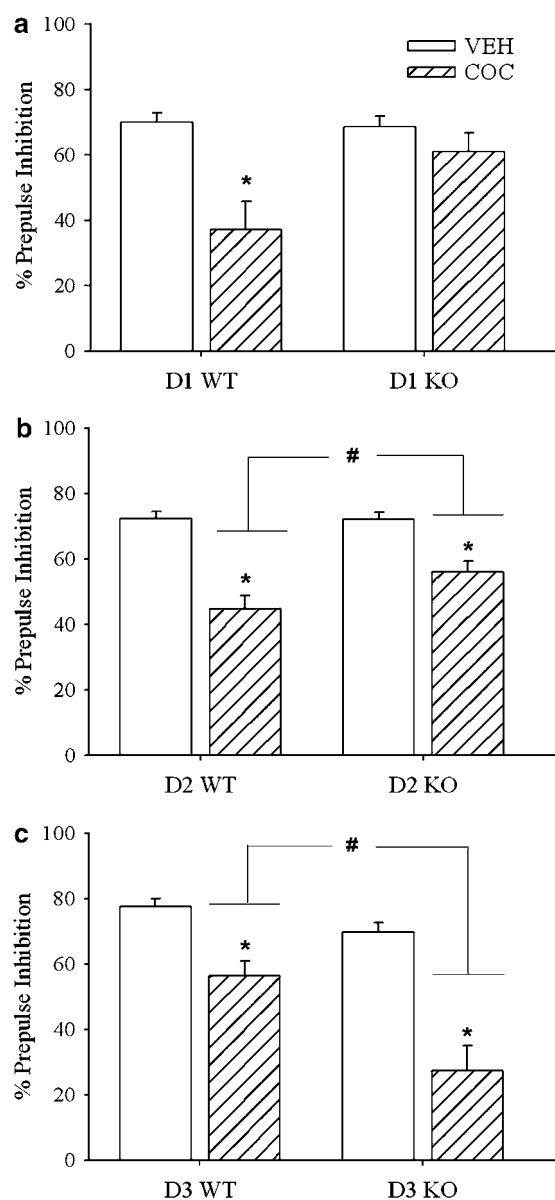
Figure 2 Prepulse inhibition of the startle response in D1R, D2R, and D3R mutant mice after vehicle (VEH) or 40.0 mg/kg cocaine (COC) treatment. (**a**) Cocaine decreased PPI in the D1R WT mice but not in the D1R KO mice. (**b**) Cocaine decreased PPI in both D2R WT and KO mice. In addition, D2R KO mice exhibited less disruption in PPI than D2R WT mice after cocaine treatment. (**c**) Cocaine decreased PPI in both D3R WT and KO mice. In addition, D3R KO mice exhibited a greater disruption in PPI than D3R WT mice after cocaine treatment. *$P < 0.01$ compared to vehicle control. #$P < 0.025$ vs WT-COC. Data are presented as mean ± SEM.

extend previous studies of mutant mice lacking the D1R, which demonstrated that D1Rs play a critical role in mediating the behavioral responses to cocaine. Both the acute, and to a lesser extent, chronic locomotor-stimulating effects of cocaine are reduced in D1R KO mice (Xu *et al*, 1994, 2000). D1R agonists and antagonists can influence locomotor and stereotyped responses to cocaine and alter cocaine self-administration patterns (Koob *et al*, 1987; Cabib *et al*, 1991; Caine and Koob, 1994; Tella, 1994; Self *et al*, 1996). Moreover, D1-like antagonists and partial

agonists attenuated the reinforcing and discriminative-stimulus effects of cocaine (Katz and Witkin, 1992; Spealman *et al*, 1997; Katz *et al*, 1999; Caine *et al*, 2000; David *et al*, 2004). Consistent with these observations in different behavioral paradigms, the present studies of PPI corroborate our previous evidence (Ralph-Williams *et al*, 2002, 2003) suggesting that activation of the D1R is essential for the PPI-disruptive effects of either cocaine or mixed DA agonists.

In addition to the essential role of the D1R in the PPI-disruptive effect of cocaine, the present results support a model in which the activation by cocaine of both D2R and D3R also contribute in modulating PPI. Mice lacking the DA D2R exhibit attenuated cocaine-induced PPI deficits, while mice administered the D2/D3R antagonist raclopride fail to show a cocaine-induced disruption in PPI. The D2R has a direct protein-to-protein link to the presynaptic DA transporter (Lee *et al*, 2007), known to regulate dopaminergic tone, which could explain the attenuated cocaine-induced PPI deficits in D2R KO mice. These observations are consistent with the proposal by Xu (1998) that D3Rs modulate the response to psychostimulants by inhibiting the cooperative effects of postsynaptic D1- and D2-family receptors (Daly and Waddington, 1992) at a systems level. In D2R KO mice, the weaker response to cocaine compared to WT animals may be attributed to the intact activation of D1R, which is partially attenuated by the opposing influence of the cocaine-induced activation of D3R. In contrast, the blockade of both D2R and D3Rs by raclopride clearly prevented the cocaine-induced activation of D1R from affecting PPI. Thus, it appears that co-activation of either D2R or D3R is required for the activation of D1R by cocaine to disrupt PPI. When only one of these D2-family receptors was inactivated by gene deletions, the cocaine-induced activation of D1R was effective in disrupting PPI. The data from the current study suggest that activation of D1R by cocaine is necessary but not sufficient to disrupt PPI in mice.

Despite the present and previous evidence that some behavioral consequences of cocaine-induced activation of D1R require the concomitant activation of either the D2 or D3 subtypes of the D2-family of DA receptors, the present findings further suggest that the permissive influences of D2R and D3R in this context are opposite in direction. That is, in contrast to the D2R KO mice, mice lacking the DA D3R exhibited exaggerated cocaine-induced PPI deficits. One explanation could be that the elevated basal extracellular DA levels in D3 KO mice (Koeltzow *et al*, 1998; Joseph *et al*, 2002) could lead to the increased sensitivity to cocaine observed here in our D3 KO mice. There also seems to be opposing effects between the D1R and the D3R on the genomic effects of cocaine. The D1R activates and the D3R inhibits gene expression of extracellular kinases and c-fos induction after acute and chronic cocaine treatment (Zhang *et al*, 2004). Our results corroborate this hypothesis, as D1R KO mice display no cocaine-induced PPI deficits while D3R KO mice exhibit exaggerated cocaine-induced PPI deficits. These results corroborate and extend previous studies of psychostimulant effects in D3R KO mice. For example, D3R KO mice exhibit an enhanced locomotor response after cocaine administration (Carta *et al*, 2000) and D3R antagonists augment cocaine (Piercey *et al*, 1992) and

amphetamine-stimulated locomotion (Waters *et al*, 1993; Pritchard *et al*, 2007). D3R KO mice also exhibit an increase in cocaine cue-conditioned hyperactivity and cocaine cue-conditioned mice had increased levels of D3R mRNA in the nucleus accumbens compared to saline controls (Le Foll *et al*, 2002). It should be noted, however, that the effects of agonists and antagonists having preferential affinities for D3R over D2R are not entirely consistent with the effects observed with the more specific genetic manipulations of these receptor subtypes (eg Corbin *et al*, 1998; Le Foll *et al*, 2002; Xi *et al*, 2005). The development of more subtype-selective D_2-family ligands or perhaps a double KO mouse in which both D2R and D3R are deleted would help to clarify the respective roles of these DA D_2-family receptor subtypes in modulating the effects of cocaine and other psychostimulants.

The contradictory literature on the behavioral effects of D2R *vs* D3R agonists and antagonists may also be a reflection of regional differences in receptor subtype expression. Comparing between the two subtypes, the D2R exhibits higher expression in dorsal striatum, substantia nigra, and ventral tegmental area, whereas the D3R exhibits higher expression in nucleus accumbens (Callier *et al*, 2003). Also, differential pre- *vs* postsynaptic expression of the receptor subtypes in brain regions important for PPI may contribute to different effects on PPI.

Since both amphetamine and cocaine are indirect DA agonists that increase synaptic DA concentrations, it is puzzling that the D2R, not the D1R, is necessary for amphetamine to disrupt PPI (Ralph *et al*, 1999), while cocaine does not disrupt PPI in D1R KO mice. The DA transporter is an obligatory target of both cocaine and amphetamine, but there is conflicting evidence on psychostimulant effects on the DA transporter. In one study these psychostimulants have no effect on locomotor activity or DA release and uptake in DA transporter KO mice (Giros *et al*, 1996). In contrast, other studies have found that DA transporter KO mice display increased amphetamine-induced conditioned place preference, and cocaine- and amphetamine-stimulated increases in DA in the nucleus accumbens (Carboni *et al*, 2001; Budygin *et al*, 2004). Confounding these reports is Yamashita *et al* (2006), where cocaine actually increased PPI in DA transporter KO mice, presumably through norepinephrine (NE) transporter blockade. Also, Tilley *et al* (2007) found that DA transporter knockdown mice exhibit increased cocaine-stimulated locomotor activity. Nevertheless, there is evidence that amphetamine elevates extracellular DA by a transporter-mediated release of non-vesicular, and possibly vesicular DA, from dopaminergic terminals, whereas cocaine mainly acts by blocking DA uptake (Ary and Komiskey, 1982; Butcher *et al*, 1988; Hurd and Ungerstedt, 1989; Floor and Meng, 1996; Sabol and Seiden, 1998). Additionally, amphetamine induces immediate early gene *c-fos* expression mainly in striosomes, whereas cocaine causes an immediate *c-fos* expression in both the striosome and matrix compartments of the dorsal striatum in the rat (Graybiel *et al*, 1990). The striosomes and matrix are the major neurotransmitter-specific compartments in the dorsal striatum (Graybiel and Ragsdale, 1978; Graybiel *et al*, 1990) and have different limbic and sensorimotor affiliations with cortex and subcortex (Donoghue and Herken-

ham, 1986; Ragsdale and Graybiel, 1990). Amphetamine and cocaine also lead to different *c-fos* expression patterns in the nucleus accumbens. Cocaine, but not amphetamine, elicited more expression of *c-fos* in the shell than in the core of nucleus accumbens (Graybiel *et al*, 1990). The nucleus accumbens is a key modulator of PPI (for review Swerdlow *et al*, 2001). The disruptive effects of amphetamine seem to correlate in time with DA increase in the nucleus accumbens in the rat (Zhang *et al*, 2000). Studies of mutant mice challenged with amphetamine and cocaine (Graybiel *et al*, 1990; Moratalla *et al*, 1996) indicate that the D1R is required to induce the expression of *c-fos* in striatal neurons. Combined with our behavioral results, these studies suggest that amphetamine and cocaine exert differential effects in rodents.

Although previous studies indicate that the D4R subtype does not influence PPI or the effect of amphetamine on PPI (Ralph *et al*, 1999), a contribution of the D4R subtype in cocaine-induced PPI deficits remains a possibility. The present results, showing the necessary role of the D1R in cocaine-induced PPI, and evidence from prior studies (Ralph *et al*, 1999; Holmes *et al*, 2001; Ralph-Williams *et al*, 2002, 2003; Ralph and Caine, 2005), indicate that the D5 DA receptor subtype has little contribution to psychostimulant-induced PPI deficits. Nevertheless, we cannot rule out a possible contribution of the D5R subtype to cocaine-induced PPI deficits.

Cocaine had slight but nonsignificant effects on startle reactivity in all experiments. Specifically, cocaine did not affect ASR in WT, D1R, D2R, or D3R mice, although D1R and D3R KO mice exhibited higher and lower startle than their WT counterparts, respectively. The only interaction of sex with cocaine treatment occurred in male D2R WT mice, where cocaine decreased ASR compared to vehicle controls. In general, some differences in ASR across experiments were due to male mice in all experiments exhibiting higher startle than female counterparts, regardless of genotype. Also, female D2R KO mice exhibited reduced startle compared to WT littermates, regardless of drug treatment. Although each of these lines has been backcrossed 7 (for D1R and D3R mice) or 14 (for D2R mice) generations and are considered to be congenic to C57Bl/6J, there is the possibility of genetic drift in each line. These differences in startle magnitude between the lines of mice should not impact the interpretability of the effects of cocaine on PPI. First, each line was bred using heterozygous matings and studies were conducted in WT and KO littermates. All statistical comparisons were carried out within each line. Second, while the startle values did differ between the WT mice of each line, the percent PPI was very similar across the lines, demonstrating the independence of PPI and startle magnitude. Moreover, cocaine disrupted PPI in all three lines of WT mice. Our results in mice corroborate the study by Yamashita *et al* (2006) and extend it with behavioral data suggesting that amphetamine and cocaine exert differential effects in rodents.

Cocaine and amphetamine both increase available NE and serotonin (5HT), although in distinct manners that may be important for modulating PPI. Cocaine blocks, while amphetamine reverses, the norepinephrine transporter (NET) and serotonin transporter (SERT), and cocaine is more potent at SERT than amphetamine (Rothman and

Baumann, 2003). In rats, the α-1 NE antagonist prazosin and the 5HT$_2$ antagonist ketanserin failed to reverse cocaine-induced PPI deficits (van der Elst et al, 2006), while the NE reuptake inhibitor desipramine, but not the 5HT reuptake inhibitor citalopram, normalized amphetamine-induced PPI disruptions (Pouzet et al, 2005). In mice, α2A-KO mice were more sensitive to amphetamine-induced PPI disruption (Lahdesmaki et al, 2004) and NET is potentially involved in cocaine-induced PPI disruptions (Yamashita et al, 2006). Future studies should include a DA uptake inhibitor with little affinity for other neurotransmitter pathways to help elucidate the mechanistic differences between amphetamine and cocaine in terms of effects on PPI.

When using genetically engineered mice, the potential exists that some compensatory mechanisms may account for the differences observed in mutants since the mice lack the DA receptor throughout development. Developing inducible DA receptor KO mice, where the receptor loss can be turned on after critical developmental periods early in life, may eliminate developmental caveats. The DA receptor KO mice are used as model systems to gain a molecular understanding of acute effects of psychostimulants, such as cocaine and amphetamine. The mutant mice help define the role the different DA receptor subtypes play in the acute effects of psychostimulants, something unobtainable with the poor subtype-specific agonists and antagonists available today. Developing double KOs (eg D3R combined with either D1R or D2R), if viable, could also help explain the contributions of each DA receptor subtype since multiple receptors seem to be involved and subtypes may modulate each other. Genetically altered mice offer a unique model to test the specificity and selectivity of different DA drugs and may provide important new concepts related to the clinical and social implications of conditions displaying dysregulated DA systems, such as Parkinson's disease, drug addiction, and schizophrenia.

The results of the present study support differential roles for the DA D1R, D2R, and D3R subtypes in modulating cocaine-induced disruption of PPI in mice. Further evidence is needed to explain fully the differences in rodent responses to psychostimulant drugs depending upon whether their DA receptors are manipulated pharmacologically or genetically. Deciphering the role that specific DA receptors play in modulating behavioral effects will help explain disorders involving DA, such as schizophrenia and drug abuse.

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