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Dopamine D1 and D3 receptors mediate reconsolidation of cocaine memories in mouse models of drug self-administration

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Abstract

Memories of drug experience and drug-associated environmental cues can elicit drug-seeking and taking behaviors in humans. Disruption of reconsolidation of drug memories dampens previous memories and therefore may provide a useful way to treat drug abuse. We and others previously demonstrated that dopamine D1 and D3 receptors play differential roles in acquiring cocaine-induced behaviors. Moreover, D3 receptors contribute to the reconsolidation of cocaine-induced conditioned place preference. In the present study, we examined effects of manipulating D1 or D3 receptors on reconsolidation of cocaine memories in mouse models of drug self-administration. We found that pharmacological blockade of D1 receptors or a genetic mutation of the D3 receptor gene attenuated reconsolidation that lasted for at least 1 week after the memory retrieval. In contrast, with no memory retrieval, pharmacological antagonism of D1 receptors or the D3 receptor gene mutation did not significantly affect reconsolidation of cocaine memories. Pharmacological blockade of D3 receptors also attenuated reconsolidation in wild-type mice that lasted for at least 1 week after the memory retrieval. These results suggest that D1 and D3 receptors and related signaling mechanisms play key roles in reconsolidation of cocaine memories in mice, and that these receptors may serve as novel targets for the treatment of cocaine abuse in humans.

Keywords

dopamine D1 and D3 receptors; reconsolidation; drug memory; antagonists; gene mutation; cocaine self-administration

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INTRODUCTION

A central feature of drug addiction is the compulsive seeking and taking of drugs despite known negative consequences. Addicts experience drug craving after long periods of abstinence and are highly susceptible to relapse (O'Brien et al, 1998; Dackis and O'Brien, 2005). Memories of drug effects or learned associations between the rewarding properties of drugs and cues are thought to precipitate craving and relapse. Reconsolidation is a process in which memory undergoes a transiently labile stage after its retrieval and needs to be consolidated again in order to be maintained (Nader et al, 2000; Miller and Sweatt, 2006; Tronson and Tayler, 2007; Alberini, 2011). Pharmacological or molecular manipulations of reconsolidation of acquired drug memories have been shown to disrupt drug-seeking and relapsing behavior in animal models (Miller and Marshall, 2005; Lee et al, 2005; 2006; Valjent et al, 2006; Taylor et al, 2009; Sanchez et al, 2010; Yan et al, 2013) and in drug addicts (Xue et al, 2012). These studies suggest that understanding the molecular basis of reconsolidation of reward memory may help to develop new medications for the treatment of drug abuse (Sorg, 2012; Tronson and Taylor, 2013).

The mesolimbic dopamine (DA) projections are a major neural substrate for mediating actions of drugs of abuse that can increase synaptic levels of DA that is required for reward and reinforcement (Hyman et al., 2006; Kalivas and O'Brien, 2008; Koob and Volkow, 2010; Luscher and Malenka, 2011). Recent studies suggest that DA is involved in reward learning and that drugs of abuse can change related neuronal circuits in the mesolimbic DA system (Ito et al, 2000; Stuber et al, 2005; Hyman et al, 2006; Wise, 2008; Volkow et al, 2009; Schultz, 2010; Torregrossa et al, 2011; Milton and Everitt, 2012). DA binds to DA receptors to trigger many molecular, physiological and behavioral changes. Five DA receptors have been identified and classified into two subfamilies (Beaulieu and Gainetdinov, 2011). The D1-like family includes D1 and D5 receptors that interact with G_s proteins. The D2-like family includes D2, D3 and D4 receptors that interact with G_i or G_0 proteins. Both D1 and D3 receptors are expressed in mesolimbic DA projection areas. We and others have shown that D1 (Xu et al, 1994a; 1994b; 2000; Anderson et al, 2003; Bachtell et al, 2005; Alleweireldt et al, 2006; Berglind et al, 2006; Caine et al, 2007; Chen and Xu, 2010) and D3 receptors (Xu et al, 1997; Pilla et al., 1999; Vorel et al., 2002; Di Ciano et al., 2003; Neisewander et al., 2004; Xi et al., 2004; 2005; 2006; Martelle et al., 2007; Micheli and Heidbreder, 2008; Heidbreder and Newman, 2010; Achat-Mendes, et al, 2010; Chen and Xu, 2010; Kong et al, 2011; Song et al, 2012a, b) mediate locomotor-stimulant and positive reinforcing effects of cocaine, as well as cue-induced reinstatement of cocaine-seeking.

D1 receptor-based medications have been tried to reduce euphoric effects of cocaine in addicts, and despite the D1 receptor's key role in mediating cocaine actions in preclinical studies, the results have not been consistent (Haney et al, 1999; 2001; Romach et al, 1999; Nann-Vernotica et al, 2001). Manipulating D1 receptor activity during reconsolidation may provide a new time window to treat cocaine abuse. Many D3 receptor agonists and antagonists have been developed and tested and several D3 receptor antagonists show promise for attenuating reinstatement of drug-seeking in preclinical studies (Micheli and Heidbreder, 2008; Heidbreder and Newman, 2010; Newman et al, 2012). Among the many

D3 receptor-preferring antagonists that have been developed and evaluated, PG01037 (*N*-{4-[4-(2,3-dichlorophenyl)-piperazin-1-yl]-*trans*-but-2-enyl}-4-pyridine-2-yl-benzamide) selectively blocked D3-agonist induced yawning and attenuates reinstatement of drug-seeking via pharmacological antagonism of D3 receptors (Collins et al, 2005; 2007; Xi et al, 2006; Martelle et al, 2007, Achat-Mendes et al, 2010; Higley et al, 2011). We previously used both a D3 receptor mutant mouse model and PG01037 and found that D3 receptors play a key role in reconsolidation of cocaine-induced conditioned place preference (CPP) (Yan et al, 2013), implying the involvement of DA signaling in reconsolidation of cocaine memory. The rodent model of intravenous drug self-administration mimics voluntary drug intake in humans and is arguably the best available preclinical model to study the neurobiological basis of drug-seeking and taking (O'Brien and Gardner, 2005; Epstein et al, 2006; Kalivas et al, 2006). In the current study, we have used both pharmacological and genetic approaches to investigate the role of D1 receptors, and to further study the role of D3 receptors in reconsolidation of cocaine memories in mouse models of drug self-administration.

EXPERIMENTAL PROCEDURES

Mice and drugs

The engineering of the D3 receptor mutant mouse model, which resulted in a complete loss of D3 receptors, has been described in a previous report (Xu et al, 1997). Homozygous D3 receptor mutant mice and their wild-type littermates were produced by crossing D3 receptor heterozygous mutant mice. Wild-type mice were used to test the effects of the D1 receptor antagonist SCH23390 on reconsolidation of cocaine memories and they were bred by crossing wild-type mice. Genotypes of all mice were determined by genomic Southern blotting (Xu et al, 1994a; Xu et al, 1997). Mice were group housed under controlled temperature and humidity conditions with a 12-h light/dark cycle. Water and food were available *ad libitum*. Roughly equal numbers of male and female mice, 10 to 18 weeks of age, were used. Mice weighed around 25–30 g at the beginning of the experiments. All procedures followed National Institutes of Health Guide for the Care and Use of Laboratory Animal and were approved by the University of Chicago Institutional Animal Care and Use Committee.

Cocaine hydrochloride and SCH23390 were purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in sterile 0.9% saline. Cocaine was self-administered through catheterized tubing into the jugular vein. PG01037 was synthesized at the National Institute on Drug Abuse-Intramural Research Program (Baltimore, MD) using previously published methods (Grundt et al, 2005; 2007). PG01037 was initially described (Grundt et al, 2005; 2007) as a highly potent (Ki=0.7 nM) D3 receptor-selective antagonist (133-fold over D2 receptors). Although PD01037 also showed actions with low affinities at histamine H1, 5-HT1A, 2A, 2C, α1 and α2 adrenergic receptors (Kumar et al, 2009), it preferentially binds to D3 receptors. PG01037 rapidly penetrates the blood brain barrier and selectively localizes in D3 receptor-rich regions, such as the nucleus accumbens (NAc), Islets of Calleja and the hippocampus (Grunt et al, 2007). Therefore, it is appropriate for *in vivo* studies. PG01037 was first characterized as a D3 receptor-selective antagonist using a D3 receptor agonist

yawning model in rats (Collins et al, 2005; 2007) and subsequently has been tested in numerous rodent and nonhuman primate models of psychostimulant abuse (Heidbreder and Newman, 2010). The selection of PD01037 dose in the present study was based on these behavioral studies and our recent report using a cocaine CPP paradigm (Yan et al, 2013) that demonstrated the 30 mg/kg dose was optimal for the antagonism of D3 receptors in *in vivo* studies. PG01037 was first dissolved in dimethyl sulfoxide (DMSO) and then diluted with sterile saline to 2% DMSO in saline (Yan et al, 2013). The selection of the SCH23390 doses was based on previous studies on acute locomotor activity (Xu et al, 1994a) and cocaine self-administration (Caine et al, 2007). SCH23390 and PG01037 were administered intraperitoneally (i.p.) in a volume of 10 ml/kg body weight.

Catheterization

The construction and implantation of tubing have been described in our previous reports (Yan et al, 2006; 2007; 2012). Indwelling catheters were constructed of micro-silicone tubing (inner diameter, 0.50 mm; outer diameter, 0.7 mm; Braintree Scientific Inc., Braintree, MA) and polyethylene tubing (inner diameter, 0.50 mm; outer diameter, 0.8 mm, Braintree Scientific Inc., Braintree, MA). D3 receptor mutant mice and different groups of wild-type mice were anesthetized with a combination of xylazine hydrochloride (10 mg/kg, i.p., Sigma-Aldrich, St Louis, MO) and ketamine hydrochloride (90 mg/kg, i.p., Sigma-Aldrich, St Louis, MO). Incisions were made on the skin of the head and ventral neck, and the right jugular vein was externalized. The end of the catheter was inserted into the jugular vein via a small incision and was secured to the vein and surrounding tissues with silk sutures (South Pointe Surgical Supply Inc., Coral Springs, FL). The exit port of the catheter passed subcutaneously to the top of the skull where it was attached to a modified 24-gauge cannula (Braintree Scientific Inc., Braintree, MA), which was secured to the mouse's skull with all-purpose Instant Krazy Glue (Walgreens, Chicago). Buprenorphine (Sigma-Aldrich, St Louis, MO) was subcutaneously administered (0.10 mg/kg) for postoperative analgesia once a day for at least 3 days. To extend catheter patency, the catheters were flushed once a day immediately after surgery or cocaine self-administration training with 0.05 ml of heparin in saline (30 Unit/ml; Fisher Scientific, Pittsburgh, PA).

Intravenous cocaine self-administration and extinction

Intravenous self-administration (IVSA) was conducted in standard mouse operant conditioning chambers (ENV-307A, Med Associates, Georgia, VT) located in a behavioral procedure room (Yan et al, 2006; 2007; 2012). The chambers were equipped with nose-poke sensors (ENV-313M, Med Associates) in two holes located on one side of the chamber 1.0 cm above the floor, and cue- and hole-lamps located, respectively, above and in each hole, and a house light located on the top of the chamber opposite the holes. During cocaine self-administration training, one hole was set as active and the other inactive. Nose-pokes in the active hole triggered pump (PHM-100, Med Associates) infusions (3 s) and turned on both cue-lamp and hole-lamp (10 s). Nose-pokes in the inactive hole and active hole during the timeout period (30 s) had no programmed consequences but were recorded. The components of the infusion line were connected from the injector to the exit port of the mouse's catheter by PE20 tubing (Instech, Plymouth Meeting, PA), which was encased in steel spring leashes

(Instech, Plymouth Meeting, PA). Swivels were suspended above the chamber. One pump/syringe set was located inside of each cubicle.

After recovery from the catheterization (3–7 days), mice were initially subjected to 3 h daily sessions of cocaine self-administration under a fixed ratio (FR) 1 schedule for 5 days, and the cocaine reinforcement schedule was then changed to an FR2 for an additional 9–10 days. A combination of an FR1 and FR2 schedules was selected for cocaine self-administration training in the current study because this schedule of reinforcement may facilitate self-administration training and shorten extinction training (Yan and Nabeshima, 2009). Based on previous reports (Caine et al, 2007; 2012) and our preliminary data, the unit dose of cocaine was used at 0.6 mg/kg/infusion over 3 s (infusion volume, 6.6 μl) for IVSA in our study.

Once stable cocaine self-administration, defined as deviations of less than 20% of the mean active responses in 3 consecutive training sessions, was established, mice were subjected to 3 h daily sessions of extinction training. Throughout the extinction session, the house light was on. The cocaine-associated cue- and hole-lamps, and the pump for cocaine infusions, were turned off. Therefore, nose-pokes into the previously active hole resulted in neither an infusion of cocaine nor cocaine-associated cues (cue- and hole-lamps) though responding was recorded. The extinction criterion was met when there were less than 15 active responses or 20% of active responses in the stable phase of self-administration in 2 consecutive sessions. In the current study, mice met the extinction criterion after 4–10 days of training.

Reconsolidation

Once the extinction criterion was met, mice were subjected to reconsolidation testing or no-retrieval control testing. For groups with the memory retrieval, mice were connected to self-administration manipulates for 10 min. Based on our preliminary data and previous reports (Sanchez et al, 2010; Wells et al, 2013), a 10-min interval was selected for the retrieval. During this period, mice were exposed to cocaine-associated cues, but with no infusion of cocaine, after active nose-pokes. If there was no active nose-poke response made in the first 6 min, non-contingent cocaine-associated cues were presented 3 times with 1-min intervals. SCH23390, PG01037, or their vehicles were i.p. administered immediately after the 10-min retrieval. All mice were returned to their home cages afterwards. 24 h, 48 h or 1 week after the 10-min retrieval, mice were tested for reconsolidation of cocaine self-administration under FR2 schedule for 3 h, during which there were cocaine-associated cues but no cocaine infusions after active nose-poke responses were presented.

For control groups with no memory retrieval, mice were taken to the behavioral testing room and they were not connected to self-administration manipulates. Mice were given an i.p. injection of SCH23390, or its vehicles, and they were returned to their home cages in the housing room. 24 h, 48 h or 1 week after the treatment of drugs or vehicles, mice were tested for reconsolidation of cocaine self-administration under FR2 schedule for 3 h, during which there were cocaine-associated cues but no cocaine infusions after active nose-poke responses were presented.

For effects of the D3 receptor gene mutation on reconsolidation of cocaine self-administration, D3 receptor mutant mice and wild-type littermates were connected to self-administration manipulates for 10 min, but were without any drug or vehicle treatment after the 10-min retrieval. Mice were returned to their home cages afterwards. No-retrieval groups of D3 receptor mutant mice and wild-type littermates were taken to the behavioral room and they were not connected to self-administration manipulates. Mice were not given drug or vehicle treatment and they were returned to their home cages. 24 h, 48 h or 1 week afterwards, mice were subjected to testing for reconsolidation of cocaine self-administration under FR2 schedule for 3 h, during which there were cocaine-associated cues but no cocaine infusions after active nose-poke responses were presented.

Data analysis

All data were expressed as the mean ± SEM. A repeated measures ANOVA was used to analyze the data from acquisition of IVSA in wild-type mice for D1 receptor antagonist treatment. A two-way ANOVA with repeated measures was used to analyze the data from acquisition of IVSA for wild-type and D3 receptor mutant mice, effects of the D1 receptor antagonist SCH23390 and D3 receptor antagonist PG01037, and the D3 receptor gene mutation on reconsolidation of cocaine self-administration. All *post hoc* ANOVAs were followed by Bonferroni multiple comparisons (Yan et al, 2013). In all cases, a significant difference was set at $P<0.05$.

RESULTS

Effects of the D1 receptor antagonist SCH23390 on reconsolidation of cocaine memory

After recovery from the catheterization, wild-type mice were subjected to 3 h daily sessions of cocaine self-administration training for 5 days under an FR1 schedule of reinforcement and then for 9 additional days under an FR2 schedule of reinforcement. A repeated measures ANOVA analysis revealed that mice started to discriminate active from inactive nose-pokes on day 4 and acquired stable cocaine self-administration after 14 days of training [Fig 1, $F(31, 403) = 9.88$, $P< 0.001$].

Starting on day 15, mice were subjected to 3 h daily sessions of extinction training for 4–10 days. Once the extinction criterion was met (Fig 2A), mice were then divided into three sub-groups. On the next day, the three sub-groups of mice were subjected to a 10-min memory retrieval followed immediately by an i.p. injection of saline or SCH23390 (0.08 or 0.22 mg/kg). 24 h, 48 h, and 1 week after the 10-min retrieval, the three sub-groups of mice were subjected to reconsolidation testing. Two-way ANOVA analysis with different SCH23390 doses and behavioral testing as fixed factors indicated that SCH23390 attenuated reconsolidation of cocaine self-administration in mice [Fig 2A, $F(2, 21) = 3.70$, $P= 0.04$]. This attenuation lasted for at least 1 week (Fig 2A). Without the 10-min memory retrieval, two-way ANOVA analysis with SCH23390 treatment and behavioral testing as fixed factors indicated that there was no significant difference in cocaine self-administration between the saline- and SCH23390-treated groups (0.22 mg/kg, i.p. administered 24 h prior to the reconsolidation testing) [Fig 2B, $F(1, 7) = 0.55$, $P= 0.48$]. Together, these results

demonstrate that the blockade of D1 receptors with SCH23390 attenuated reconsolidation of cocaine memory in mouse models of drug self-administration.

Effects of a D3 receptor gene mutation on reconsolidation of cocaine memory

To further study the role of D3 receptors in reconsolidation of cocaine memory, we first used D3 receptor mutant mice and wild-type littermates. These mice were subjected to 3 h daily sessions of cocaine self-administration training for 5 days under an FR1 schedule of reinforcement and then for 10 additional days under an FR2 schedule of reinforcement. As shown in Fig 3, wild-type mice started to discriminate active from inactive nose-pokes on day 6 and D3 receptor mutant mice on day 4. Both groups of mice acquired stable cocaine self-administration following the 15 day training [$F(14, 840) = 11.18$, $P<0.001$]. Two-way ANOVA analysis with genotypes and behavioral testing as fixed factors suggested that there was a significant difference in active nose-pokes between D3 receptor mutant mice and their wild-type littermates [$F(3, 60) = 41.48$, $P< 0.001$]. The *post-hoc* analysis with Bonferroni multiple comparisons indicated that D3 receptor mutant mice did more active nose-pokes than their wild-type littermates on day 8 ($P<0.05$). In contrast, there was no significant difference in inactive nose-pokes between D3 receptor mutant mice and wild-type littermates ($P>0.05$).

From day 16 on, D3 receptor mutant mice and wild-type littermates were subjected to 3 h daily sessions of extinction training until the extinction criterion was met (Fig 4A). On the next day, both groups of mice were subjected to a 10-min memory retrieval in operant chambers. 24 h, 48 h and 1 week after the 10-min retrieval, these mice were tested for reconsolidation. Two-way ANOVA analysis with genotypes and behavioral testing as fixed factors suggested that the mutation of D3 receptor gene in mice disrupted the reconsolidation of cocaine memory [Fig 4A, $F(1, 23) = 9.89$, $P= 0.005$]. This disruption lasted for at least 1 week (Fig 4A). In the absence of the 10-min memory retrieval, although the D3 receptor gene mutation showed a tendency toward reduced active nose-pokes for cocaine-seeking behavior when tested 24 h later, two-way ANOVA analysis with genotypes and testing as fixed factors indicated that D3 receptor mutation did not affect cocaine self-administration with no memory retrieval [Fig 4B, $F(1, 20) = 0.94$, $P= 0.34$]. These results suggest that the mutation of the D3 receptor gene disrupted reconsolidation of cocaine memories.

Effects of the D3 receptor selective antagonist PG01037 on reconsolidation of cocaine memory

To further confirm the role of D3 receptors in reconsolidation of cocaine memories, we examined the effects of the selective D3 receptor antagonist PG01037 on reconsolidation in wild-type mice and D3 receptor mutant littermates. Both groups of mice were subjected to 3 h daily sessions of cocaine self-administration training for 5 days under an FR1 schedule of reinforcement and then for 10 additional days under an FR2 schedule of reinforcement (Fig 5A). Starting on day 16, mice were subjected to 3 h daily sessions of extinction training. Once the extinction criterion was met (Fig 5A), each group of mice was divided into two sub-groups. On the next day, all groups of mice were subjected to a 10-min memory retrieval followed immediately by an i.p. injection of PG01037 (30 mg/kg) or its vehicle. 24

h, 48 h and 1 week after the 10-min retrieval, these mice were tested for reconsolidation. Two-way ANOVA analysis with PG01037 treatment and behavioral testing as fixed factors indicated that PG01037 at 30 mg/kg (i.p.) blocked the reconsolidation of cocaine memories in wild-type mice [Fig 5B, $F(1, 22) = 12.27$, $P= 0.0004$], and that reconsolidation in D3 receptor mutant mice was attenuated after vehicle treatment [$F(1, 23) = 13.50$, $P= 0.0013$]. In contrast, the PG01037 treatment in D3 receptor mutant mice did not significantly affect the attenuation of the reconsolidation of cocaine memories caused by the D3 receptor gene mutation. Taken together, these results suggest that D3 receptors play a critical role in reconsolidation of cocaine memory in mouse models of drug self-administration.

DISCUSSION

Memories of drug experience and drug-associated cues can elicit drug craving and relapse in humans. Emerging studies demonstrated that molecular manipulations of the *N*-methyl-*D*-aspartate receptor, and the extracellular signal-regulated kinase and protein kinase A-mediated signaling events in the context of reconsolidation of drug-induced reward memory can reduce drug craving and seeking behavior in animal models (Lee et al, 2005; 2006; Miller and Marshall, 2005; Valjent et al, 2006; Taylor et al, 2009; Sanchez et al, 2010) and in drug addicts (Xue et al, 2012). These findings suggest that identifying novel molecular targets of reconsolidation may aid the treatment of drug abuse (Sorg, 2012; Tronson and Taylor, 2013). We and others have demonstrated that DA D1 and D3 receptors play differential roles in acquiring cocaine-induced behaviors (Xu et al, 1994a; 1994b, 2007; Caine et al, 2007; Chen and Xu, 2010; Kong et al, 2011). Moreover, D3 receptors contribute to the reconsolidation of cocaine-induced CPP (Yan et al, 2013). In this study, we further studied dopaminergic mechanisms of reconsolidation cocaine memories. Our findings demonstrate that D1 and D3 receptors play critical roles in reconsolidation of cocaine memories, and that these receptors may serve as novel targets for the treatment of cocaine abuse in humans.

Role of D1 receptors in reconsolidation of cocaine memory

We previously used D1 receptor mutant mice and found that they do not acquire cocaine-induced CPP at several doses (Chen and Xu, 2010) and acquire little cocaine self-administration even after extended training (Caine et al, 2007). Although the D1 receptor mutant mouse model was very useful in studying functions of D1 receptors in the acquisition of cocaine-induced CPP and operant behaviors as well as underlying molecular mechanisms, this mouse model cannot be used to evaluate the role of D1 receptors in reconsolidation of cocaine memory. Consequently, we used a pharmacological approach to address this issue in the current study. We first trained wild-type mice to acquire stable cocaine self-administration behavior (Fig 1). Following extinction training and after the extinction criterion has been met (Fig 2A), we administered i.p. SCH23390 immediately following the 10-min memory retrieval in these mice. We found that SCH23390, at both the 0.08 and 0.22 mg/kg doses, attenuated reconsolidation of cocaine self-administration (Fig 2A). Such attenuation in reconsolidation remained at least 1 week after the memory retrieval (Fig 2A). In the absence of the 10-min retrieval, mice in the control group showed normal levels of reconsolidation (Fig 2B). These results suggest that D1 receptors contribute to the

reconsolidation of cocaine self-administration in mice. To the best of our knowledge, this is the first demonstration that D1 receptors contribute to reconsolidation of cocaine-induced reward memory. D1 receptor-based medications have been tried to treat cocaine addicts yet the results have not been consistent (Haney et al, 1999; 2001; Romach et al, 1999; Nann-Vernotica et al, 2001). Our current results provide preclinical evidence for a potential new time window for developing D1 receptor-based treatment for cocaine abuse.

Role of D3 receptors in reconsolidation of cocaine memory

We previously used D3 receptor mutant mice and found that they exhibit potentiated acquisition of cocaine-induced CPP at lower, but not higher doses of cocaine compared to their wild-type littermates (Chen and Xu, 2010; Kong et al, 2011). Others have shown that D3 receptor mutant mice exhibit enhanced (Song et al, 2012a) or relatively normal acquisition of cocaine self-administration (Caine et al, 2012). In the current study, we found that D3 receptor mutant mice did more active nose-pokes than their wild-type littermates during acquisition training (Fig 3). Following extinction training and after the extinction criterion has been met, we found that the mutation of the D3 receptor gene in mice reduced reconsolidation of cocaine self-administration (Fig 4A). Such reduction in reconsolidation lasted for at least 1 week after the memory retrieval (Fig 4A). Moreover, in the absence of the retrieval, there was no significant difference in cocaine-seeking between wild-type and D3 receptor mutant mice (Fig 4B). This is consistent with our previous studies using the D3 receptor mutant mice and a CPP paradigm (Yan et al, 2013), and it further suggests that D3 receptors participate in mechanisms related to reconsolidation of cocaine-induced reward memory.

PG01037 is an antagonist which shows high affinity and selectivity for D3 receptors *in vitro* and *in vivo* (Grundt et al, 2005; 2007; Micheli and Heidbreder, 2008; Heidbreder and Newman, 2010). We previously found that PG01037 administration immediately following a 3-min memory retrieval disrupted reconsolidation of cocaine-induced CPP in wild-type mice and such disruption remained at least 1 week after the retrieval (Yan et al, 2013). With no memory retrieval, PG01037 did not affect cocaine-induced CPP (Yan et al, 2013). To expand this finding and those using the D3 receptor mutant mice, following acquisition and extinction training, we administered PG01037 immediately following the 10-min memory retrieval in wild-type and D3 receptor mutant mice. PG01037 but not vehicle attenuated reconsolidation of cocaine self-administration at the 30 mg/kg dose in wild-type mice (Fig 5). Moreover, such attenuation in reconsolidation remained at least 1 week after the retrieval (Fig 5). PG01037 treatment in D3 receptor mutant mice did not significantly affect the attenuation of the reconsolidation of cocaine memories caused by the D3 receptor gene mutation, although it does appear that there was some minor pharmacological effect when reconsolidation was tested at 48 h time point, possibly due to non-specific antagonism of PG01037 at DA D2 and other receptors.

We note that under no retrieval conditions, D3 receptor mutant mice showed a tendency toward a reduction in nose-pokes compared to that exhibited by wild-type littermates (Fig 4B). One possible explanation is that, due to the lack of D3 receptors, these mutant mice show deficits in memory retrieval for cocaine-seeking. Supporting this possibility is the fact

that a D3 receptor antagonist, YQA14, reduces cocaine-seeking behavior in mice (Song et al, 2012b). The fact that D3 receptor mutant mice showed a loss of reconsolidation after memory retrieval (Fig 4A) and a non-significant reduction in nose-pokes without the retrieval (Fig 4B), combined with results from the pharmacological studies described above, is consistent with the interpretation that D3 receptors contribute to reconsolidation of cocaine-induced reward memory as analyzed in mouse models of drug self-administration.

Potential mechanisms and conclusions

Our current studies using both pharmacological and genetic approaches suggest that D1 and D3 receptors play key roles in reconsolidation of cocaine-induced reward memories. We and others have demonstrated that D1 and D3 receptors play differential roles in acquiring cocaine-induced behaviors (Xu et al, 1994b; 1997; 2000; Caine et al, 2007; Chen and Xu, 2010; Kong et al, 2011) and that underlying molecular mechanisms are also different (Zhang et al, 2002; Zhang et al, 2004; Jiao et al, 2007; Liu et al, 2009; Chen and Xu, 2010). D3 receptors are known to have higher affinities for DA than D1 receptors (Sokoloff et al, 1992). It has been thought that D2 family receptors including D3 receptors can respond to tonic DA release while D1 receptors are activated when there is phasic DA release (Goto et al., 2007; Grace et al., 2007). A large percentage of D3 receptor-bearing neurons co-express D1 receptors, especially in the NAc (Surmeier et al, 1996; Schwartz et al, 1998). D3 receptors are activated by basal levels of DA to inhibit adenylyl cyclase and related signaling including extracellular signal-regulated kinase and Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα). When DA release is enhanced and DA levels increase, CaMKIIα phosphorylates D3 receptors at the serine 229 site in the third intracellular loop (Liu et al, 2009). As a result, the inhibitory tone of D3 receptors on cocaine-induced behavior and related signaling is removed. This allows other DA receptors including D1 receptors to fully mediate behavioral activation and related signaling induced by cocaine. We found that inhibition of functions of either D1 or D3 receptors attenuates reconsolidation of cocaine memories. This result suggests that responses to both tonic and phasic DA release are needed for the reconsolidation process.

D1 and D3 receptors are expressed in brain reward circuits including the basolateral amygdala, the prefrontal cortex, and NAc (Beaulieu and Gainetdinov, 2011). These different brain regions play an important role in reconsolidation of drug memories (Théberge et al, 2010; Otis et al, 2013). D1 and D3 receptors expressed in these brain regions may participate in the reconsolidation of cocaine-induced reward memories. Indeed, abnormal levels of D1 receptor availability have been linked to an increased risk of cocaine taking in cocaine-dependent subjects (Martinez et al, 2009). Moreover, D3 receptors may be upregulated in these places in the brains of cocaine and methamphetamine abusers (Staley and Mash, 1996; Boileau et al, 2012). It is also intriguing to consider that D1–D3 receptor heteromers (Marcellino et al, 2008, Ferre et al, 2014) in these discrete brain regions may play a role in drug memory reconsolidation. Despite a need for additional studies, our current results suggest that pharmacological blockade of either D1 or D3 receptors in the context of drug memory reconsolidation may be therapeutic for the treatment of cocaine craving and relapse in clinical settings.

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ABBREVIATIONS

CaMKIIα	Ca^{2+}/calmodulin-dependent protein kinase IIα
CPP	conditioned place preference
DMSO	dimethyl sulfoxide
DA	dopamine
FR	fixed ratio
i.p	intraperitoneally
IVSA	intravenous self-administration
PG01037	N-{4-[4-(2,3-dichlorophenyl)-piperazin-1-yl]-trans-but-2-enyl}-4-pyridine-2-yl-benzamide
NAc	nucleus accumbens

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- Pharmacological blockade of D1 or D3 receptors disrupts reconsolidation of cocaine memory
- Genetic mutations in D3 receptors attenuate reconsolidation of cocaine-induced reward memory
- Dopamine D1 and D3 receptors may serve as new targets for combating cocaine abuse

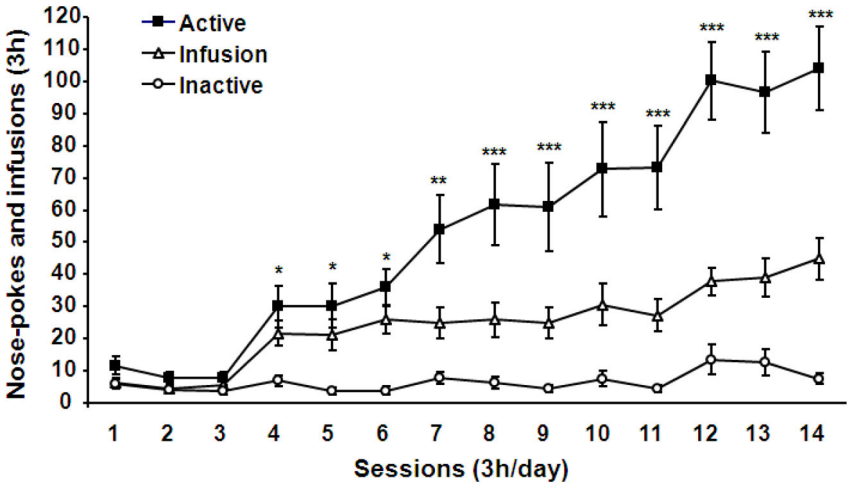

Fig. 1.

Acquisition of intravenous cocaine self-administration in wild-type mice. Day 1–5: FR1 schedule of cocaine reinforcement, and day 6–14: FR2 schedule of cocaine reinforcement. N = 16 mice. Data represent mean±SEM. *P<0.05, **P<0.01, and ***P<0.001 active versus inactive nose-pokes.

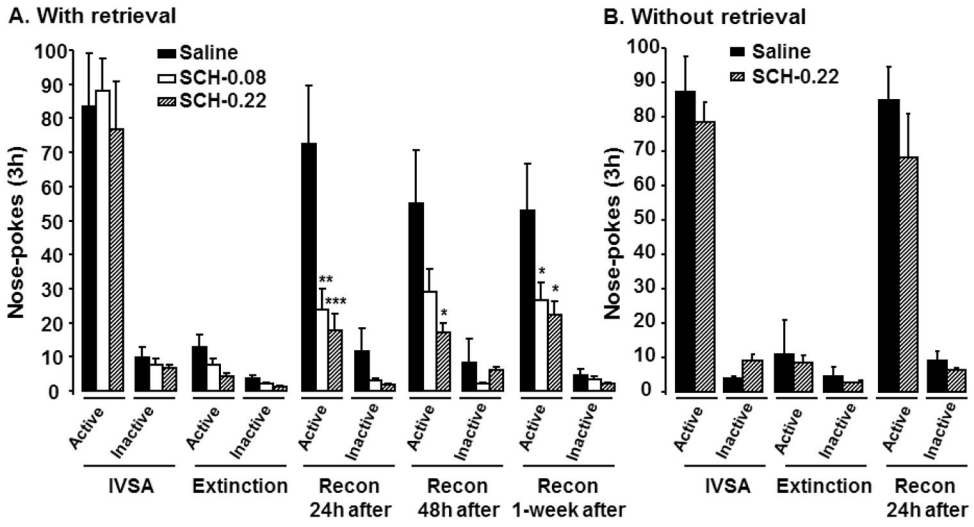

Fig. 2.

Effects of the D1 receptor antagonist SCH23390 on reconsolidation of cocaine self-administration in wild-type mice. Wild-type mice were subjected to 3 h daily self-administration training and showed stable IVSA. Mice then went through 3 h extinction training daily. Extinction data were from the last day of training. Once the extinction criterion was met, mice were subjected to a 10-min retrieval. Reconsolidation was tested 24 h, 48 h or 1 week after the retrieval **(A)**, or 24 h after no retrieval manipulations **(B)**. SCH23390 was administered i.p. immediately after the retrieval or during no retrieval manipulations. N = 6–8 mice for each group. Data represent mean ± SEM. *P<0.05, **P<0.01, and ***P<0.001 SCH23390 versus saline treatment. Recon: reconsolidation. SCH-0.08: SCH23390-0.08 mg/kg. SCH-0.22: SCH23390-0.22 mg/kg.

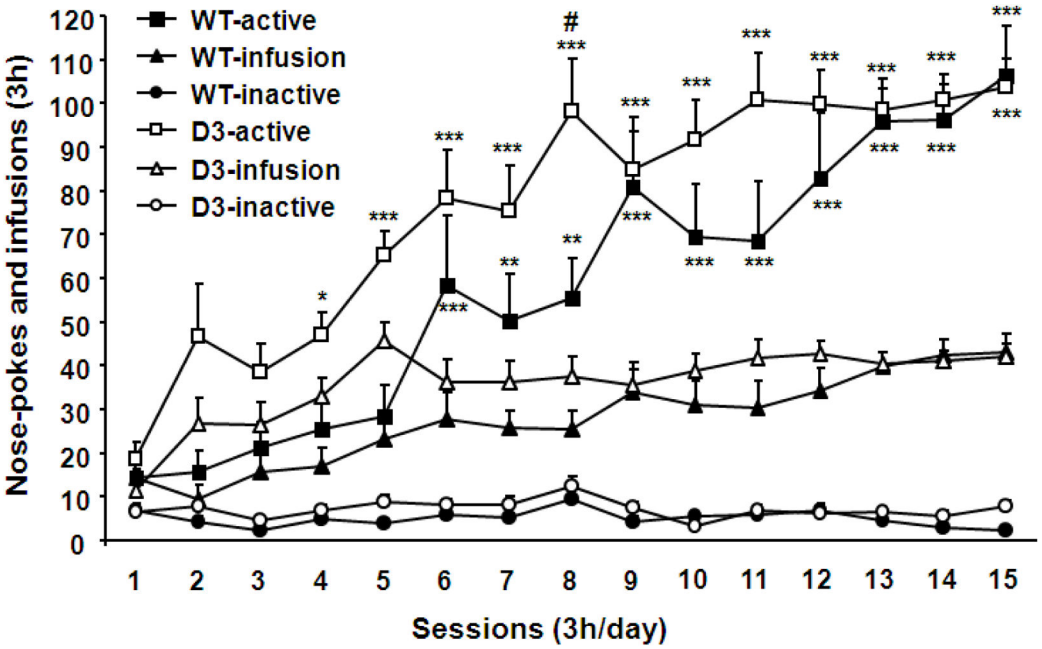

Fig. 3.

Acquisition of intravenous cocaine self-administration in D3 receptor mutant mice and wild-type littermates. Day 1–5: FR1 schedule of cocaine reinforcement, and day 6–15: FR2 schedule of cocaine reinforcement. N = 14–18 mice for each group. Data represent mean ± SEM. *P<0.05, **P<0.01, and ***P<0.001 active versus inactive nose-pokes. $^{\#}$P<0.05 active nose-pokes in D3 receptor mutant versus those in wild-type mice.

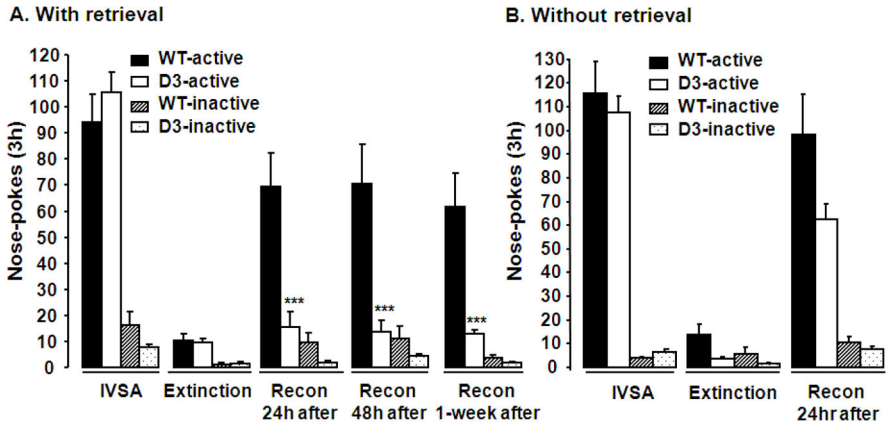

Fig. 4.

Effects of a D3 receptor gene mutation on reconsolidation of cocaine self-administration. D3 receptor mutant mice and wild-type littermates were trained to stably self-administer cocaine. Mice were then subjected to extinction training. Extinction data were from the last day of training. Once the extinction criterion was met, mice were connected to IVSA manipulates for 10 min, during which mice could trigger the presentation of drug-associated cues, but were with no cocaine infusions. Reconsolidation was tested 24 h, 48 h or 1 week after the retrieval **(A)**, or 24 h after no retrieval manipulations **(B)**. N = 8–13 for each group. Data represent mean ± SEM. ***P<0.001 active nose-pokes in D3 receptor mutant versus those in wild-type mice. Recon: reconsolidation.

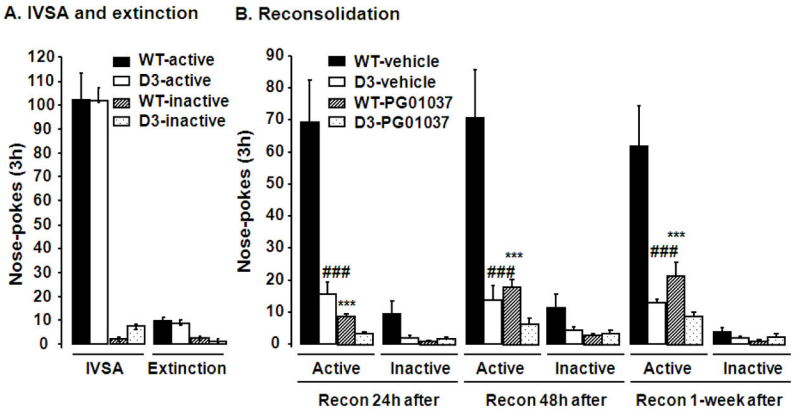

Fig. 5.

Effects of the D3 receptor antagonist PG01037 on reconsolidation of cocaine self-administration. Wild-type and D3 receptor mutant mice were subjected to daily IVSA training and showed stable acquisition **(A)**. Once the extinction criterion was met following daily extinction training **(A)**, mice were subjected to a memory retrieval for 6–10 min. Reconsolidation was tested 24 h, 48 h or 1 week after the retrieval **(B)**. PG01037 (30 mg/kg) or its vehicle was administered i.p. immediately after the retrieval. N = 8–13 for each group. Data represent mean ± SEM. ***P<0.001 PG01037 versus vehicle treatment in wild-type mice. $^{###}$P<0.001 D3 receptor mutant versus wild-type mice with vehicle treatment. Recon: reconsolidation. Pharmacological blockade of D1 or D3 receptors disrupts reconsolidation of cocaine memory Genetic mutations in D3 receptors attenuate reconsolidation of cocaine-induced reward memory Dopamine D1 and D3 receptors may serve as new targets for combating cocaine abuse