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Dopamine D_3 receptor deletion or blockade attenuates cocaine-induced conditioned place preference in mice

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ABSTRACT

The dopamine (DA) D_3 receptor (D_3R) has received much attention in medication development for treatment of addiction. However, the functional role of the D_3R in drug reward and addiction has been a matter of debate. We recently reported that D_3 receptor-knockout ($D_3^{-/-}$) mice display increased vulnerability to cocaine self-administration, which we interpret as a compensatory response to attenuated cocaine reward after D_3R deletion. Here we report that $D_3^{-/-}$ mice displayed attenuated cocaine-induced conditioned place response (CPP) compared to wild-type mice. Similarly, blockade of brain D_3Rs by YQA-14, a novel DA D_3 receptor antagonist, significantly and dose-dependently inhibits acquisition and expression of cocaine-induced CPP in WT mice, but not in $D_3^{-/-}$ mice. These findings suggest that: 1) D_3Rs play an important role in mediating cocaine's rewarding effects; and 2) YQA-14 is a highly potent and selective D_3R antagonist *in vivo*, which deserves further study as a candidate for treatment of cocaine addiction.

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1. Introduction

The mesocorticolimbic dopamine (DA) system, which projects from midbrain DA neurons to the forebrain nucleus accumbens (NAc) and prefrontal cortex (PFC), constitutes a central component of the brain's reward circuitry. Cocaine elevates extracellular DA in the NAc by blocking the presynaptic DA transporter (DAT) (Wise, 1996; Sulzer, 2011). However, the *receptor* mechanisms underlying cocaine reward and addiction are not fully understood.

Five DA receptor subtypes (D_1–D_5) have been identified in the brain, which are classified as D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) based on their respective pharmacological profiles (Beaulieu and Gainetdinov, 2011). Since genetic deletion of D_1 or D_2 receptors abolishes or largely reduces the reinforcing properties of cocaine (Caine et al., 2002, 2007; Holmes et al., 2004), it is generally believed that the D_1 and D_2 receptor subtypes are critically involved in cocaine reward. In contrast, the role of the D_3 receptor (D_3R) in cocaine's actions remains somewhat uncertain. Given that the D_3R has extremely high affinity for DA (Sokoloff et al., 1992) and enriched distribution in the mesolimbic DA system (Bouthenet et al., 1991; Levesque et al., 1992; Diaz et al., 1995), it has been proposed that the D_3R plays an important role in mediating DA's or cocaine's actions (Sokoloff et al., 1992). This is supported by findings that the D_3R-preferring agonists 7-OH-DPAT or PD128908 potentiate, while the D_3R antagonists nafadotride, SB-277011A, NGB-2904 and YQA-14 attenuate cocaine's rewarding efficacy in the rat self-administration and electrical brain-stimulation reward models (Caine and Koob, 1993; Caine et al., 1997; Xi et al., 2005, 2006; Song et al., 2012a). Further, 7-OH-DPAT substitution maintains self-administration in rodents and non-human primates previously self-administering cocaine (Caine and Koob, 1993; Nader and Mach, 1996), suggesting that D_3R activation produces reinforcing effects. This is further supported by findings that selective D_3R blockade by SB-277011A or NGB-2904 inhibits cocaine-induced reinstatement (relapse) to drug-seeking behavior (Vorel et al., 2002; Xi et al., 2005, 2006). Such data suggest that the D_3Rs, at least in part, mediate cocaine reward and relapse to cocaine-seeking behavior.

However, this view has been challenged by the following: First, the D_3R-preferring agonists used in many of the above-cited experiments (7-OH-DPAT, PD-128908) have low (10–20 fold) selectivity for D_3Rs over D_2Rs (Seeman and Van Tol, 1994; Pugsley et al., 1995), and are reported to produce similar biological effects in both WT and $D_3^{-/-}$ mice (Boulay et al., 1999; Xu et al., 1999), suggesting

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D_2R-mediated effects. Second, blockade of D_3R antagonists by SB-277011A or NGB2904 has no effect on cocaine self-administration under low fixed-ratio (FR1 or FR2) reinforcement (Xi et al., 2005, 2006); and third, $D_3^{-/-}$ mice exhibit enhanced conditioned place preference (CPP) response to very low doses (1, 2.5 mg/kg) of cocaine (Kong et al., 2011) and reduced CPP extinction (Chen and Xu, 2010), suggesting that D_3R activation may produce an inhibitory effect on cocaine's action. The reasons for these conflicting findings are unclear. We have recently reported that another strain of $D_3^{-/-}$ mice provided by JAX® Laboratory (Accili et al., 1996) displays increased vulnerability to cocaine as assessed by enhanced cocaine-taking and cocaine-seeking behavior compared to wild-type (WT) mice (Song et al., 2012b). However, this enhanced vulnerability to cocaine was accompanied by diminished DA-elevating responses to cocaine, suggesting that the enhanced cocaine-taking and cocaine-seeking behaviors could be compensatory behavioral responses to attenuated cocaine reward after D_3R deletion (Song et al., 2012b).

To further test this hypothesis, we here used the CPP paradigm with the same strain of $D_3^{-/-}$ mice as we used in the above-cited cocaine self-administration study (Song et al., 2012b) to compare CPP response to a commonly used dose of cocaine (20 mg/kg) between WT and $D_3^{-/-}$ mice. We then investigated whether pharmacological blockade of D_3Rs with YQA-14, a novel highly potent and selective D_3R antagonist (Song et al., 2012a), alters CPP response to cocaine. YQA-14 shows similar pharmacological properties than SB-277011A. *In vitro* receptor binding assays suggest that YQA14 has two binding sites on human cloned D_3 receptors with $K_{i\text{-High}}$ (0.68×10^{-4} nM) and $K_{i\text{-Low}}$ (2.11 nM), and displays >150-fold selectivity for D_3 over D_2 receptors and >1000-fold selectivity for D_3 over other DA receptors (Song et al., 2012a).

2. Materials and methods

2.1. Animals

Male wild-type (WT) and D_3R knockout ($D_3^{-/-}$) mice with C57BL/6J genetic backgrounds were bred at the National Institute on Drug Abuse (NIDA) from three $D_3^{+/-}$ breeding pairs purchased from the Jackson Laboratory (Bar Harbor, ME, USA). This strain of $D_3^{-/-}$ mice expresses a truncated D_3R, including the extracellular N-terminal, the first intracellular loop and part of the second intracellular loop (a total of 148 residues), while lacking the downstream sequences from the second intracellular loop (from residue 149) (Accili et al., 1996). Genotyping was performed in our laboratory according to protocol from Charles River Laboratories International Inc. (Wilmington, MA, USA). All mice used in the present experiments were matched for age (8–14 weeks) and weight (25–35 g). They were housed individually in a climate-controlled animal colony room on a reversed light–dark cycle (lights on at 7:00 PM, lights off at 7:00 AM) with free access to food and water. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Academy of Sciences, and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health.

2.2. Apparatus

The CPP apparatus (MED-CPP-3013, Med Associates, Georgia, VT, USA) composed of two large compartments ($17.4 \times 12.7 \times 12.7$ cm^3) and a center corridor ($11.7 \times 12.7 \times 12.7$ cm^3). One compartment was black with a stainless steel grid floor consisting of rods (3.2 mm diameter) placed 7.9 mm apart. The other compartment was white with 6.35^2 mm stainless steel mesh floor. The center corridor was neutral gray, with a smooth polyvinyl chloride floor. Each compartment had a Plexiglas top with a light on it. Infrared beams detected movement of animals from one CPP compartment to another, and were connected to a computer. Data were acquired using SOF-700RA-4 software (Med Associates, Georgia, VT, USA).

2.3. Procedure

We used an unbiased conditioned place preference procedure. The procedure is illustrated in Fig. 1.

2.3.1. Pre-conditioned phase

On days 1–3, mice were placed in the center corridor and provided free access to the other two compartments for 20 min daily. The time spent in each compartment was recorded. This habituation was to eliminate biased mice (defined operationally as spending over 800 s in either compartment).

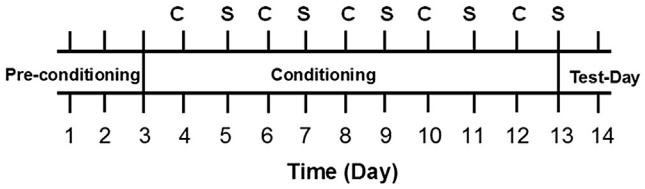

Fig. 1. The cocaine-induced conditioned place preference procedure used for these experiments. C = Cocaine; S = Saline.

2.3.2. Cocaine-conditioning phase

On each of the next 10 days (days 4–13) mice received vehicle (saline) or one dose of cocaine (5, 10, 20 mg/kg, i.p.) on alternating days in 4 separate groups of mice corresponding to the four cocaine doses, after which each mouse was confined to a randomly designated treatment-appropriate CPP compartment for 15 min.

2.3.3. YQA14-conditioning phase

We used additional two groups of mice ($n = 12$ per group) to determine whether YQA14 alone produces CPP or CPA. After pre-conditioning, mice received vehicle (25% beta-cyclodextrin) or one dose of YQA14 (25, 30 mg/kg, i.p.) on each of the next 10 days (days 4–13) on alternating days. Then each mouse was confined to a randomly designated treatment-appropriate CPP compartment for 15 min.

2.3.4. Test phase

On day 14, the mice were again placed in the center corridor and provided free access to the other two compartments for 15 min. In this phase, no cocaine or saline was given. The computer recorded the time spent in the each chamber. The preference (i.e., CPP score) was assessed by time (sec) spent in the cocaine- or YQA14-paired compartment minus time spent in the vehicle-paired compartment.

2.4. Experiments

2.4.1. Exp. 1: dose-orderly cocaine-induced CPP

In this experiment, we used 3 groups of WT mice ($n = 12$–16 per group) to observe cocaine-induced dose-dependent CPP response (5, 10, 20 mg/kg). After 10 days of cocaine-saline conditioning, the CPP scores were evaluated on the following day (48 h after the last cocaine injection or 24 h after last saline injection) (see Fig. 1 for time-line of experiment).

2.4.2. Exp. 2: cocaine-induced CPP in WT and $D_3^{-/-}$ mice

Given that 20 mg/kg cocaine produced the most robust and reliable CPP response, we compared the CPP response to 20 mg/kg cocaine between WT ($n = 16$) and $D_3^{-/-}$ ($n = 17$) mice to study whether D_3R deletion alters learning and memory associated with acute cocaine reward.

2.4.3. Exp. 3: YQA14-induced CPP in WT mice

In addition, we also evaluated whether YQA14, at the doses of 25 or 50 mg/kg, produces CPP or conditioned place aversion (CPA) in two groups of WT mice.

2.4.4. Exp. 4: effects of YQA-14 on acquisition of cocaine-induced CPP in WT and $D_3^{-/-}$ mice

We then investigated whether blockade of D_3R by YQA-14 alters cocaine-induced CPP, as seen by D_3R deletion. Three groups of WT mice ($n = 9$–12 per group, between-subjects design) and three groups of $D_3^{-/-}$ mice ($n = 6$–12 per group) were used to study the effects of chronic daily YQA-14 administration on acquisition of cocaine-induced CPP. During the 10 days of conditioning, each mouse was given vehicle (25% 2-hydroxypropyl-β-cyclodextrin) or one dose of YQA-14 (25 or 50 mg/kg) 30 min prior to the daily cocaine or saline injection. The cocaine dose was 20 mg/kg, and each animal received a total of 10 daily YQA-14 injections. On the test day, CPP was measured in the absence of any drug treatment (no YQA-14, no vehicle, no cocaine, no saline).

2.4.5. Exp. 5: effects of YQA-14 on expression of cocaine-induced CPP in WT and $D_3^{-/-}$ mice

Three groups of WT mice ($n = 7$–8 per group) and three groups of $D_3^{-/-}$ mice ($n = 7$–9 per group) were used to study the effects of a single injection of YQA-14 on expression of cocaine-induced CPP on the CPP test day. The cocaine dose was 20 mg/kg. YQA-14 (25 or 50 mg/kg i.p.) was given 30 min prior to testing on the test day in the absence of cocaine or saline injection.

2.5. Drugs

Cocaine HCl (Sigma Chemical Co., Saint Louis, MO, USA) was dissolved in physiological saline. YQA-14 was synthesized at the Beijing Institute of

Pharmacology and Toxicology and dissolved in vehicle, i.e., 25% 2-hydroxypropyl-β-cyclodextrin (Sigma, St Louis, MO).

2.6. Data analysis

The CPP score was defined as time spent in the cocaine- or YQA14-paired compartment minus time spent in the vehicle-paired compartment and is presented as mean ± S.E.M. One-way analysis of variance (ANOVA) was used to analyze differences in CPP scores between WT and $D_3^{-/-}$ mice or between different drug dose groups. Individual group comparisons were performed using Student–Newman–Keuls method. Paired *t*-tests were used to analyze the statistical significance of cocaine-induced CPP before (preconditioning) and after cocaine conditioning.

3. Results

3.1. Cocaine induced dose-dependent CPP

Fig. 1 shows the time-line for experimental procedures of the initial cocaine- or YQA14-induced CPP experiment. Each animal received 5 days of cocaine or YQA14 injections and 5 days of vehicle (saline or 25% beta-cyclodextrin) treatment during the conditioning phase. Fig. 2A shows the dose-orderly cocaine-induced CPP observed in WT mice. Since 20 mg/kg cocaine-induced CPP in both the vehicle control groups in Exp. 3 and Exp. 4 is substantially comparable, we pooled both vehicle group data to increase the power for statistical analysis. Paired *t*-tests revealed statistically significant increases in cocaine-induced CPP after 10 mg/kg ($t = 4.67$, $P < 0.001$) or 20 mg/kg ($t = 4.30$, $P < 0.001$), but not 5 mg/kg ($t = 1.96$, $P > 0.05$) cocaine, compared to vehicle control group.

3.2. $D_3^{-/-}$ mice display attenuated CPP response to cocaine

Fig. 2B illustrates that, compared to pre-conditioning, both WT and $D_3^{-/-}$ groups of mice showed statistically significant increases in cocaine-induced CPP (WT: $t = 3.30$, $P < 0.01$; $D_3^{-/-}$: $t = 3.36$, $P < 0.01$). However, when compared to WT mice, $D_3^{-/-}$ mice displayed a statistically significant reduction in cocaine-induced CPP (one-way ANOVA, $F_{1,15} = 4.59$, $P < 0.05$).

3.3. Chronic YQA-14 pretreatment inhibits acquisition of cocaine-induced CPP in WT mice, but not in $D_3^{-/-}$ mice

Fig. 3 illustrates that repeated YQA-14 (25 or 50 mg/kg, i.p., for 10 days) pretreatment significantly inhibited acquisition of cocaine-induced CPP in a dose-dependent manner in WT mice (one-way ANOVA, $F_{2,37} = 3.85$, $P < 0.05$), but not in $D_3^{-/-}$ mice ($F_{2,32} = 0.20$, $P > 0.05$). Post hoc individual group comparisons revealed a statistically significant reduction in cocaine-induced CPP in WT mice after 50 mg/kg ($t = 6.04$, $P < 0.05$), but not 25 mg/kg ($t = 2.49$, $P > 0.05$), YQA-14, compared to vehicle. However, in $D_3^{-/-}$ mice, 20 mg/kg cocaine produced similar levels of CPP response in the absence or presence of either dose of YQA-14 pretreatment.

3.4. Acute YQA-14 pretreatment inhibits expression of cocaine-induced CPP in WT mice, but not in $D_3^{-/-}$ mice

Then, we examined whether an acute single injection of YQA-14 on the test day alters expression of cocaine-induced CPP in the two mouse strains. Fig. 4 illustrates that YQA-14 (25, 50 mg/kg, 20 min before test) pretreatment significantly inhibited expression of cocaine-induced CPP only in WT (Fig. 4A, $F_{2,\ 29} = 4.90$, $P < 0.05$), but not in $D_3^{-/-}$ mice (Fig. 4B: $F_{2,\ 32} = 2.55$, $P > 0.05$). Cocaine, at 20 mg/kg, still produced a statistically significant increase in CPP in the presence of either dose of YQA-14 pretreatment in $D_3^{-/-}$ mice.

3.5. YQA-14 itself failed to produce CPP or CPA in WT mice, but moderately inhibited locomotor behavior

Lastly, to determine whether such a reduction in cocaine-induced CPP was due to YQA14-induced CPA or locomotor inhibition, we used the same CPP procedures as those for cocaine to measure YQA14-induced CPP/CPA and locomotion. We found that YQA14 did not produce CPP or CPA by itself (Fig. 5A: 25 mg/kg YQA14, $t = 0.15$, $P > 0.05$; 50 mg/kg YQA14, $t = 0.15$, $P > 0.05$, compared to pre-test). However, an acute single injection of YQA14 produced an immediate, moderate reduction in locomotion ($F_{2,\ 25} = 6.135$, $P < 0.01$), an effect that lasted for about 30 min.

4. Discussion

The major findings of the present study are that $D_3^{-/-}$ mice display attenuated CPP response to cocaine, and that D_3R blockade by YQA-14 dose-dependently inhibits acquisition and expression of cocaine-induced CPP in WT mice, but not $D_3^{-/-}$ mice. The attenuated CPP response to cocaine in $D_3^{-/-}$ mice is unlikely to have been due to locomotor impairment, since $D_3^{-/-}$ mice did not show significant differences in basal locomotion as compared to WT mice.

We note that systemic administration of YQA-14 a short-time (30 min), moderate reduction in basal locomotion. However, we don't think such a reduction in locomotion may underlie or contribute to the attenuation of cocaine-induced CPP because: 1)

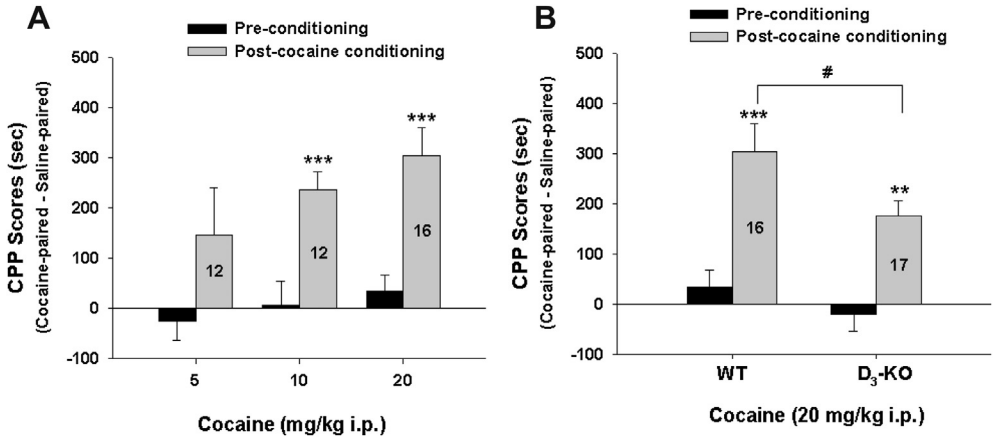

Fig. 2. Cocaine-induced CPP in WT mice and $D_3^{-/-}$ mice. A: Dose-dependent response of cocaine-induced CPP; B: Attenuated CPP response to 20 mg/kg cocaine in $D_3^{-/-}$ mice, compared to WT mice. $^{**}P < 0.01$, $^{***}P < 0.001$, compared to preconditioning; $^{\#}P < 0.05$, compared to WT group.

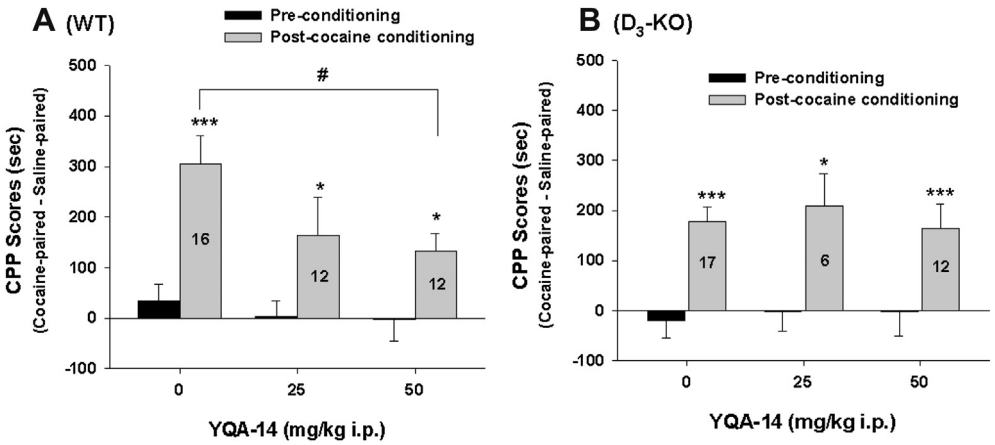

Fig. 3. Effects of YQA-14 on acquisition of cocaine-induced CPP in wild type and $D_3^{-/-}$ mice. Chronic YQA-14 pretreatment (one injection per day for 10 days) during the conditioning phase dose-dependently inhibited cocaine-induced CPP in WT mice (A), but not in $D_3^{-/-}$ mice (B). $^{*}P < 0.05$, $^{***}P < 0.001$, compared to preconditioning; $^{\#}P < 0.05$, compared to vehicle control group.

no YQA-14 was given on the test day in evaluation of the effects of chronic YQA-14 on acquisition of cocaine-induced CPP (Exp. 4);) 2) YQA-14 was given 30 min before the CPP test in evaluation of the effects of acute YQA-14 on expression of cocaine-induced CPP (Exp. 5); 3) the CPP experiment is not an operant behavior test or not highly locomotor-sensitive; 4) all animals can still freely move around after drug injection; and finally, 5) when YQA14 was co-administered with cocaine (Exp. 4), cocaine-enhanced locomotor behavior may functionally counteract YQA-14-induced reduction in locomotion. Overall, the present finding that YQA-14 moderately inhibits locomotion observed in mice is consistent with our previous finding in rats (Song et al., 2012a).

We have previously reported that YQA-14 does not maintain self-administration in rats previously self-administering cocaine (Song et al., 2012a). In addition, YQA-14 itself produced neither CPP nor CPA. Such findings suggest that YQA-14 by itself has neither rewarding nor aversive properties, consistent with the findings with other D_3R antagonists such as SB-277011A and NGB-2904 (Vorel et al., 2002; Xi et al., 2006). These findings also suggest that the antagonism of cocaine-induced CPP is unlikely due to YQA-14-induced aversion or malaise.

The present finding that D_3R deletion or blockade attenuates cocaine-induced CPP suggests that D_3Rs play an important role in mediating cocaine reward. Overall, this is consistent with our recent findings that $D_3^{-/-}$ mice display attenuated responses to cocaine (attenuated NAc DA response to cocaine), which causes a compensatory increase in cocaine intake after D_3R deletion (Song et al., 2012b). It is also consistent with our recent findings that D_3R blockade by YQA-14 or SB-277011A inhibits cocaine self-administration in WT mice, but not $D_3^{-/-}$ mice (Song et al., 2012a).

However, the present findings appear to conflict with two recent reports demonstrating that another strain of $D_3^{-/-}$ mice (Xu et al., 1997) displays increased CPP to low doses of cocaine (1 or 2.5 mg/kg) (Kong et al., 2011) or no change in CPP to high doses of cocaine (10 or 20 mg/kg) (Chen and Xu, 2010). Although the reasons underlying these differences are unclear, several points may be relevant. First, different strains of $D_3^{-/-}$ mice with different genetic backgrounds display different behavioral responses to cocaine. For example, the presently used JAX strain of $D_3^{-/-}$ mice (with C57BL/6J genetic background) displays enhanced cocaine-self-administration (Song et al., 2012b), while the strain of D_3-mutant mice (with mixed 129 SvJ and C57BL/6J background) used by Xu and colleagues does not (Caine et al., 2012). Paradoxically, the strain of D_3-mutant mice used by Xu and colleagues displays identical locomotor and body temperature responses to D_3R-preferring agonists (PD 128907, 7-OH-DPAT) or antagonists (Boulay et al., 1999; Xu et al., 1999). Second, different doses of cocaine were used in the present study as compared to Kong et al. (2011). In general, cocaine-induced CPP behavior is variable, depending largely upon cocaine dose. In a majority of studies, 10 mg/kg

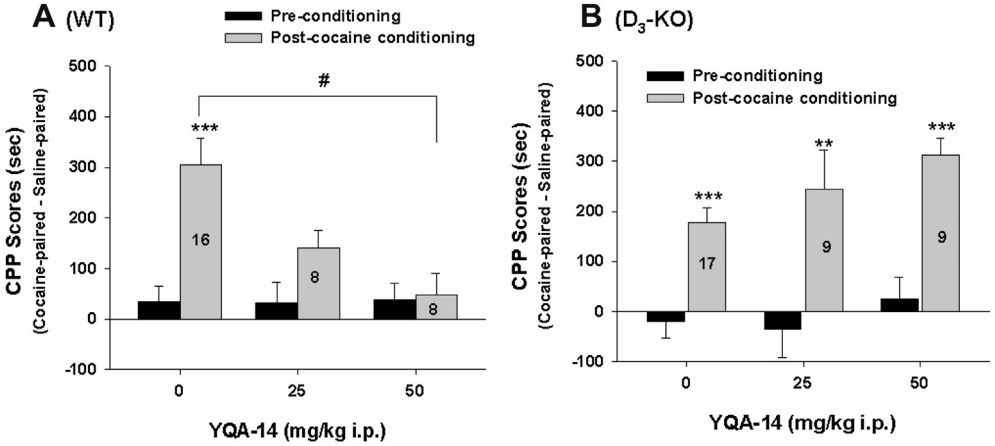

Fig. 4. Effects of YQA-14 on expression of cocaine-induced CPP in WT and $D_3^{-/-}$ mice. A. A single injection of YQA-14 on CPP test day dose-dependently inhibited expression of 20 mg/kg cocaine-induced CPP in WT mice (A), but not in $D_3^{-/-}$ mice (B). $^{**}P < 0.01$, $^{***}P < 0.001$, compared to pre-conditioning; $^{\#}P < 0.05$, compared to vehicle control group.

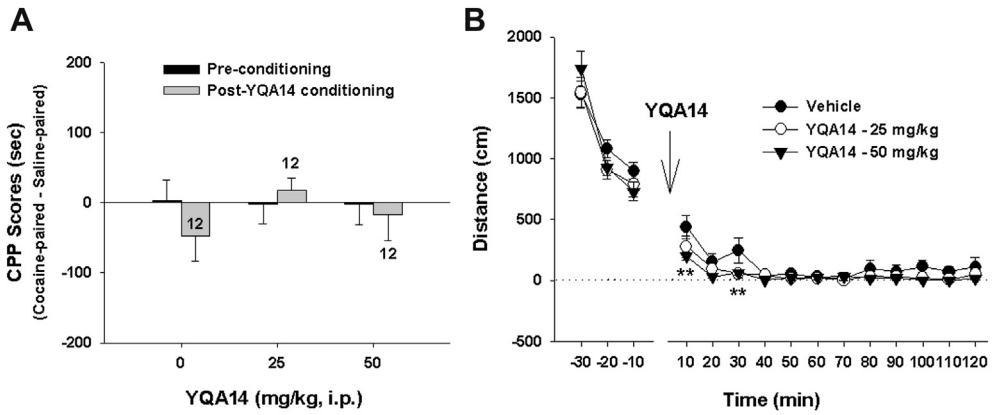

Fig. 5. YQA14 alone failed to produce CPP or CPA (A), while moderately inhibited locomotor behavior (B). $^{**}P < 0.01$ compared to vehicle control group.

(or higher) cocaine is required to produce robust and reliable CPP. In contrast to Xu and colleagues' finding that 1 mg/kg or 2.5 mg/kg cocaine produced significant CPP in both WT and D_3-mutant mice after a single injection of cocaine, we did not see a statistically significant CPP response to 5 mg/kg cocaine after 5 days of cocaine conditioning (Fig. 2A). Third, different CPP procedures were used in these studies. We used a standard CPP procedure – a total of 10 days of CPP conditioning consisting of 5 days of cocaine and 5 days of saline injections, followed by a single test at 48 h after the last cocaine injection or 24 h after the last saline injection (Fig. 1). However, in the work by Xu and colleagues, a mixed and somewhat idiosyncratic CPP procedure was used – CPP test after each daily cocaine injection with multiple CPP tests during cocaine conditioning (Chen and Xu, 2010; Kong et al., 2011). It is not unreasonable to expect that such major differences in CPP procedure may well affect CPP response to cocaine. Clearly, more studies are required to address this issue, and more importantly, comparison of cocaine-induced CPP between these two different strains of $D_3^{-/-}$ mice using a standard CPP procedure.

We note that our findings in $D_3^{-/-}$ mice are congruent with our pharmacological studies that YQA-14, a highly potent and selective D_3R antagonist (Song et al., 2012a), significantly inhibits acquisition and expression of cocaine-induced CPP only in WT mice, but not in $D_3^{-/-}$ mice. In addition, this strain of $D_3^{-/-}$ mice also displays increased cocaine self-administration, which we have interpreted as a compensatory behavioral response to cocaine, secondary to diminished DA response to cocaine (Song et al., 2012b), consistent with the present study.

The mechanisms underlying this attenuated cocaine reward after D_3R deletion or blockade are not fully understood. We have recently reported that the presently used $D_3^{-/-}$ mice display a significant increase in basal DA release in the NAc (due to disinhibition after presynaptic D_3R loss) and a significant reduction in NAc DA response to cocaine compared to WT mice (Song et al., 2012b). In addition, $D_3^{-/-}$ mice also display attenuated locomotor response to cocaine, most likely due to reduced DA response to cocaine. Given the important role of DA in mediating cocaine reward (Wise, 1996), we have suggested that a blunted DA response to cocaine may underlie the attenuated cocaine reward after D_3R loss or blockade (Song et al., 2012b).

Finally, the present findings in $D_3^{-/-}$ mice are also consistent with previous studies in rats demonstrating that activation of D_3Rs increases, while blockade of D_3Rs attenuates, cocaine's rewarding efficacy. Previous studies have shown that D_3R-preferring agonists decrease the rate of cocaine self-administration in a manor similar to that produced by a larger unit dose of cocaine, shifting the cocaine self-administration dose-effect function to the left, and increasing the PR break-point for cocaine self-administration (Caine and Koob, 1993, 1995), while the D_3R antagonists SB-277011A, NGB-2904 or YQA-14 inhibit cocaine self-administration under FR (Song et al., 2012a) and PR (Xi et al., 2005, 2006) reinforcement conditions. In addition, the D_3-preferring agonist 7-OH-DPAT was reported to substitute for cocaine in self-administration studies in rats (Caine and Koob, 1993; Nader and Mach, 1996) and in drug discrimination studies in non-human primates (Lamas et al., 1996; Spealman, 1996), and sensitizes locomotion by itself (Fuchs et al., 2002). These findings suggest that D_3R activation produces reinforcing and psychomotor stimulating effects. The importance of D_3Rs in cocaine reward and addiction is further supported by the finding that brain D_3R mRNA and receptor expression are up-regulated in the NAc in humans after cocaine overdose (Staley and Mash, 1996; Mash, 1997; Mash and Staley, 1999) and in rats after acute or chronic cocaine administration (Le Foll et al., 2002; Neisewander et al., 2004).

In conclusion, the present findings suggest that brain D_3Rs play an important role in mediating cocaine's rewarding and psychostimulant effects and that YQA-14, a highly selective D_3R antagonist *in vivo*, deserves further study as a candidate in medication development for treatment of cocaine abuse and addiction.

Disclosure/conflict of interest

The authors declare no conflicting financial interests.

Author contributions

R.S. conducted the experiments, analyzed the data and wrote the first draft of the manuscript. H.-Y.Z. conducted mouse genotyping and analyzed the data. X.-Q.P. conducted the experiments. R.-B.S. and J.L. overall supervised the experiments and revised the manuscript. R.-F.Y. provided YQA-14. Z.-X.X. designed the experiments, analyzed the data, finalized the figures, and revised the manuscript. E.L.G. overall supervised the experiments and revised the manuscript.

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