Neuroscience 231 (2013) 373–383

SELF-ADMINISTRATION OF PROPOFOL IS MEDIATED BY DOPAMINE D1 RECEPTORS IN NUCLEUS ACCUMBENS IN RATS

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Abstract—As a widely used intravenous short-acting anesthetic, propofol is recently indicated by clinical and animal studies for its abuse potential, but the mechanism underlying propofol abuse is largely unknown. This study examined the contribution of dopamine receptor subtype (D1 and D2 receptors) and neuroanatomical locus (i.e. nuclear accumbens) in the maintenance of propofol self-administration in rats. After the acquisition and maintenance of self-administration of propofol (1.7 mg/kg/infusion) under a fixed ratio (FR1) schedule of reinforcement over 14 days, rats were treated by either intraperitoneal injection or intra-nucleus accumbens (NAc) injection of D1 receptor antagonist (SCH23390) or D2 receptor antagonists (spiperone and eticlopride) 10 min prior to the subsequent propofol self-administration. We demonstrated (i) systemic administration of SCH23390 (10, 30, 100 μg/kg, i.p.) dose-dependently decreased the rate of propofol-maintained self-administration, suggesting a critical role of the D1 receptor in mediating propofol self-administration; (ii) the blockade of the propofol self-administration by SCH23390 was specific since spiperone and eticlopride did not affect propofol self-administration and SCH23390 at these doses did not affect food-maintained responding under an FR5 schedule; (iii) intra-accumbenal injection of SCH23390 (2.5 μg/site) but not eticopride (3.0 μg/site) attenuated the propofol self-administration, localizing nuclear accumbal D1 receptors as a critical locus in the reinforcement of propofol. Together, these findings provide the first direct evidence that D1 receptors in nuclear accumbens play an important role in the maintenance of propofol self-administration.

Key words: propofol, addiction, dopamine receptor, nuclear accumbens, reward.

INTRODUCTION

Propofol is widely used as an intravenous short-acting anesthetic and is recently implicated for abuse potential to induce pleasant effects (Zacny et al., 1993) and dependence (Roussin et al., 2007; Bonnet et al., 2008). For example, the injection frequency of propofol by physicians has been found higher than 100 times per day (Follette and Farley, 1992; Soyka and Schutz, 1997). It has been hypothesized that chronic occupational exposure to aerosolized propofol, might cause sensitization and create a risk factor for addiction (McAuliffe et al., 2006). Anesthesiologists tend to have the highest rate of propofol abuse. A recent survey of propofol abuse in academic anesthesia programs in the United States show that seven die of propofol abuse (28%) of the 25 reported individuals abusing propofol (Wischmeyer et al., 2007). Furthermore, propofol abuse potential has been demonstrated in animals using conditioned place preference (Pain et al., 1996, 1997) and self-administration paradigm (Weerts et al., 1999; LeSage et al., 2000). However, the neurochemical mechanism underlying propofol abuse and dependence remains largely unknown.

Dopamine signaling plays a critical role in the positive reinforcement action of abused drugs (Schultz, 1998; Phillips et al., 2003). Dopamine exerts its effects through two distinct subfamilies, the D1-like (D1R including D1 and D5) and the D2-like (D2R including D2, D3 and D4) receptors (Kling-Petersen et al., 1995; Abrahams et al., 1998). The dopamine signal is essential to the development and expression of addictive behaviors of various drugs of abuse, including psychostimulants such as cocaine (Kosten et al., 2002) and amphetamine (Velazquez-Sanchez et al., 2011), opioid (Gardner, 2011) and cannabinoid (Seif et al., 2011) and alcohol (Soderpalm and Ericson, 2011). Thus, the dopamine signal represents a common mechanism of drug abuse in the brain (Self et al., 1996; Alleweireldt et al., 2002; Olsen and Duvauchelle, 2006). The nucleus accumbens (NAc), a major terminal area of

☆ Supported in part by NSFC (30972840 and 81271469) and RFDP (20113321110003) to Q.L., NSF of Zhejiang Province (Z2101211) to Q.L., WZKJ (H20100060) to Q.L. and NSF of Zhejiang Province (D2080515) to W.Z.
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Abbreviations: ANOVA, analysis of variance; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; NAc, nucleus accumbens.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2012.11.002

the mesolimbic dopamine systems, is a crucial component of the neuronal circuitry mediating reward-related behaviors (Everitt and Wolf, 2002) and is critically involved in development as well as the maintenance of addictive behaviors, since genetic or pharmacological disruption of dopamine receptors in the NAc eliminated or reduced the rewarding effects of abusive drugs (Bolanos and Nestler, 2004). Consistent with this notion, propofol at the sub-anesthetic and anesthetic doses has been shown to increase dopamine concentration in the NAc of rats (Wise, 2004). This increase in dopamine concentrations is also observed after the intake of alcohol and other drugs, and is thought to reinforce substance intake (Heinz et al., 2004). Moreover, large doses of propofol can enhance the dopamine levels in the NAc by inhibiting the reuptake of dopamine (Keita et al., 1996). These observations led us to propose that dopamine receptors in NAc mediate propofol self-administration.

To test this hypothesis, we investigate whether propofol-maintained reinforcement and self-administration are mediated by dopamine D1-like and D2-like receptors (D1R and D2R) in the NAc. Specifically, using a self-administration paradigm, we examined the effects of the D1R antagonist (SCH23390) and the D2R antagonists (spiperone and eticlopride) on propofol-reinforcement after intraperitoneal or intra-NAc injections in rats.

EXPERIMENTAL PROCEDURES

Animals

Two hundred and thirty-two adult male Sprague–Dawley rats (280–300 g) were purchased from the Experimental Animal Center of the Zhejiang Province (Hangzhou, China). Seven rats were not successfully trained to maintain propofol self-administration and in two rats it was found that the guide cannulae were thrown out. These nine rats were not included in the study. Rats were housed individually in home cages in a temperature-controlled ventilated colony room with a reversed 12-h light/dark cycle (lights on at 07:00 pm). Ad libitum food and water were provided in the home cage. The Institutional Animal Care and Use Committee of Zhejiang Province/The Ningbo Addiction Research and Training Center approved of all experimental procedures. Animal care was provided by trained vivarium staff members at the Laboratory of the Ningbo Addiction Research and Treatment Center (Ningbo, China). At the completion of the experiment, some rats were killed by carbon dioxide inhalation after pentobarbital anesthesia prior to the removal of their brains for the analysis of cannula placement.

Drugs

Propofol (10 mg/ml; Diprivan, Astrazeneca, Italy) was prepared immediately before use and injected intravenously. The propofol dosage (1.7 mg/kg/injection) used for the self-administration experiment was selected on the basis of a previous study (LeSage et al., 2000). The dopamine D2 receptor antagonists spiperone and eticlopride were obtained from Sigma Chemical Co. (Sigma, MA, USA). The dopamine D1 selective receptor antagonist R-(2)-8-chloro-2,3,4,5-tetrahydro-3,1-methyl-5-phenyl-11-3-benzyozepine-7-ol (SCH23390) was obtained from Research Biochemicals International (Natick, MA, USA). SCH23390 was dissolved in diluted water and brought to pH 4.5 using 0.1 M NaOH. Eticlopride was dissolved in diluted water. Twenty percent intraplid was used as the control whenever propofol was used.

Operant behavioral apparatus

The procedure for propofol training took place in a custom-made operant box (Ningbo Addiction Research and Treatment Center, China) has been described previously (Zhou et al., 2007). Briefly, the operant box was equipped with two nose-poke operanda located 5 cm above the floor with a green LED light inside each nose-poke hole. Control was defined as animals receiving infusion of either intralipid (compared with propofol) or saline (compared with D1 and D2 receptor drugs, such as SCH23390 and eticopride). In addition, active nose poke (with food-reward) versus inactive nose poke (with no food-reward) was also recorded to assess the general motor activity versus specific addictive property. Propofol solution was delivered through Tygon tubing attached to a syringe pump at a speed of 1.2 ml/min. Experimental procedure was a computer-assisted system using a MED Associates interface and running self-programmed software written in Borland Delphi 6.0.

Surgery

Implantation of intravenous catheters and guide cannula into NAc were performed as we described previously (Zhou et al., 2007). Briefly, under sodium pentobarbital anesthesia, rats were surgically implanted with chronic indwelling intravenous catheters which were flushed daily with 0.2 ml saline–heparin solution (25 U/ml heparin). The rats were treated post-surgically with penicillin B to prevent infection and allowed to recover for at least 7 days (Zhou et al., 2007). For intra-NAc injection, the bilateral guide cannulae (20-gauge, Small Parts Inc., Roanoke, VA, USA) were implanted in the NAc (1.5 mm anterior to bregma, 2.0 mm lateral to midline, and 6.7 mm ventral to the surface of the cortex) as described previously (Zhou et al., 2007). Guide cannula was attached to the skull via dental acrylic.

Microinjection procedure

Animals that had been trained for 14 days received microinjections beginning on the day 15. All injections into the NAc were delivered through infusion cannulas (33 gauge, extending 0.5 mm beyond the tip of the guide cannulas) by using a microinjection pump (MD-1001, Bioanalytical System Inc., West Lafayette, IN, USA) in a volume of 0.5 μl/site over 5 min.

Propofol self-administration training

Rats were trained to self-administer drugs as described (Zhou et al., 2007). For each daily 3-h training session, the rats were moved from their home cages to the operant chambers and their connectors were attached to the infusion lines. Each session started with the illumination of the green light inside the active nose-poke hole. The rats first received propofol infusion (1.7 mg/kg per infusion) or intralipid (0.17 ml/kg per infusion) as vehicle control following completion of the ratio requirement (fixed ratio = 1) in the active nose-poke. Each infusion was paired with a 5-s illumination of the house light and in combination with the noise of the infusion pump. A timeout period was imposed for 30 s, during which the responding produced no programmed consequences but was still recorded.

Illumination of the green light in the active nose-poke signaled the end of the 30-s time-out period. Responding in the inactive nose poke produced no programmed consequences. The sessions ended after 3 h or 50 propofol injections whichever occurred first. After 1.70 mg/kg propofol maintained self-administration, the dose of propofol was reduced to 0.56 or 1.0 mg/kg per infusion and continued for at least five sessions until the responding stabilized. In these studies, we changed the session parameters (by allowing total injecting number beyond 50 and the sessions ended after 3 h) to allow more propofol injections to maintain the same total amount of propofol consumed per session. Rats were returned to their individual home cages shortly after the session.

Animal treatments

Experiment #1: Propofol self-administration. Twenty-four rats received propofol (1.7 mg/kg) and six rats received intralipid (vehicle control) for one 3-h daily session for a total of 14 days. Another set of 48 rats were first given access to 1.70 mg/kg propofol per infusion. After the number of infusions per session stabilized, the dose of propofol was reduced to 0.56 or 1.0 mg/kg per infusion and continued at least five sessions or until responding stabilized.

Experiment #2: The effect of systemic administration of dopamine D1 and D2 receptor antagonists on the maintenance of propofol self-administration. Sixty rats (n = 6 per group) were tested for the effects of dopamine D1 and D2 receptor antagonists, given systemically, on the maintenance of propofol self-administration. Rats were trained for propofol (1.7 mg/kg per infusion) self-administration for 14 days. Ten minutes prior to the session on day 15, rats received vehicle control or an intraperitoneal injection of D1 receptor antagonist SCH23390 (10, 30 and 100 μg/kg, respectively) or D2R antagonists spiperone (10, 30 and 100 μg/kg, respectively) or eticlopride (10, 30 and 100 μg/kg, respectively).

Experiment #3: The effect of systemic injection of D1 and D2 antagonists on the maintenance of sucrose-self administration and on motor activity. Rats were trained to nose poke for sucrose pellets under a fixed ratio (FR5) schedule of reinforcement daily for 1-h sessions for 7 days. In this sucrose self-administration paradigm, rats received a 45-mg sucrose pellet that was delivered via a sucrose cup (Dustless precision pellets, Bio-Serv, NJ, USA). During the session, each of the five nose-pokes in the active hole resulted in the delivery of a sucrose pellet only. Nose pokes in the inactive hole had no programmed consequence. Active nose-poke responses, inactive nose-poke responses, and the number of sucrose pellets that were earned during each training session were recorded by a computer. Ten minutes prior to the session, 32 rats received an intraperitoneal injection of D1 receptor antagonist SCH23390 (10, 30 and 100 μg/kg, respectively) or vehicle. To further assess possible nonspecific effects of SCH23390 on general activity, we examined the effects of intraperitoneal administration of SCH23390 on locomotion in a novel open field. Naive 32 rats were treated with SCH23390 (10, 30 or 100 μg/kg, i.p.) and motor activity (total distance traveled) was recorded and analyzed as the measure of locomotion using MED Associates SOF-811 Open-field Activity Software.

Experiment #4: The effect of intra-accumbenal injection of D1 and D2 receptor antagonists on the maintenance of propofol self-administration. Thirty-six rats that had received bilateral guide cannula were trained to self-administer propofol for 14 days. On the 15th day, rats received microinjections (n = 6 per group) into the NAc of either vehicle control or the D1 receptor antagonist SCH23390 (0.5 or 2.5 μg/side) or eticlopride (0, 1.0 or 3.0 μg/side). Ten minute later, rats were placed into operant chambers and tested for the self-administration of propofol.

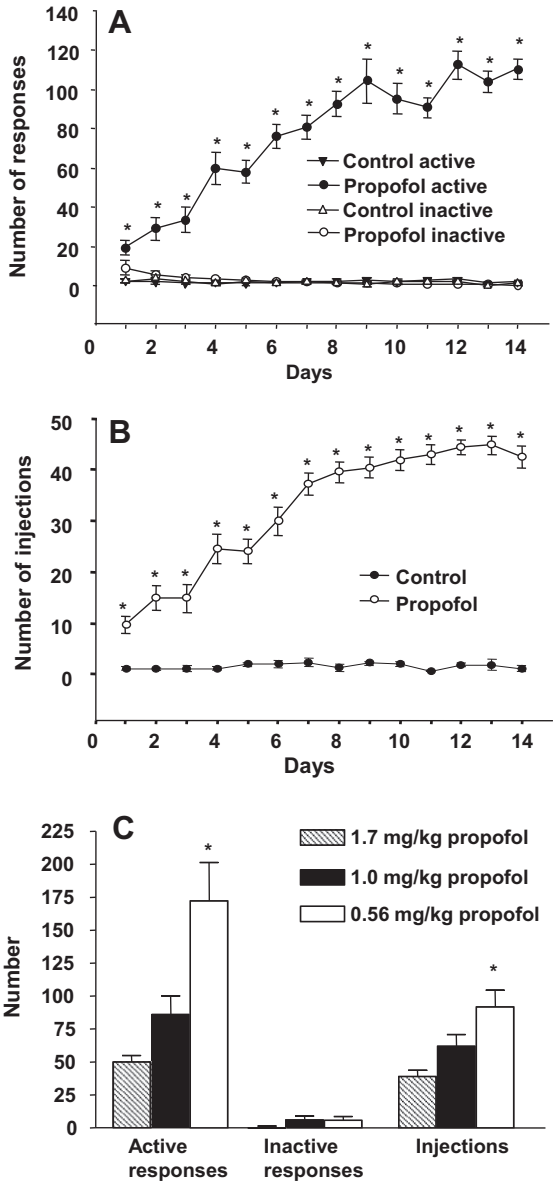

Fig. 1. Self-administration maintained by propofol. The number of active responses (nose-pokes) and inactive responses (panel A) as well as the number of injections (panel B) maintained by propofol compared to vehicle control (intralipid) were shown. The number of active nose-pokes responses ($F(2, 17) = 11.335$, $P < 0.05$) and injections ($F(2, 17) = 8.876$, $P < 0.05$) of propofol-maintained self-administration increase significantly with the diminution of propofol (panel C). Mean ± SEM. * indicates significant difference between the propofol and control groups at the same time points at $P < 0.05$.

Histological analysis

Rats were anesthetized and transcardially perfused, first with phosphate-buffered saline, and then with 4% polyformalin solution. The brains were sectioned in the coronal plane to a thickness of 50 μm on a Cryostat Microtome (Leica CM1850; Leica, Wetzlar, Germany). The placement of the cannula tip for

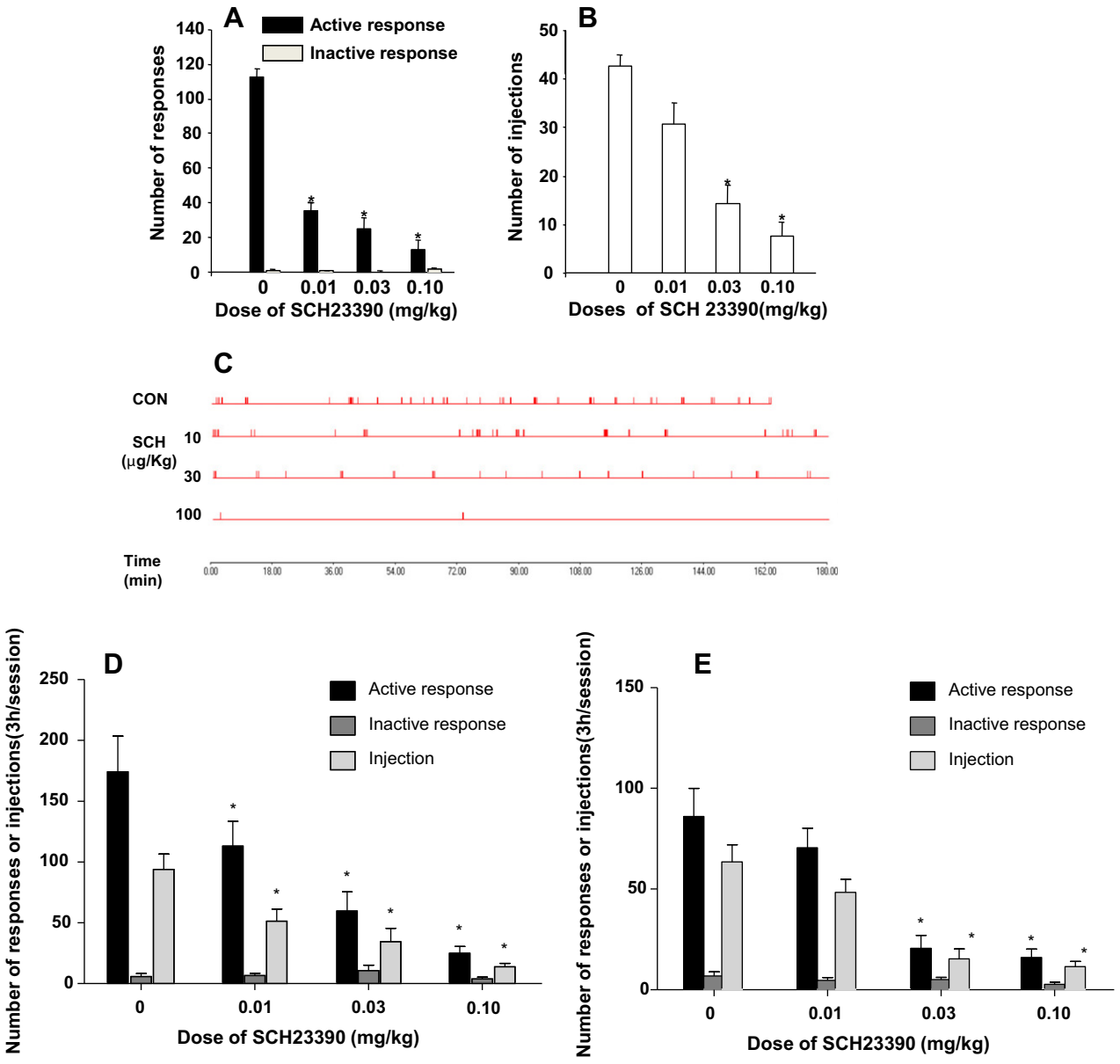

Fig. 2. The effects of systemic administration of SCH23390 (SCH) on the maintenance of the propofol-maintained self-administration. The number of active and inactive responses (panel A) and the number of injections were recorded (panel B). Representative individual response records of propofol self-administration on day 15 after systemic administration of vehicle (CON) or SCH23390 (SCH) (panel C) were recorded. Each vertical tick mark indicates an injection. Mean ± SEM. * indicates significant difference between SCH and CON at the same time points at $P < 0.05$.

infusion was located using light microscopy and mapped onto a schematic diagram of the rat brain as in a previously published method (Zhou et al., 2007). Only animals with proper functioning of the i.v. cannula and a correct location of the microdialysis probe were included in the study.

Statistical analysis

Only rats with proper functioning of the i.v. cannula and a correct location of the microdialysis probe were included in the statistical analysis. The number of infusions or responses for active and inactive holes during self-administration was analyzed using a one-way analysis of variance (ANOVA) or two-way (hole, treatment) ANOVA with repeated measure. A Newman–Keuls multiple comparison with an alpha level of 0.05 was used for post hoc comparisons between group means.

RESULTS

Rats developed a reliable propofol self-administration during the training session

Rats developed a reliable self-administration of propofol after 2–3 days of training. Active responses and the number of self-infusions were used to measure propofol reinforcement behavior. The mean number of active and inactive nose-pokes for the 14 consecutive daily self-administration sessions is shown in Fig. 1A. A repeated measure showed a significant increase in the number of responses active nose-pokes for propofol ($F(1, 46) = 294.989$, $P < 0.05$), but no difference in the number of responses for active nose-pokes for intralipid

($F(1, 10) = 0.532, P = 0.483$). However, it did not alter the number of inactive responses of propofol and intralipid ($P > 0.05$). As shown in Fig. 1B, propofol significantly increased the number of self-infusions compared to the vehicle control ($F(1, 28) = 126.200$, $P < 0.05$). As shown in Fig. 1C, one-way ANOVA for repeat measurement suggested that the number of active nose-poke responses ($F(2, 17) = 11.335$, $P < 0.05$) and injections ($F(2, 17) = 8.876$, $P < 0.05$) of propofol-maintained self-administration increased significantly with the diminution of propofol. These data suggest that propofol functions as a positive reinforcer in rats.

Systemic injection of dopamine D1 receptor antagonists, but not D2 receptor antagonists, attenuated propofol self-administration in rats

Rats were trained to self-administer propofol for 14 days. On day 15, rats were pretreated with either saline (control) or D1R antagonist SCH23390 (10–100 μg/kg) 10 min before propofol self-administration. SCH23390 dose-dependently inhibited the number of active responses (Fig. 2A, $F(3, 23) = 60.415$, $P < 0.05$) and the infusions of the propofol self-administration (Fig. 2B, $F(3, 23) = 20.900$, $P < 0.05$) during the session compare to the vehicle-treated group. The inhibitory effect of SCH23390 was revealed in the representative time course of injection shown in Fig. 2C. Self-administration maintained with 0.56 mg/kg (Fig. 2D) and 1.00 mg/kg (Fig. 2E) propofol also was inhibited by SCH23390. As the lower dose propofol self-administration was maintained, SCH23390 decreased the number of active responses and injections more manifestly (Fig. 2D&E). However, these treatments did not affect the number of inactive nose-poke responses ($P > 0.05$).

On day 15, rats received either vehicle control (saline) or D2R antagonists spiperone (10–100 μg/kg) or eticlopride (10–100 μg/kg) 10 min before propofol self-administration. In contrast to the D1 receptor antagonist, systemic injection of the D2R antagonist spiperone did not affect the number of active (Fig. 3A, one-way ANOVA, $F(3, 23) = 1.163$, $P > 0.05$), inactive nose-poke responses (Fig. 3A, $F(3, 23) = 0.975$, $P > 0.05$), and infusions of propofol (Fig. 3B, $F(3, 23) = 1.123$, $P > 0.05$). Similarly, another D2 receptor antagonist eticopride failed to alter the number of active nose-poke responses (Fig. 3C, $P > 0.05$) and the infusions of propofol (Fig. 3D, $P > 0.05$).

Dopamine D1 antagonists at the doses abolishing propofol self-administration did not affect sucrose self-administration and locomotor activity in rats

To address whether the effect of the D1R antagonist is selective for propofol self-administration or general

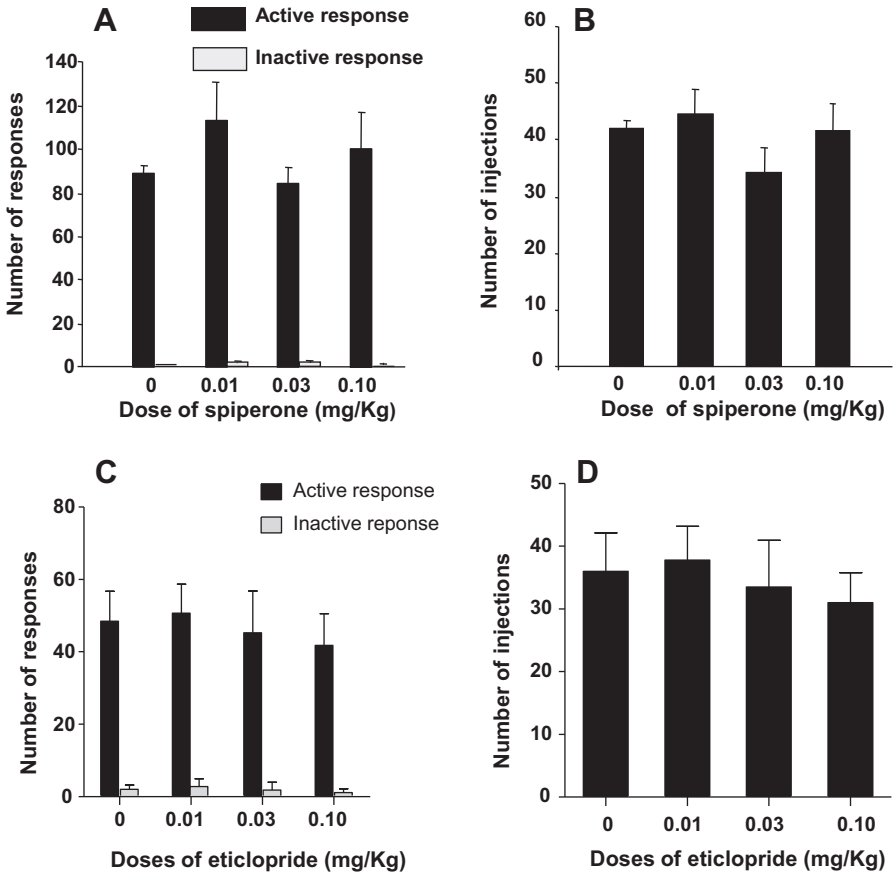

Fig. 3. The effects of systemic administration of spiperone and eticlopride on the propofol self-administration. The number of active responses (panels A, C) and the number of injections (panels B, D) were recorded. Mean ± SEM. * indicates significant difference between spiperone and the control groups at the same time points at $P < 0.05$.

suppression on the instrumental and motor behaviors, we also assessed the effect of SCH23390 on active nose-poke response to sucrose as well as locomotor activity in rats. One-way ANOVA for repeated measures revealed that active nose-poke responses maintained by food were significantly altered compared to inactive nose-poke responses (Fig. 4A, $F(1, 12) = 929.874$, $P < 0.05$). The One-way ANOVA revealed the significant effect of SCH 23390 treatment on the active nose-poke responses ($F(3, 27) = 48.681$, $P < 0.05$), but not inactive nose-poke responses ($F(3, 27) = 1.638$, $P > 0.05$) and pellets earned ($F(3, 27) = 71.785$, $P < 0.05$) (shown in Fig. 4B). Multiple comparisons showed that SCH 23390 at 0.1 mg/kg significantly attenuated the active responses compared to vehicle. However, SCH 23390 at 0.01 or 0.03 mg/kg did not alter the active responses. Additionally, SCH23390 treatment failed to alter locomotor activity in a novel context (Fig. 4C, $F(3, 31) = 0.477$, $P > 0.05$) relative to vehicle treatment. Thus, SCH23390 (0–0.30 mg/kg) abolished propofol self-administration without affecting nature reward or instrumental performance or motor activity.

Intra-accumbenal infusion of D1 but not D2 receptor antagonists abolished propofol self-administration in rats

Fig. 5A shows a histological reconstruction to illustrate the injection site of NAc. As in experiment 1, one-factor repeated measures ANOVA revealed that NAc-cannulated rats exhibited stable propofol-maintained self-administration (Fig. 5B, $F(1, 34) = 506.841$, $P < 0.05$). Post Hoc Test indicated that SCH23390 dose-dependently inhibited propofol-maintained active responses (Fig. 5C, $F(2, 17) = 27.721$, $P < 0.05$),not inactive responses (Fig. 5C, $F(2, 17) = 2.974$, $P > 0.05$), and injections (Fig. 5D, $F(2, 17) = 15.846$, $P < 0.05$). However, intra-NAc infusion of the D2R antagonist eticlopride did not affect propofol-produced self-administration (Fig. 6A, B, $P > 0.05$). These data

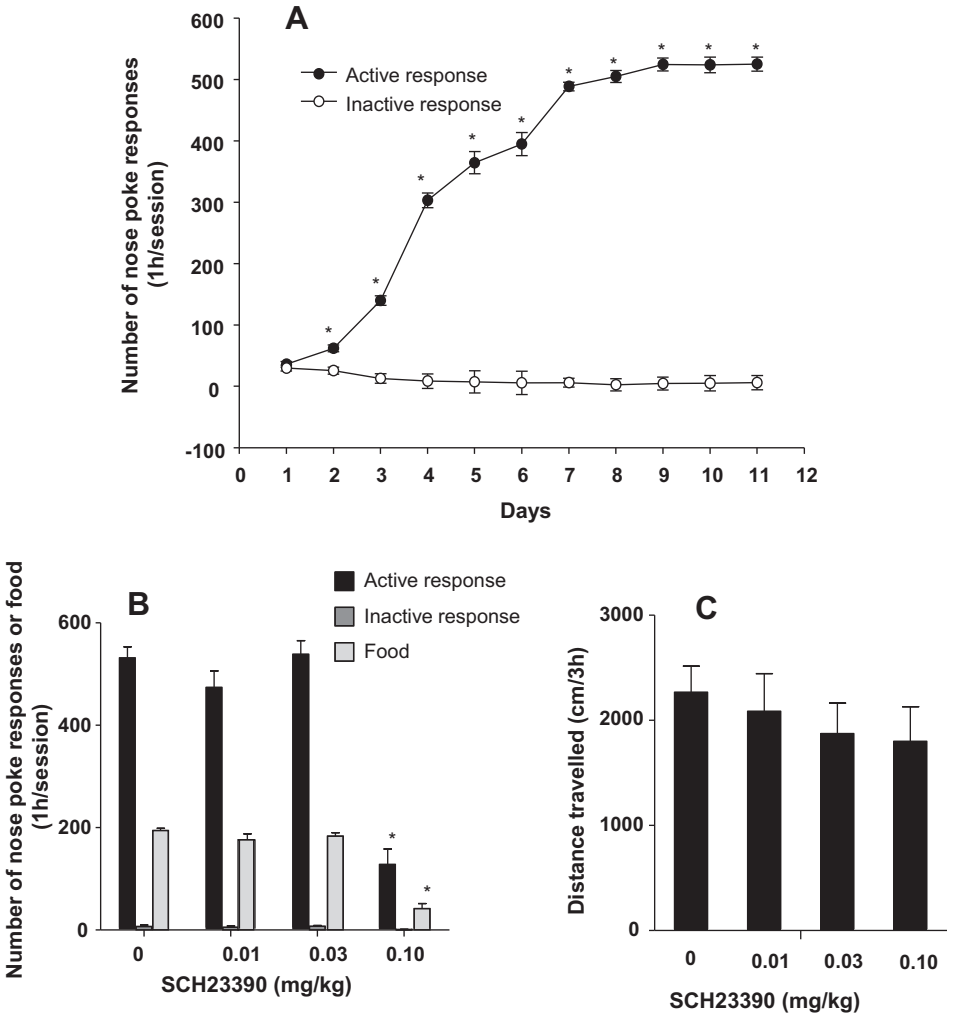

Fig. 4. Food maintained and locomotor activity testing. The number of active responses (nose-pokes) and inactive responses (panel A) maintained by food in rats were recorded. Effects on the food-maintained self-administration, SCH23390 (panel B) was systemically administered. As shown in panel C SCH23390 failed to attenuate the motor activity of rats. Mean ± SEM. * indicates significant difference between SCH23390 and the control groups at the same time points at $P < 0.05$.

demonstrate that D1Rs in NAc mediate propofol self-administration in rats.

DISCUSSIONS

Propofol has a powerful positive reinforcing property of addictive drugs

Propofol has been recently shown to have self-administrative property and maintain responding (Weerts et al., 1999; LeSage et al., 2000; Roussin et al., 2007). Consistent with these findings, we show that rats developed strong self-administration behavior within a 15 day training session. The finding that rats acquired the propofol self-administration behavior is consistent with previous results showing that the active response and infusions increased over the training session of propofol self-administration under fixed ratio schedule (LeSage et al., 2000). Other studies have reported a similar pattern of self-administration with barbiturates, ethanol and cocaine, the pattern typically observed after the use of psychomotor stimulants (Winger et al., 1975). It is noted that propofol self-administration is dose-dependent, with 1.0 and 1.7 mg/kg (but not 0.56 mg/kg) to elicit self-administration behavior. Furthermore, the time to acquire propofol (1.0 mg/kg) self-administration is about 4.2 days, significantly shorter than the time to acquire morphine self-administration at the same dose (i.e. 20–30 days) (Mierzejewski et al., 2003), indicating strong potency of propofol's addictive property. This minimal required dose of propofol to elicit self-administration is comparable to psychostimulants cocaine (Mierzejewski et al., 2003). Thus, the data suggest that propofol may

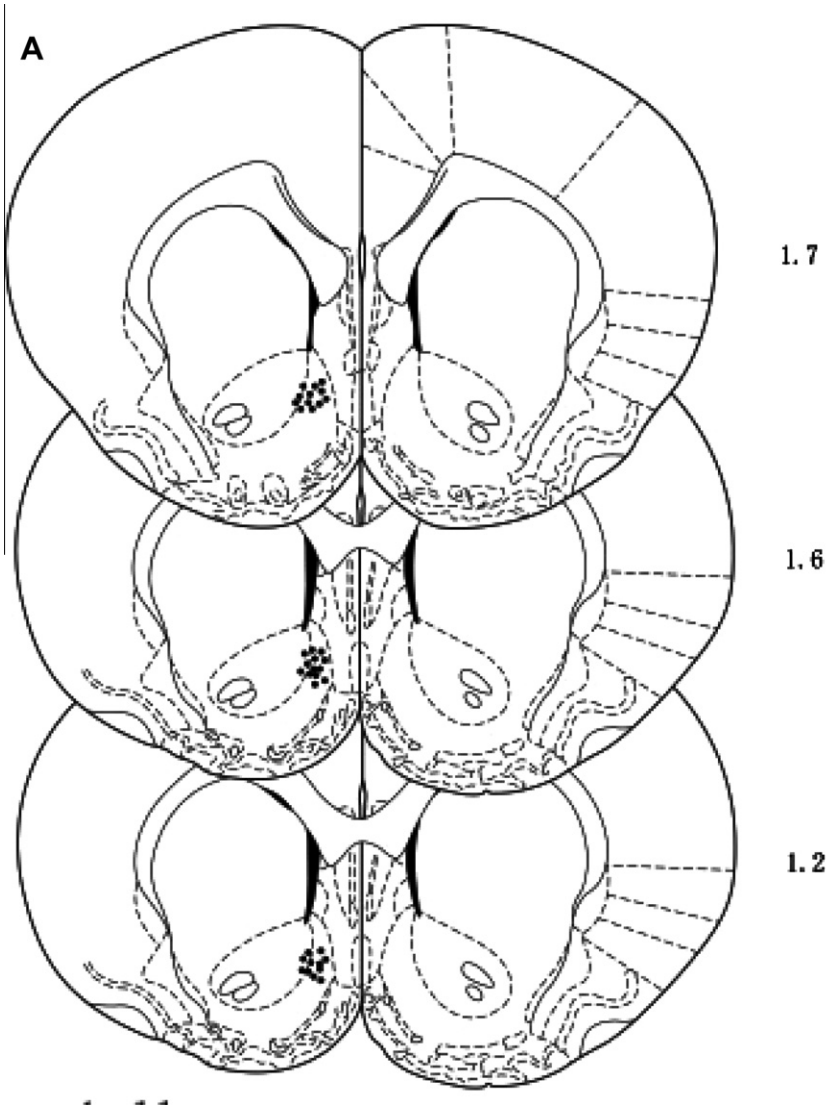

Fig. 5. Effects of intra-NAc injection of SCH23390 (SCH) on the maintenance of the propofol-maintained self-administration. Panel A shows a histological reconstruction to illustrate the injection site of NAc. The number of active responses (nose-pokes) and inactive responses (panel B) maintained by propofol in rats that had received bilateral guide cannulae were recorded. The number of active responses (panel C) and the number of injections (panel D) after intra-NAc injection of SCH were also recorded. Mean ± SEM. * indicates significant difference between propofol and the control groups at the same time points at $P < 0.05$ in panel A or between SCH23390 and control groups at the same time points at $P < 0.05$ in panels B and C.

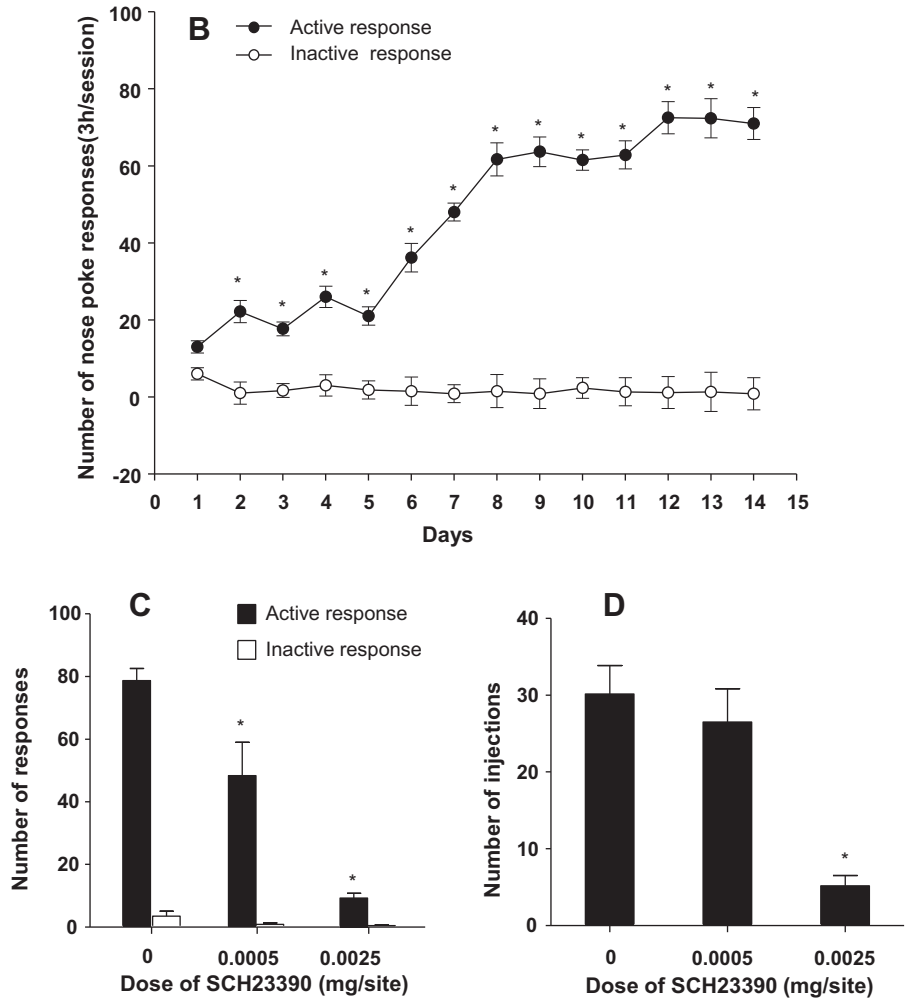

Fig. 5. (*continued*)

have the reinforcement effects and drug dependence. From the minimal dose, time to acquire self-administration and training sessions required for eliciting self-administration, propofol appears to have similar potency of addictive property to psychostimulants and morphine.

Dopamine D1 (but not D2) receptors play a critical role in the reinforcement property of propofol

Dopamine is a critical neurotransmitter for positively reinforcing stimuli (Wise, 2004). Abused drugs including cocaine, amphetamine, heroine and nicotine are associated with elevated mesocorticolimbic dopamine in the NAc. However, the contribution of dopaminergic system to the addictive property of propofol is not known. Furthermore, while two major types of dopamine receptors, including the D1R and D2R families, have been associated with the drug abuse, increasing evidence supports a critical role of D1Rs in the development of drug abuse of psychostimulants (Caine et al., 2000; Mutschler and Bergman, 2002; Barrett et al., 2004; Bachtell et al., 2005). The main finding of the present study is that systemic or intra-NAc injection with D1R antagonist (but not D2R antagonists) inhibited the rate of propofol self-administration. Furthermore, the present study showed that propofol-maintained self-administration was attenuated selectively by the dopamine D1R antagonist SCH23390, but not by dopamine D2R antagonists spiperone and eticopride. We conclude that dopamine D1R, not D2R, is involved in the rewarding effect of propofol. The selective effect of D1R but not D2R antagonists in blocking propofol self-administration agree with the previous results showing that the selective dopamine D1R antagonist SCH23390 blocked cocaine-conditioned place preference in rats (Nazarian et al., 2004). These findings are consistent with previous studies that systemic administration of neuroleptics or antagonists of D2Rs fails to impair morphine-maintained conditioned place preference (Mackey and van der Kooy, 1985; Shippenberg and Herz, 1988).

Many previous studies have indeed shown that D1R antagonists increase cocaine self-administration during a continuous intake paradigm as a compensatory increase to the blockade D1Rs (e.g. Caine and Koob, 1993). However, we believe that the different effects of D1R antagonists on propofol self-administration (e.g.

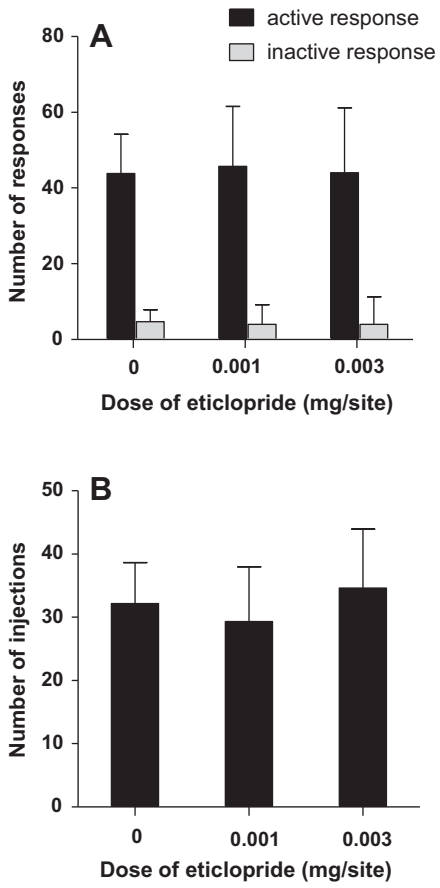

Fig. 6. Effects of intra-NAc injection of eticlopride on the maintenance of the propofol-maintained self-administration. The number of active responses (panel A) and the number of injections (panel B) were recorded.

attenuation) and on cocaine-self administration (i.e. enhancement) likely reflect the different mechanisms underlying cocaine- and propofol-self-administration. One of the critical differences is the different involvement of dopamine release in these two drug self-administrations. In contrast to the marked increase in extracellular dopamine by cocaine, propofol increases extracellular dopamine levels in the NAc only in subanesthetic and anesthetic doses (Pain et al., 2002), but at the dose lower than subanesthetic doses, propofol in fact reduces the stimulated dopamine release in the NAc. Moreover, we found that D2R antagonist did not block the effect of propofol, consistent with the findings in a previous study (Schulte et al., 2000). In contrast, pretreatment with the D2R antagonist eticlopride increased the rates of self-administration of high doses of cocaine (Caine et al., 1999). These distinct pharmacological actions of propofol and cocaine may explain their different behaviors in response to D1R antagonists (i.e. decrease in propofol and increase in cocaine). On the other hand, propofol shares some common features (such as alteration of dopamine and glutamate signaling in NAc) with other types of drugs of abuse, including heroine, nicotine, alcohol and of other positive reinforcers such as sucrose. Consistent with this notion, we found that the effect of D1R antagonists on propofol is largely in agreement with the finding on heroine (McFarland and Ettenberg, 1995), opioids (Shippenberg et al., 1993), nicotine (Spina et al., 2006) and sucrose (Grimm et al., 2011). In these studies, D1R antagonists consistently attenuate intake of these drugs of abuse or positive reinforced sucrose. Our data agree with these studies by showing that the selective dopamine D1R antagonist SCH23390 (with systemic injection or intra-NAc injection) reduced self-administration in rats. These findings demonstrate a critical role of D1Rs in establishing the self-administration of propofol and other drugs of abuse. Thus, propofol interacts directly or indirectly with D1Rs to develop a self-administrative pattern.

It should be noted that the blocking effects were specific for responding maintained by propofol since SCH 23390 at the doses (10 and 30 μg/kg) that blocked propofol administration failed to alter nose-poke responding maintained by food pellets or food consumption. However, the highest dose (100 μg/kg) of SCH23390 also attenuated active nose-poke responses of food-maintained self-administration and food consumption, which is consistent with the early results indicating a non-selective effect of SCH23390 at this dose (Woolverton and Virus, 1989; Glowa and Wojnicki, 1996; Weissenborn et al., 1996; Caine et al., 2000; Platt et al., 2001). The doses of SCH 23390 used in the current study may also reflect propofol interactions with additional receptors such as 5-HT2C receptors since SCH23390 has also been shown to act at 5-HT2C receptors (Millan et al., 2001) and that 5-HT2C agonists have reduced food-maintained responding in squirrel monkeys (Brady and Barrett, 1985; McKearney, 1990). Lastly, we noted that systemic administration of SCH23390 failed to alter distance traveled of rats. Together, the present results demonstrate a critical role for D1Rs in the reinforcing properties of propofol.

Propofol interacts with dopamine D1 receptors in nuclear accumbens to develop self-administration

The brain region that plays an important role in mediating propofol-maintained self-administration is unknown. Several studies have indicated the NAc may be an important locus whereby propofol elicits self-administration. For example, the *in vivo* infusion study showed that sub-anesthetic and anesthetic doses of propofol increased extracellular DA levels in the NAc (Pain et al., 2002). However, large doses of propofol decrease the dopamine release in the rat NAc by a mechanism independent of D2R, GABAA and NMDA receptors (Schulte et al., 2000). Thus, that excitability of dopamine transmission in VTA may increase the dopamine release in the terminal in the NAc.

To clarify whether NAc D1Rs mediate propofol-induced reinforcement, we used a microinjection pump for intra-NAc infusion of SCH23390, and found that nanomolar administration of D1R antagonist into the NAc, as did systemic administration of the antagonist, decreased the rate of self-administration, whereas systemic administration or intra-NAc infusion of the D2R antagonist eticlopride did not alter the rate of self-

administration. Therefore, our finding demonstrates that NAc D1Rs mediate propofol-maintained self-administration behavior. The exact mechanism by which D1R in NAc modifies propofol self-administration is unclear at this point. NAc D1Rs are predominantly located on neurons that project to the VTA. Interestingly, propofol elicited a robust increase in Delta FosB expression in the NAc (Xiong et al., 2011) and (likely dopamine) increases glutamate transmission in the VTA. Thus, propofol may increase excitation of VTA dopamine neurons via increasing in afferent glutamatergic transmission. These effects induced by propofol were eliminated by the D1R antagonist, indicating that propofol increases glutamate transmission via the activation of presynaptic D1Rs (Sun et al., 2008). Consequently, the elimination of excitation of VTA dopamine neurons by D1R antagonist can lead to the inhibitory action on propofol self-administration by D1R antagonists in the NAc.

CONCLUSIONS

The present results demonstrated that systemic or intra-NAc administration with D1R (but not D2R) antagonist attenuated the propofol reinforcement. Thus, D1Rs in NAc play an important role in the maintenance of propofol self-administration.

Acknowledgment—We wish to thank Dianne O. Hardy from the Population Council (New York, USA) for her critical comments on this manuscript.

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(Accepted 3 November 2012)
(Available online 29 November 2012)