Published in final edited form as:

Addict Biol. 2013 July ; 18(4): 665–677. doi:10.1111/j.1369-1600.2012.00486.x.

Blockade of Dopamine D₃ Receptors in the Nucleus Accumbens and Central Amygdala Inhibits Incubation of Cocaine Craving in Rats

Zheng-Xiong Xi^{1,*}, Xia Li^{1,#}, Jie Li¹, Xiao-Qing Peng¹, Rui Song^{1,2}, József Gaál³, and Eliot L. Gardner¹

¹Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA

³MegaPharma Kft, Budapest, Hungary

Abstract

Cue-induced drug seeking progressively increases over time of withdrawal from drug self-administration in rats, a phenomenon called “incubation of craving”. The underlying mechanisms have been linked to increased expression of brain-derived neurotrophic factor (BDNF) and GluR2-lacking AMPA receptors in the mesolimbic dopamine (DA) system, and also to increased extracellular signal-regulated kinase (ERK) activation in the central amygdala (CeA). However, it remains unclear whether any DA mechanism is also involved in incubation of craving. Recent research demonstrates that cue-induced cocaine seeking appears to parallel increased DA D₃, but not D₁ or D₂, receptor expression in the nucleus accumbens (NAc) of rats over time of withdrawal, suggesting possible involvement of D₃ receptors (D₃R) in incubation of cocaine craving. Here we report that systemic or local administration of SB-277011A, a highly selective D₃R antagonist, into the NAc (core and shell) or the CeA, but not the dorsal striatum or basolateral amygdala, significantly inhibits expression of incubation of cocaine craving in rats after 2–30 days of withdrawal from previous cocaine self-administration, but had no effect on sucrose-seeking behavior in rats after 10–30 days of withdrawal. These data suggest that DA D₃Rs in both the NAc and the CeA plays an important role in incubation of cocaine craving in rats, and supports the potential utility of D₃R antagonists in the treatment of cocaine addiction.

Keywords

Cocaine; dopamine; D₃ receptor; SB-277011A; incubation; craving; relapse; drug-seeking

Introduction

Drug-associated cues induce craving and relapse to drug-seeking behavior in humans (Childress et al. 1999; Garavan et al. 2000; Grant et al. 1996). However, the underlying neural mechanisms remain unclear. Shaham and colleagues first reported that cocaine-

*Correspondence to: Zheng-Xiong Xi, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA. zxixi@intramural.nida.nih.gov.

²Current Address: Beijing Institute of Pharmacology and Toxicology, Beijing, 100850, China

[#]Current address: Department Psychiatry, School of Medicine, University of California San Diego, CA 92093, USA

Disclosure/Conflict of Interest

The authors declare no conflicting financial interests.

Author Contributions: Z.-X.X. designed and supervised the experiments, analyzed the data, finalized the figures and wrote the paper. X.L., J.L., X.-Q.P., and R.S. conducted the experiments and analyzed the data. E.L.G. supervised the study and revised the paper.

associated cue-induced drug-seeking behavior progressively increases over time of withdrawal from cocaine self-administration in rats, a phenomenon termed “incubation of craving” (Grimm et al. 2001; Lu et al. 2004b; Lu et al. 2005; Pickens et al. 2011). Mechanistic studies suggest that increased brain-derived neurotrophic factor (BDNF) expression in the mesolimbic dopamine (DA) system (Grimm et al. 2003), increased extracellular signal-regulated kinase (ERK) activation in the central nucleus of the amygdala (CeA) (Lu et al. 2005), and increased GluR2-lacking AMPA receptor expression in the nucleus accumbens (NAc) (Conrad et al. 2008) may underlie incubation of cocaine craving. However, it remains unclear whether a dopamine (DA) mechanism is also involved in incubation of cocaine craving.

Neuroimaging studies in humans suggest that drug-associated visual cues elicit DA release in the dorsal striatum and increase cocaine craving (Volkow et al. 2006; Wong et al. 2006), suggesting a correlation between DA activity and cocaine craving (Volkow et al. 2008). In experimental animals, cocaine-associated cues maintain cocaine self-administration (Ito et al. 2004), potentiate cocaine seeking (Ciccocioppo et al. 2004), and increase extracellular DA in the NAc (Aragona et al. 2009; Day et al. 2007; Ito et al. 2000; Stuber et al. 2008), dorsal striatum (DS) (Ito et al. 2002), and amygdala (Carelli et al. 2003; Harmer and Phillips 1999; Weiss et al. 2000), although other studies failed to show an increase in NAc DA (Bradberry et al. 2000; Brown and Fibiger 1992; Di Ciano et al. 2001). In addition, a recent study suggests that cue-induced incubation of cocaine craving appears to parallel an increase in DA D3, but not D1 or D2, receptor expression in the NAc and ventral caudate-putamen in rats after prolonged (45 days), but not short (1–8 days), withdrawal from cocaine self-administration (Conrad et al. 2010), suggesting a possible role for increased DA-D3R signaling in incubation of cocaine craving. However, direct evidence for this is currently lacking.

In the present study, we first investigated whether systemic administration of SB-277011A, a highly selective D3R antagonist (Reavill et al. 2000), inhibits incubation of cocaine craving. We then investigated loci of action in rat brain by microinjections of SB-277011A into specific brain regions, including NAc shell and core, dorsal striatum, CeA and basal lateral amygdala (BLA), loci which have been shown to be importantly involved in incubation of craving.

MATERIALS AND METHODS

Animals

A total of 102 male Long-Evans rats (Charles River, Raleigh, NC) were used, weighting 250–300 gm at the beginning of the experiments. They were housed in a fully-accredited animal facility and were maintained on a reversed 12 hr light/dark cycle (lights on at 7:00 P.M., lights off at 7:00 A.M.) with food and water available in the home cage. The experimental procedures followed the *Guide for the Care and Use of Laboratory Animals* (1996) and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse.

Surgery

A total 85 of animals were prepared for experimentation by surgical catheterization of the right external jugular vein for intravenous cocaine self-administration. These animals were divided into 8 groups: incubation test group (between-subject design, n=24), incubation test group (within-group design, n=10), systemic SB-277011A treatment group (n=8) and 5 additional groups for intracranial SB-277011A treatment (NAc-shell, NAc-core, DS, BLA, CeA). The jugular catheters were constructed of microrenathane (Braintree Scientific Inc.,

Braintree, MA, USA), and catheterization was performed under sodium pentobarbital anaesthesia (65 mg/kg, i.p.) using standard aseptic surgical techniques. A catheter was inserted into the right atrial auricle through the jugular vein, passed under the skin and fixed to the top of the skull, where it was mated to a connector device (a modified 24 gauge cannula; Plastics One, Roanoke, VA, USA) to which the catheter coming from the infusion pump could be connected for i.v. drug infusions. To determine loci of action in rat brain, five additional groups of rats were also surgically implanted with intracranial guide cannulae (20 gauge, 14 mm; Plastics One, Roanoke, VA, USA) into the NAc shell (AP +1.7 mm, ML $\pm$ 2.0 mm, DV -5.0 mm, 6° angle from vertical), NAc core (AP 1.2 mm, ML $\pm$ 2.20 mm, DV -4.5 mm, 6° angle from vertical), DS (AP 1.0 mm, ML $\pm$ 2.4 mm, DV -3.0 mm, 6° angle from vertical), CeA (-2.3 mm, ML 4.5, DV -7.5 mm, 2° angle), or BLA (-2.8 mm, ML 5.3, DV 7.8 mm, 2° angle) with intracranial target coordinates based on the atlas of Paxinos and Watson (1986). Both the self-administration cannulae and intracranial guide cannulae were fixed to the skull with 4 stainless steel jeweler's screws (Small Parts Inc., Miami Lakes, FL, USA) and dental acrylic. During experimental sessions, the self-administration catheter was connected to an injection pump via tubing encased in a protective metal spring from the head-mounted connector to the top of the experimental chamber. To help prevent clogging, the catheters were flushed daily with a gentamicin-heparin-saline solution (0.1 mg/ml gentamicin, 30 IU/ml heparin; ICN Biochemicals, Cleveland, OH, USA).

Self-administration apparatus

Intravenous (i.v.) self-administration experiments were conducted in operant response test chambers (32 × 25 × 33 cm) from Med Associates Inc. (Georgia, VT, USA). Each test chamber had 2 levers: 1 active and 1 inactive, located 6.5 cm above the floor. Depression of the active lever activated the infusion pump; depression of the inactive lever was counted but had no consequence. A cue light and a speaker were located 12 cm above the active lever. The house light was turned on at the start of each 3 hr test session. Scheduling of experimental events and data collection was accomplished using Med Associates software.

Self-administration procedure

After recovery from surgery, each rat was placed into a test chamber (day time - dark phase) and allowed to lever-press for i.v. cocaine (1 mg/kg/infusion) delivered in 0.08 ml over 4.6 sec, on a fixed ratio 1 (FR1) reinforcement schedule. Each cocaine infusion was associated with presentation of a stimulus light and tone. During the 4.6 sec infusion time, additional responses on the active lever were recorded but did not lead to additional infusions. Each session lasted 3 hr. FR1 reinforcement was used for 3–5 days until stable cocaine self-administration was established: a minimum of 20 presses on the active lever per test session and stability criteria of less than 10% variability in inter-response interval, less than 10% variability in number of infusions taken, and less than 10% variability in number of presses on the active lever for at least 3 consecutive days. Subjects were then allowed to continue cocaine (0.5 mg/kg/infusion) self-administration under FR2 reinforcement. This dose of cocaine was chosen based on previous studies showing that 0.5 mg/kg/infusion of cocaine lies within the middle range of the descending limb of the cocaine dose-response self-administration curve, where reliable dose-dependent effects are observed (Xi et al. 2005). In addition, we chose 0.5 mg/kg, rather than 1 mg/kg, of cocaine in order to increase the work demand (the lever responses) on the animals for the same amount of drug intake. In our experience, this approach increases the sensitivity of assessing changes in drug-seeking behavior. To avoid cocaine overdose, each animal was limited to a maximum of 50 cocaine injections per 3hr session.

Tests for incubation of cocaine craving

After stable cocaine self-administration was achieved, two groups of rats were used to assess cocaine cue-induced incubation of craving) in two different ways. One group of rats (n=24) was used to compare extinction responses in different sub-groups of rats (between-group design) after 2, 10, 30, or 60 days (6 rats in each sub-group) of withdrawal from cocaine self-administration. Another group of rats (n=10) was used to examine contextual cue-induced cocaine seeking (within-group design) after 7, 14, 21 and 28 days of withdrawal. On the test day, rats were re-placed into the same self-administration chambers, and cocaine cue-induced drug-seeking behavior (i.e., extinction responding) was assessed under extinction conditions during which cocaine and cocaine-associated discrete cue-light and tone were unavailable, and lever pressing resulted in no consequence. Each extinction session lasted 3 hr.

Here we used two different experimental designs and different periods of withdrawal to assess cue-induced incubation of cocaine craving for a couple of reasons. First, we used the same between-subject designs as used by others (Lu et al., 2004) to determine whether a similar incubation phenomenon can be observed in rats in which different cocaine self-administration procedures (0.5–1.0 mg/kg/infusion, 3 hrs per session) are used, as compared to the procedures used previously by Shaham's group (0.75 mg/kg/infusion, 1 hr per session $\times$ 5 sessions per day). If incubation was observed, we then determined how long it lasted. Second, we also used the within-subjects design to study whether a similar incubation phenomenon can be observed in a single group of rats with repeated measures after different periods of withdrawal. We then observed the effects of SB-277011A on incubation of cocaine craving after various periods of withdrawal from previous cocaine self-administration using both the between-subjects and within-subjects designs.

Effects of SB-277011A on incubation of cocaine craving

We first evaluated the effects of systemic administration of SB-277011A (6, 12, 24 mg/kg, i.p., 30 min prior to testing) or vehicle (25% β -cyclodextrin) on incubation of cocaine craving in one group of rats (within-group design, n=8) at 21–28 days after cocaine withdrawal. We then observed the effects of SB-277011A (24 mg/kg) on incubation of cocaine craving after different periods (2, 10, 30, 60 days) of withdrawal in different subgroups of rats (between-subjects design, 6 rats each subgroup). After each test, animals were returned to their home cages. The time interval between tests was 2–3 days.

To study loci of action, we used microinjections to determine whether local administration of SB-277011A into the NAc shell, NAc core, dorsal striatum, CeA, or BLA alters incubation of cocaine craving. Five separate groups of rats were used, with measurements taken at 21–28 days of withdrawal from cocaine self-administration. On the test day, each animal randomly received bilateral intracranial microinjections of vehicle or one of two doses of SB-277011A (1.5, 3.0 μ g/ μ l/side, 30 min prior to test). A total of 0.5 μ l was injected bilaterally into the above brain regions over 1 min, and the injectors were then kept in place for an additional 1 min. After each test, animals were returned to their home cages for 2–3 days before being tested again. The doses of SB-277011A were based on pilot preliminary observations and our previous study (Xi et al. 2004). Cannula placements were verified after the completion of the experiments by standard histological and anatomic localization techniques.

Effects of systemic administration of SB-277011A on sucrose-seeking behavior

To determine whether the inhibition of cocaine craving produced by SB-277011A can generalize to other non-drug-induced reward seeking, we observed the effects of SB-277011A on sucrose cue-induced incubation of sucrose craving. Additional 17 rats were

divided into two groups. One group ($n=8$) was used to study sucrose cue-induced incubation of craving over time (within-subjects design), while another group ($n=9$) was used to study the effects of SB-277011A on sucrose-seeking behavior. The procedures for oral sucrose self-administration were identical to the procedures for cocaine self-administration except for the following: 1) no surgery was performed on the animals in the sucrose experiment; 2) active lever presses led to delivery of 0.1 ml of 5% sucrose solution into a liquid food tray on the operant chamber wall; and 3) each daily sucrose self-administration session lasted for 1 hr. The effects of SB-277011A (24 mg/kg, i.p.) on sucrose seeking were observed at 10 or 30 days after sucrose withdrawal.

Effects of microinjections of SB-277011A into the NAc or CeA on locomotor behavior

To determine whether the attenuated cocaine seeking was induced by sedation or impaired locomotion after SB-277011A administration, we observed the effects of microinjections of SB-277011A into the NAc core or CeA on spontaneous locomotion activity. Before drug administration, each animal was placed in a locomotor detection chamber (Accuscan Instruments, Inc., Columbus, OH, USA) for 3 days (4 hr per day) for environmental habituation. On each test day, rats were randomly given either saline or SB-277011A (3 μ g/side, 30 min prior to testing). Animals were then placed into the locomotor chambers to observe the effects of microinjections of SB-277011A into the NAc core or CeA on locomotion. Data were collected in 30 min intervals using the VersaMax data analysis system (version 3.0) (Accuscan Instruments, Inc., Columbus, OH, USA). Total distance was used to evaluate the effects of SB-277011A on locomotor behavior.

Statistical Analysis

Data are presented as means $\pm$ SEMs of active and inactive lever presses on each test day after different durations of withdrawal. One-way ANOVA was used to determine the significance of extinction responses (active lever presses) over time of withdrawal and the effects of SB-277011A on extinction responses and incubation of cocaine craving. Two-way ANOVA was used to determine the significance of SB-277011A-induced changes in locomotion. Whenever a significant main effect was found, individual group comparisons were carried out using the Student-Newman Keuls method.

RESULTS

Time-dependent incubation of cocaine craving in rats

Figure 1A shows mean numbers of cocaine infusions, active lever responses and inactive lever responses during the initial 10 days of cocaine self-administration, demonstrating that the majority of rats (34 of 36) rapidly acquired stable cocaine self-administration behavior after 1–2 weeks of training. Then, the animals were divided into two groups to measure cue-induced incubation of cocaine craving in rats in the absence of cocaine and cocaine-associated discrete cues (i.e., light and tone). One group of rats ($n=24$) was used to study incubation of cocaine craving after 2, 10, 30 or 60 days of withdrawal using between-subjects design (6 rats per subgroup) (Figure 1B). Another group of rats ($n=10$) was used to study incubation of cocaine craving after 2, 7, 14, 21, 28 days of withdrawal using within-subjects design (Figure 1C). There was a significant and time-dependent increase in cocaine-seeking behavior (active lever responses) after prolonged withdrawal from cocaine self-administration compared to that after 2 days of withdrawal (Fig. 1B, $F_{3,21}=13.60$, $p<0.001$; Fig. 1C, $F_{4,36}=12.02$, $p<0.001$). There was no significant difference in inactive lever response over time of withdrawal (Figs. 1B, 1C). Individual group comparisons for the data shown in Fig. 1B revealed a significant increase in active lever response after 10 days ($q=3.88$, $p<0.05$), 30 days ($q=7.25$, $p<0.001$), but not 60 days ($q=0.57$, $p=n.s.$), when compared to that after 2 days, of withdrawal. Individual group comparisons for the data

shown in Fig. 21C revealed a significant increase in active lever responses after 7 days ($q=4.32$, $p<0.01$), 14 days ($q=6.56$, $p<0.001$), 21 days ($q=7.15$, $p<0.001$), or 28 days ($q=9.05$, $p<0.001$), compared to that after 2 days, of withdrawal from cocaine self-administration.

Systemic administration of SB-277011A inhibits cue-induced cocaine seeking

Figure 1D shows that systemic administration of SB-277011A (6, 12, 24 mg/kg) dose-dependently inhibited cocaine seeking (active lever responses) in a separate group of rats after 3–4 weeks of withdrawal (drug main effect, $F_{3,21}=8.53$, $p<0.001$). Individual group comparisons revealed a significant reduction in active lever pressing after 24 mg/kg ($q=7.12$, $p<0.001$), but not 6 mg/kg ($q=2.71$, $p=0.068$) or 12 mg/kg ($q=3.35$, $p=0.069$) SB-277011A, when compared to vehicle control group. Systemic administration of SB-277011A did not alter inactive lever responses. Then, we further assessed whether the same effective dose of SB-277011A also inhibits cue-induced cocaine seeking in different groups of rats after 2, 10, 30, 60 days of withdrawal, respectively. Figure 1E shows that SB-277011A significantly inhibited cue-induced cocaine seeking after 2, 10 or 30 days, but not 60 days, of withdrawal from previous cocaine self-administration ($F_{1,3} = 87.72$, $P<0.001$).

Systemic administration of SB-277011A failed to alter cue-induced sucrose seeking

To determine whether SB-277011A selectively inhibits cue-induced cocaine seeking, we also studied the effects of SB-277011A on cue-induced sucrose-seeking behavior. Unexpectedly, we did not see sucrose cue-induced incubation of sucrose craving, but saw a reduction of sucrose seeking over time (2, 10, 30 days) of withdrawal from previous sucrose self-administration (Fig. 2A, $F_{2,21}=26.89$, $p<0.001$). Then, we further assessed whether SB-277011A, at the effective dose that inhibited cocaine seeking, also inhibited sucrose-seeking behavior. We found that systemic administration of SB-277011A did not alter sucrose-seeking behavior (Fig. 2B) measured at 10 days ($F_{1,9}=0.006$, $p=n.s.$) or 30 days ($F_{1,9}=0.01$, $p=n.s.$) after sucrose withdrawal.

Microinjections of SB-277011A into the NAc or CeA, but not dorsal striatum or BLA, inhibit contextual cue-induced cocaine seeking

To determine loci of action in the brain, SB-277011A was locally microinjected into different brain regions. Microinjections of SB-277011A into the NAc shell (Fig. 3A, $F_{2,16}=19.11$, $p<0.001$), NAc core (Fig. 3B, $F_{2,10}=12.77$, $p<0.01$), but not dorsal striatum (Fig. 3C, $F_{2,40}=1.43$, $p=n.s.$), significantly inhibited cue-induced cocaine seeking measured at 3–4 weeks after withdrawal from cocaine self-administration. Microinjections of SB-277011A into the CeA (Fig. 4A, $F_{2,16}=11.79$, $p<0.001$), but not the BLA (Fig. 4B, $F_{2,14}=1.76$, $p=n.s.$), also significantly and dose-dependently inhibited cue-induced cocaine seeking.

Microinjections of SB-277011A into the NAc or CeA fail to alter locomotion

Finally, to determine whether the inhibition of cocaine-seeking behavior produced by microinjections of SB-277011A was due to sedation or locomotor impairment after drug administration, SB-277011A was locally administered into the NAc core or CeA, two brain loci where SB-277011A significantly and dose-dependently inhibited cue-induced cocaine seeking. Figure 5 shows that SB-277011A, at the effective dose (3 μ g/side) that significantly inhibited cocaine seeking above, failed to alter locomotor activity when microinjected into either of these brain regions (Fig. 5A, treatment main effect, $F_{1,6} = 0.91$, $P>0.05$; Fig. 5B, $F_{1,6} = 2.04$, $P>0.05$).

Discussion

The present study demonstrates that re-exposure to cocaine-associated (contextual) cues induces time-dependent increases in cocaine-seeking behavior in rats up to 30 days, but not at 60 days, of forced withdrawal from cocaine self-administration. This effect was dose-dependently blocked by systemic administration or local microinjection of SB-277011A into the NAc (shell or core) or CeA, but not into the dorsal striatum or BLA, suggesting an important role for NAc and CeA DA D3Rs in incubation of cocaine craving.

Two animal models are commonly used to evaluate cue-induced drug-seeking behavior. One is *conditioned cue-induced reinstatement of drug-seeking* behavior (See 2005; Shalev et al. 2002), in which operant responding for cocaine self-administration is paired with discrete conditioned cues (light and tones). Drug-seeking behavior is then extinguished in the *absence* of cocaine and cocaine-paired cues. Rats are then tested for cue-induced reinstatement of drug-seeking behavior during which the previously-reinforced operant response leads only to delivery of the conditioned cues (light and tones). The second model is *incubation of craving*, in which animals are forcibly withdrawn from cocaine self-administration without behavioral extinction of the previously-reinforced operant responding. After varying periods of withdrawal time in their home cages, animals are re-exposed to the environmental contextual cues previously paired with drug self-administration, i.e., the self-administration chambers. Subsequent lever responding (drug-seeking) leads either to no consequence (no drug, no conditioned cue light and tones) (Grimm et al. 2003) or leads to delivery of conditioned cues only (but no drug) (Grimm et al. 2001; Lu et al. 2005). The former procedure can be used to study contextual cue-induced drug-seeking behavior. Strikingly, there is a progressive increase in drug seeking over duration of withdrawal. Since the time course of the increase in cocaine seeking is somewhat similar to that of the expression of psychostimulant sensitization after withdrawal (Kalivas and Stewart 1991; Robinson and Berridge 1993) and that of drug craving in humans during abstinence (Gawin and Kleber 1986; Satel et al. 1991), it has been termed “cue-induced incubation of cocaine craving” (Grimm et al. 2001; Grimm et al. 2003; Lu et al. 2005).

In the present study, we found that contextual cue-induced incubation of craving persisted only for about 30 days of withdrawal, much shorter than the previously reported 60–90 days of withdrawal (Grimm et al. 2001; Grimm et al. 2003). This could be due to different experimental conditions during cocaine self-administration and testing including different cocaine doses (0.5 mg/kg/infusion in the present study *versus* 0.75 mg/kg/infusion in the above cited studies), different durations of cocaine self-administration session(s) (3 hrs *versus* 6 hrs), different durations of testing (3 hrs *versus* 30 min), and more importantly, different cues (contextual cues only *versus* contextual cues + conditioned cues) during testing. In addition, we did not see an increase in cue-induced sucrose seeking as previously reported (Grimm et al. 2011; Grimm et al. 2005); rather, we observed a time-dependent reduction in cue-induced sucrose seeking. Again, differences in experimental conditions including sucrose concentration (5% in the present study *versus* 10% in the above cited studies), duration of self-administration (1 hr *versus* 6 hr), and the presence of different cues (contextual cues only *versus* contextual cues + conditioned cues) during testing may have contributed to the different results.

Importantly, the reduction in cocaine seeking after SB-277011A administration was unlikely due to sedative effects or locomotor impairment because 1) systemic administration of SB-277011A alters neither basal locomotion (Pak et al. 2006; Song et al. 2012) nor cue-induced sucrose seeking (present study); 2) local administration of SB-277011A into the NAc or CeA failed to alter locomotor activity (Liang et al. 2011) (present study); and 3)

systemic or local administration of SB-277011A failed to alter inactive lever responding for cocaine or sucrose seeking (present study).

The mechanisms underlying incubation of cocaine craving are incompletely understood. Early studies suggested that incubation of cocaine craving is associated with increased BDNF expression in the VTA, NAc, and amygdala (Grimm et al. 2003). This increased BDNF had a similar time course to that of incubation of craving. Microinjections of BDNF into the VTA or NAc shell cause delayed and long-lasting increases in cocaine seeking after withdrawal (Lu et al. 2004a). The mechanism(s) by which BDNF potentiates incubation of cocaine craving are unclear. Since pharmacological inhibition of intracellular ERK reverses the behavioral effect of VTA BDNF injections (Lu et al. 2004a), it has been suggested that intracellular ERK signaling mechanisms may be involved. This is supported by evidence that cue-induced incubation of cocaine craving parallels increased ERK phosphorylation in the CeA, but not in the BLA, over 30 days of cocaine withdrawal (Lu et al. 2005). Further, activation of CeA ERK by NMDA increases, while inhibition of ERK by U0126 attenuates cue-induced incubation of cocaine craving.

Wolf and colleagues have recently suggested that an enhanced NAc GluR2-lacking AMPA receptor mechanism is involved in incubation of cocaine craving (Conrad et al. 2008). Due to the high conductance of GluR2-lacking AMPA receptors, it was proposed that increased synaptic AMPA receptors should increase reactivity of NAc neurons to cocaine-associated cues, and thereby intensify cocaine craving and relapse to cocaine-seeking behavior. Supportingly, blockade of GluR2-lacking AMPA receptors inhibited cue-induced incubation of cocaine craving (Conrad et al. 2008).

The mesocorticolimbic DA system projecting from the VTA to the NAc, amygdala and prefrontal cortex is critically involved in drug reward and addiction, including cue-elicited reinstatement of drug-seeking behavior (Kalivas and McFarland 2003; See et al. 2003; Shalev et al. 2002). However, it has been unclear whether a DA receptor mechanism is implicated in cue-induced incubation of cocaine craving. As noted above, cocaine-conditioned cues significantly increase extracellular DA in the NAc (Aragona et al. 2009; Day et al. 2007; Ito et al. 2000; Stuber et al. 2008), dorsal striatum (Ito et al. 2002), and amygdala (Carelli et al. 2003; Harmer and Phillips 1999; Weiss et al. 2000), suggesting that enhanced DA transmission may be involved in cue-induced craving and relapse to drug-seeking behavior. Consistent with this hypothesis, it was recently reported that enhanced D3R (but not D1R or D2R) expression in the NAc appears to be associated with the incubation of cocaine craving (Conrad et al. 2010). In that study, cue-induced incubation of cocaine craving was found to parallel an increase in D3R binding and cell surface expression in the NAc core in rats after prolonged (45 days), but not brief (1–8 days), withdrawal from cocaine self-administration (Conrad et al. 2010). In contrast, increased cell surface D1 receptor expression was observed only in the NAc shell on day 1, but not day 45, after withdrawal, while *decreased* cell surface D2R expression in NAc was observed on both day 1 and day 45 after cocaine withdrawal (Conrad et al. 2010). A similar increase in D3R binding and mRNA expression was also observed in the NAc of human cocaine addicts (Mash and Staley 1999; Staley and Mash 1996), in rats after prolonged (31–32 days) but not brief (1–8 days) cocaine self-administration (Neisewander et al. 2004), and in mice behaviorally sensitized to cocaine-associated cues (Le Foll et al. 2002).

In the present study, we found that systemic administration of SB-277011A significantly inhibited cue-induced cocaine seeking and incubation of cocaine craving, i.e., a progressive increase of cue-induced cocaine seeking over time of withdrawal. These findings, combined with previous findings that cue-induced incubation of cocaine craving parallel an increase in D3R expression (Conrad et al. 2010; Neisewander et al. 2004; Staley and Mash 1996),

support an important role for NAc D3Rs in cue-induced incubation of cocaine craving. However, we should point out that this D3R mechanism is not specific to incubation of cocaine craving because blockade of D3Rs also inhibited cue-induced cocaine seeking as measured after 2 days of withdrawal during which no incubation of craving was observed. This is consistent with previous reports that blockade of D3Rs also inhibits cue reactivity as discussed below.

To further determine the loci of action in the brain, SB-277011A was locally administered into different brain regions. We found that microinjections of SB-277011A into the NAc core or shell significantly inhibited cue-induced incubation of cocaine craving. We note that SB-277011A was more potent and effective when it was locally administered into the NAc core than when administered into the NAc shell. This finding appears to be congruent with views that the NAc core and shell play different roles in addictive behaviors. NAc core inactivation (by the GABA receptor agonists muscimol and baclofen) inhibits (Di Ciano et al. 2008; Fuchs et al. 2004), while NAc shell inactivation fails to alter cue-induced reinstatement of drug-seeking behavior (Fuchs et al. 2004) or produces an increase in cue-induced reinstatement of drug-seeking behavior (Di Ciano et al. 2008). Similarly, NAc core inactivation decreases conditioned reinforcement as measured under second-order reinforcement (Di Ciano and Everitt 2004; Ito et al. 2000), while NAc shell inactivation has no effect on responding for drug-paired conditioned reinforcers (Ito et al. 2004; Parkinson et al. 1999). In contrast, inactivation of the NAc shell decreases stress-induced reinstatement (McFarland et al. 2004), and blockade of NAc shell DA receptors decreases cocaine-induced reinstatement (Anderson et al. 2003; Bachtell et al. 2005; Xi et al. 2004). Such data suggest that NAc core may be essential in cue-induced responding, while NAc shell may be important for cocaine- or stress-induced reinstatement of drug-seeking behavior. The present findings support an important role of the NAc, particularly NAc core, in incubation of contextual cue-triggered cocaine-seeking behavior.

Neuroimaging studies in humans suggest that drug-associated visual cues elicit cocaine craving along with DA release in the dorsal striatum (Volkow et al. 2006; Wong et al. 2006), suggesting a possible role for enhanced dorsal striatum DA transmission in cocaine craving (Volkow et al. 2008). However, in the present study, we did not see significant effects of SB-277011A on contextual cue-induced cocaine seeking when it was locally administered into the dorsal striatum. This is consistent with previous reports demonstrating that microinjections of SB-277011A into the dorsal striatum fail to alter foot-shock stress-induced reinstatement of cocaine-seeking behavior (Xi et al. 2004) and that dorsal striatum inactivation fails to affect conditioned cue-induced reinstatement of cocaine seeking (Di Ciano et al. 2008). We note that the present findings appear to conflict with other reports demonstrating that the dorsal striatum is importantly involved in habitual drug-seeking behavior in rats after chronic (30–60 days) cocaine self-administration (Belin and Everitt 2008; Ito et al. 2002; Vanderschuren et al. 2005). Thus, the different durations of cocaine self-administration (10–15 days in the present study *versus* 30–60 days in the above cited studies) could be an important factor underlying the different results observed between the present study and others. That is, chronic cocaine self-administration may cause transition from initial voluntary drug use to habitual and ultimately compulsive drug-taking and drug-seeking behavior by chronic cocaine-induced neuroadaptive changes in locus of control over behavior from the ventral striatum (NAc) to more dorsal striatum (Everitt et al. 2008).

In addition to the NAc, the amygdala has also been shown to be importantly involved in drug reward and relapse to drug-seeking behavior. Cocaine injections or exposure to cocaine-associated cues activates the amygdala in animals and humans as assessed by neuroimaging and c-fos expression studies (Grant et al. 1996; Kilts et al. 2004; Neisewander et al. 2000). Amygdala lesions or microinjections of DA receptor antagonists into the

amygdala inhibit the reinforcing and discriminative stimulus effects of cocaine, as well as the conditioned motivational effects of cocaine-associated cues (Berglund et al. 2006; Brown and Fibiger 1993; Caine et al. 1995; Hurd et al. 1997; McGregor and Roberts 1993; See et al. 2001). Other studies suggest dissociable roles for the CeA and BLA in mediating cocaine-related behavior. Microinjections of psychostimulants into the CeA, but not the BLA, produce a conditioned place preference, whereas selective lesions of the BLA do not affect cocaine self-administration (Grimm and See 2000; Meil and See 1997; Yun and Fields 2003). Such data suggest an important role for the CeA in processing the primary rewarding effects of psychostimulants (O'Dell et al. 1999). In contrast, both the CeA and the BLA appear to be involved in responding for conditioned reinforcers (Burns et al. 1993; Ciccocioppo et al. 2004; Fuchs et al. 2006; Fuchs and See 2002; Kruzich and See 2001; Neisewander et al. 2000; Robledo et al. 1996).

In the present study, we found that microinjections of SB-277011A into the CeA, but not the BLA, significantly inhibited contextual cue-induced cocaine seeking in a dose-dependent manner. This is consistent with a previous report demonstrating that a NMDA-ERK signaling mechanism in the CeA, but not in the BLA, is critically involved in incubation of cocaine craving (Lu et al., 2005). It is also consistent with findings that the CeA receives the greatest dopaminergic innervation (Asan 1997; Fallon et al. 1992) and has higher D3 receptor expression than other subregions of the amygdala complex (Herroelen et al. 1994; Murray et al. 1994; Suzuki et al. 1998; Tupala et al. 2001).

In conclusion, the present study demonstrates a progressive increase in cue-induced cocaine seeking in rats over the 30-day period after withdrawal from cocaine self-administration. This increase of craving was dose-dependently inhibited by systemic administration or local microinjections of SB-277011A into the NAc or CeA, but not the dorsal striatum or BLA. These data suggest that, in addition to previously suggested BDNF-/glutamate-ERK mechanisms, enhanced DA-D₃R mechanisms in both the NAc and amygdala may also play an important role in cue-induced incubation of cocaine craving and relapse to drug-seeking behavior. Such findings add to the significant and growing body of literature suggesting that highly selective DA D₃ receptor antagonists may have a useful role in the treatment of drug addiction.

Acknowledgments

This work was supported by the U.S. National Institute on Drug Abuse Intramural Research Program.

References

- Anderson SM, Bari AA, Pierce RC. Administration of the D1-like dopamine receptor antagonist SCH-23390 into the medial nucleus accumbens shell attenuates cocaine priming-induced reinstatement of drug-seeking behavior in rats. *Psychopharmacology (Berl)*. 2003; 168:132–8. [PubMed: 12491029]
- Aragona BJ, Day JJ, Roitman MF, Cleaveland NA, Wightman RM, Carelli RM. Regional specificity in the real-time development of phasic dopamine transmission patterns during acquisition of a cue-cocaine association in rats. *Eur J Neurosci*. 2009; 30:1889–99. [PubMed: 19912327]
- Asan E. Ultrastructural features of tyrosine-hydroxylase-immunoreactive afferents and their targets in the rat amygdala. *Cell Tissue Res*. 1997; 288:449–69. [PubMed: 9134859]
- Bachtell RK, Whisler K, Karanian D, Self DW. Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. *Psychopharmacology (Berl)*. 2005; 183:41–53. [PubMed: 16163523]
- Belin D, Everitt BJ. Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron*. 2008; 57:432–41. [PubMed: 18255035]

- Berglind WJ, Case JM, Parker MP, Fuchs RA, See RE. Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience*. 2006; 137:699–706. [PubMed: 16289883]
- Bradberry CW, Barrett-Larimore RL, Jatlow P, Rubino SR. Impact of self-administered cocaine and cocaine cues on extracellular dopamine in mesolimbic and sensorimotor striatum in rhesus monkeys. *J Neurosci*. 2000; 20:3874–83. [PubMed: 10804227]
- Brown EE, Fibiger HC. Cocaine-induced conditioned locomotion: absence of associated increases in dopamine release. *Neuroscience*. 1992; 48:621–9. [PubMed: 1603333]
- Brown EE, Fibiger HC. Differential effects of excitotoxic lesions of the amygdala on cocaine-induced conditioned locomotion and conditioned place preference. *Psychopharmacology (Berl)*. 1993; 113:123–30. [PubMed: 7862818]
- Burns LH, Robbins TW, Everitt BJ. Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. *Behav Brain Res*. 1993; 55:167–83. [PubMed: 8357526]
- Caine SB, Heinrichs SC, Coffin VL, Koob GF. Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Res*. 1995; 692:47–56. [PubMed: 8548319]
- Carelli RM, Williams JG, Hollander JA. Basolateral amygdala neurons encode cocaine self-administration and cocaine-associated cues. *J Neurosci*. 2003; 23:8204–11. [PubMed: 12967981]
- Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP. Limbic activation during cue-induced cocaine craving. *Am J Psychiatry*. 1999; 156:11–8. [PubMed: 9892292]
- Ciccocioppo R, Martin-Fardon R, Weiss F. Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. *Nat Neurosci*. 2004; 7:495–6. [PubMed: 15048121]
- Conrad KL, Ford K, Marinelli M, Wolf ME. Dopamine receptor expression and distribution dynamically change in the rat nucleus accumbens after withdrawal from cocaine self-administration. *Neuroscience*. 2010; 169:182–94. [PubMed: 20435100]
- Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*. 2008; 454:118–21. [PubMed: 18500330]
- Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat Neurosci*. 2007; 10:1020–8. [PubMed: 17603481]
- Di Ciano P, Blaha CD, Phillips AG. Changes in dopamine efflux associated with extinction, CS-induced and d-amphetamine-induced reinstatement of drug-seeking behavior by rats. *Behav Brain Res*. 2001; 120:147–58. [PubMed: 11182163]
- Di Ciano P, Everitt BJ. Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. *Neuropharmacology*. 2004; 47(Suppl 1):202–13. [PubMed: 15464138]
- Di Ciano P, Robbins TW, Everitt BJ. Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. *Neuropsychopharmacology*. 2008; 33:1413–25. [PubMed: 17712353]
- Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW. Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:3125–35. [PubMed: 18640910]
- Fallon JH, Di Salvo J, Loughlin SE, Gimenez-Gallego G, Seroogy KB, Bradshaw RA, Morrison RS, Ciofi P, Thomas KA. Localization of acidic fibroblast growth factor within the mouse brain using biochemical and immunocytochemical techniques. *Growth Factors*. 1992; 6:139–57. [PubMed: 1375040]
- Fuchs RA, Evans KA, Parker MC, See RE. Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)*. 2004; 176:459–65. [PubMed: 15138757]

- Fuchs RA, Feltenstein MW, See RE. The role of the basolateral amygdala in stimulus-reward memory and extinction memory consolidation and in subsequent conditioned cued reinstatement of cocaine seeking. *Eur J Neurosci.* 2006; 23:2809–13. [PubMed: 16817884]
- Fuchs RA, See RE. Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. *Psychopharmacology (Berl).* 2002; 160:425–33. [PubMed: 11919670]
- Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA. Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. *Am J Psychiatry.* 2000; 157:1789–98. [PubMed: 11058476]
- Gawin FH, Kleber HD. Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. *Arch Gen Psychiatry.* 1986; 43:107–13. [PubMed: 3947206]
- Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A. Activation of memory circuits during cue-elicited cocaine craving. *Proc Natl Acad Sci U S A.* 1996; 93:12040–5. [PubMed: 8876259]
- Grimm JW, Barnes J, North K, Collins S, Weber R. A general method for evaluating incubation of sucrose craving in rats. *J Vis Exp.* 2011:e3335. [PubMed: 22083029]
- Grimm JW, Fyall AM, Osincup DP. Incubation of sucrose craving: effects of reduced training and sucrose pre-loading. *Physiol Behav.* 2005; 84:73–9. [PubMed: 15642609]
- Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature.* 2001; 412:141–2. [PubMed: 11449260]
- Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci.* 2003; 23:742–7. [PubMed: 12574402]
- Grimm JW, See RE. Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. *Neuropsychopharmacology.* 2000; 22:473–9. [PubMed: 10731622]
- Harmer CJ, Phillips GD. Enhanced dopamine efflux in the amygdala by a predictive, but not a non-predictive, stimulus: facilitation by prior repeated D-amphetamine. *Neuroscience.* 1999; 90:119–30. [PubMed: 10188939]
- Herroelen L, De Backer JP, Wilczak N, Flamez A, Vauquelin G, De Keyser J. Autoradiographic distribution of D3-type dopamine receptors in human brain using [3H]7-hydroxy-N, N-di-n-propyl-2-aminotetralin. *Brain Res.* 1994; 648:222–8. [PubMed: 7922537]
- Hurd YL, McGregor A, Ponten M. In vivo amygdala dopamine levels modulate cocaine self-administration behaviour in the rat: D1 dopamine receptor involvement. *Eur J Neurosci.* 1997; 9:2541–8. [PubMed: 9517459]
- Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J Neurosci.* 2000; 20:7489–95. [PubMed: 11007908]
- Ito R, Dalley JW, Robbins TW, Everitt BJ. Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. *J Neurosci.* 2002; 22:6247–53. [PubMed: 12122083]
- Ito R, Robbins TW, Everitt BJ. Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nat Neurosci.* 2004; 7:389–97. [PubMed: 15034590]
- Kalivas PW, McFarland K. Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology (Berl).* 2003; 168:44–56. [PubMed: 12652346]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev.* 1991; 16:223–44. [PubMed: 1665095]
- Kilts CD, Gross RE, Ely TD, Drexler KP. The neural correlates of cue-induced craving in cocaine-dependent women. *Am J Psychiatry.* 2004; 161:233–41. [PubMed: 14754771]
- Kruzich PJ, See RE. Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J Neurosci.* 2001; 21:RC155. [PubMed: 11425882]

- Le Foll B, Frances H, Diaz J, Schwartz JC, Sokoloff P. Role of the dopamine D3 receptor in reactivity to cocaine-associated cues in mice. *Eur J Neurosci.* 2002; 15:2016–26. [PubMed: 12099907]
- Liang J, Zheng X, Chen J, Li Y, Xing X, Bai Y, Li Y. Roles of BDNF, dopamine D(3) receptors, and their interactions in the expression of morphine-induced context-specific locomotor sensitization. *Eur Neuropsychopharmacol.* 2011; 21:825–34. [PubMed: 21277174]
- Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y. A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal. *J Neurosci.* 2004a; 24:1604–11. [PubMed: 14973246]
- Lu L, Grimm JW, Hope BT, Shaham Y. Incubation of cocaine craving after withdrawal: a review of preclinical data. *Neuropharmacology.* 2004b; 47(Suppl 1):214–26. [PubMed: 15464139]
- Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y. Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. *Nat Neurosci.* 2005; 8:212–9. [PubMed: 15657599]
- Mash DC, Staley JK. D3 dopamine and kappa opioid receptor alterations in human brain of cocaine-overdose victims. *Ann N Y Acad Sci.* 1999; 877:507–22. [PubMed: 10415668]
- McFarland K, Davidge SB, Lapish CC, Kalivas PW. Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. *J Neurosci.* 2004; 24:1551–60. [PubMed: 14973230]
- McGregor A, Roberts DC. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. *Brain Res.* 1993; 624:245–52. [PubMed: 8252397]
- Meil WM, See RE. Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. *Behav Brain Res.* 1997; 87:139–48. [PubMed: 9331482]
- Murray AM, Ryoo HL, Gurevich E, Joyce JN. Localization of dopamine D3 receptors to mesolimbic and D2 receptors to mesostriatal regions of human forebrain. *Proc Natl Acad Sci U S A.* 1994; 91:11271–5. [PubMed: 7972046]
- Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF. Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *J Neurosci.* 2000; 20:798–805. [PubMed: 10632609]
- Neisewander JL, Fuchs RA, Tran-Nguyen LT, Weber SM, Coffey GP, Joyce JN. Increases in dopamine D3 receptor binding in rats receiving a cocaine challenge at various time points after cocaine self-administration: implications for cocaine-seeking behavior. *Neuropsychopharmacology.* 2004; 29:1479–87. [PubMed: 15100700]
- O'Dell LE, Sussman AN, Meyer KL, Neisewander JL. Behavioral effects of psychomotor stimulant infusions into amygdaloid nuclei. *Neuropsychopharmacology.* 1999; 20:591–602. [PubMed: 10327428]
- Pak AC, Ashby CR Jr, Heidbreder CA, Pilla M, Gilbert J, Xi ZX, Gardner EL. The selective dopamine D3 receptor antagonist SB-277011A reduces nicotine-enhanced brain reward and nicotine-paired environmental cue functions. *Int J Neuropsychopharmacol.* 2006; 9:585–602. [PubMed: 16942635]
- Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J Neurosci.* 1999; 19:2401–11. [PubMed: 10066290]
- Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. *Trends Neurosci.* 2011; 34:411–20. [PubMed: 21764143]
- Reavill C, Taylor SG, Wood MD, Ashmeade T, Austin NE, Avenell KY, Boyfield I, Branch CL, Cilia J, Coldwell MC, Hadley MS, Hunter AJ, Jeffrey P, Jewitt F, Johnson CN, Jones DN, Medhurst AD, Middlemiss DN, Nash DJ, Riley GJ, Routledge C, Stemp G, Thewlis KM, Trail B, Vong AK, Hagan JJ. Pharmacological actions of a novel, high-affinity, and selective human dopamine D(3) receptor antagonist, SB-277011-A. *J Pharmacol Exp Ther.* 2000; 294:1154–65. [PubMed: 10945872]

- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev.* 1993; 18:247–91. [PubMed: 8401595]
- Robledo P, Robbins TW, Everitt BJ. Effects of excitotoxic lesions of the central amygdaloid nucleus on the potentiation of reward-related stimuli by intra-accumbens amphetamine. *Behav Neurosci.* 1996; 110:981–90. [PubMed: 8919000]
- Satel SL, Southwick SM, Gawin FH. Clinical features of cocaine-induced paranoia. *Am J Psychiatry.* 1991; 148:495–8. [PubMed: 2006696]
- See RE. Neural substrates of cocaine-cue associations that trigger relapse. *Eur J Pharmacol.* 2005; 526:140–6. [PubMed: 16253228]
- See RE, Fuchs RA, Ledford CC, McLaughlin J. Drug addiction, relapse, and the amygdala. *Ann N Y Acad Sci.* 2003; 985:294–307. [PubMed: 12724166]
- See RE, Kruzich PJ, Grimm JW. Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. *Psychopharmacology (Berl).* 2001; 154:301–10. [PubMed: 11351937]
- Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev.* 2002; 54:1–42. [PubMed: 11870259]
- Song R, Yang RF, Wu N, Su RB, Li J, Peng XQ, Li X, Gaal J, Xi ZX, Gardner EL. YQA14: a novel dopamine D3 receptor antagonist that inhibits cocaine self-administration in rats and mice, but not in D3 receptor-knockout mice. *Addict Biol.* 2012; 17:259–73. [PubMed: 21507153]
- Staley JK, Mash DC. Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci.* 1996; 16:6100–6. [PubMed: 8815892]
- Stuber GD, Klanker M, de Ridder B, Bowers MS, Joosten RN, Feenstra MG, Bonci A. Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. *Science.* 2008; 321:1690–2. [PubMed: 18802002]
- Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Sedvall G. D3 dopamine receptor mRNA is widely expressed in the human brain. *Brain Res.* 1998; 779:58–74. [PubMed: 9473588]
- Tupala E, Hall H, Bergstrom K, Sarkioja T, Rasanen P, Mantere T, Callaway J, Hiltunen J, Tiihonen J. Dopamine D(2)/D(3)-receptor and transporter densities in nucleus accumbens and amygdala of type 1 and 2 alcoholics. *Mol Psychiatry.* 2001; 6:261–7. [PubMed: 11326293]
- Vanderschuren LJ, Di Ciano P, Everitt BJ. Involvement of the dorsal striatum in cue-controlled cocaine seeking. *J Neurosci.* 2005; 25:8665–70. [PubMed: 16177034]
- Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J Neurosci.* 2006; 26:6583–8. [PubMed: 16775146]
- Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C. Dopamine increases in striatum do not elicit craving in cocaine abusers unless they are coupled with cocaine cues. *Neuroimage.* 2008; 39:1266–73. [PubMed: 18024160]
- Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben-Shahar O. Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. *Proc Natl Acad Sci U S A.* 2000; 97:4321–6. [PubMed: 10760299]
- Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brasic JR, Kimes AS, Maris MA, Kumar A, Contoreggi C, Links J, Ernst M, Rousset O, Zuckin S, Grace AA, Lee JS, Rohde C, Jasinski DR, Gjedde A, London ED. Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. *Neuropsychopharmacology.* 2006; 31:2716–27. [PubMed: 16971900]
- Xi ZX, Gilbert J, Campos AC, Kline N, Ashby CR Jr, Hagan JJ, Heidbreder CA, Gardner EL. Blockade of mesolimbic dopamine D3 receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. *Psychopharmacology (Berl).* 2004; 176:57–65. [PubMed: 15083257]
- Xi ZX, Gilbert JG, Pak AC, Ashby CR Jr, Heidbreder CA, Gardner EL. Selective dopamine D3 receptor antagonism by SB-277011A attenuates cocaine reinforcement as assessed by progressive-ratio and variable-cost-variable-payoff fixed-ratio cocaine self-administration in rats. *Eur J Neurosci.* 2005; 21:3427–38. [PubMed: 16026480]

Yun IA, Fields HL. Basolateral amygdala lesions impair both cue- and cocaine-induced reinstatement in animals trained on a discriminative stimulus task. *Neuroscience*. 2003; 121:747–57. [PubMed: 14568033]

Figure 1.

Lever responses during cocaine self-administration or after different periods of withdrawal in the absence or presence of SB-277011A pretreatment. **A**: the mean numbers of cocaine infusions, and active and inactive lever responses during the initial 10 days of cocaine self-administration. And then these 34 animals were divided into two groups to observe cue-induced incubation of cocaine craving measured in four different groups of rats ($n=24$, 6 rats per subgroup) after 2, 10, 30, 60 days of withdrawal (**B**) or in a single group of rats ($n=10$) after 2, 7, 14, 21, 28 days of withdrawal (**C**). Systemic administration of SB-277011A (6, 12, 24 mg/kg, i.p., 30 min prior to test) dose-dependently inhibited cue-induced cocaine-seeking behavior (**D**, **E**). * $P<0.05$, *** $P<0.001$, compared to day 2 of withdrawal (**B**, **C**) or vehicle (**D**, **E**) control group.

Figure 2.

Cue-induced sucrose seeking and the effects of SB-277011A on sucrose-seeking behavior.

A: Contextual cue-induced sucrose seeking over different periods (2, 10, 30 days) of withdrawal measured in one group of rats. **B:** Systemic administration of SB-277011A (24 mg/kg, i.p., 30 min prior to test) had no effect on cue-induced sucrose-seeking behavior measured at 10 days or 30 days after withdrawal in a separate group of rats. *** $p < 0.001$, compared to day 2 of withdrawal (**A**).

Figure 3.

Effects of local microinjection of SB-277011A (0, 1.5, 3 µg/0.5 µl/side) into the NAc shell (A), NAc core (B), or dorsal striatum (C) on incubation of cocaine craving. Upper panels show locations of drug microinjections in the brain. * $p < 0.05$, *** $p < 0.001$, compared to vehicle control group.

Figure 4.

Effects of local microinjection of SB-277011A (0, 1.5, 3 µg/0.5 µl/side) into the BLA (A) or CeA (B) on incubation of cocaine craving. Upper panels show locations of drug microinjections in the brain. * $p < 0.05$, *** $p < 0.001$, compared to vehicle control group.

Figure 5.
Effects of microinjections of SB-277011A into the NAc core (**A**) or CeA (**B**) on locomotor behavior in rats.