Dopamine D<sub>1</sub> receptor antagonism in the prelimbic cortex blocks the reinstatement of heroin-seeking in an animal model of relapse

Ronald E. See
Department of Neurosciences, Medical University of South Carolina, Charleston, SC, USA

Abstract
In brain regions that have been implicated in the reinstatement of drug-seeking, the prelimbic cortex has emerged as a critical regulator of relapse behaviours. Here, the effects of prelimbic cortex dopamine (DA) D<sub>1</sub> receptor antagonism on drug-seeking produced by heroin-paired cues, or by a single priming dose of heroin are examined. Rats lever-pressed daily for i.v. heroin discretely paired with a conditioned stimulus during 3-h sessions for a period of 2 wk, followed by extinction and reinstatement of drug-seeking by previously heroin-paired cues (tone + light) or heroin-priming injections (0.25 mg/kg) in the absence of heroin reinforcement. Intracranial infusion of the DA D<sub>1</sub> receptor antagonist, SCH 23390 (0.02–2.0 μg/side), into the prelimbic cortex potently and dose dependently attenuated heroin-seeking in response to either cue presentations or a priming dose of heroin. These results suggest that DA D<sub>1</sub> receptors regulate prefrontal cortex pathways necessary for the reinstatement of heroin-seeking.

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Introduction
Relapse to drug-taking following periods of abstinence constitutes the major impediment in the treatment of addiction. Relapse can be triggered by stimuli previously associated with the drug (Childress et al. 1999), exposure to the drug itself (Jaffe et al. 1989), or stress (Sinha et al. 2000). Using the reinstatement model of relapse in animals, exposure to conditioned cues, stress, or a drug-priming injection will reinduce extinguished drug-seeking as defined by responding on a previously drug-paired operandum in the absence of drug reinforcement (Shaham et al. 2003). A number of studies have explored the neural circuitry for relapse to drug-seeking, primarily for psycho-stimulants and opiates (Feltenstein & See, 2008; Shalev et al. 2002). While several key brain regions have been implicated in the reinstatement of drug-seeking, the prelimbic region of the medial prefrontal cortex has emerged as a critical component of the relapse circuitry across different modalities of reinstatement and different classes of abused drugs (Kalivas & Volkow, 2005). The results from animal models of relapse coincide well with in-vivo brain-imaging studies, in which the prefrontal cortex shows activation in human drug addicts during presentation of drug-associated cues and other trigger factors (Volkow et al. 2004).

While it has been established that the prelimbic cortex is important for drug-seeking, the specific cortical neurotransmitter regulation during reinstatement has not been well explored. Prefrontal dopamine (DA) innervation is a critical regulator of cognitive and motivational domains (Seamans & Yang, 2004), and prefrontal cortex DA dysfunction has been suggested to play a major role in psychopathologies, including addiction (Volkow et al. 2002). Some evidence has implicated medial prefrontal DA activity during reinstatement of cocaine-seeking in that DA receptor blockade reduced cocaine-primed (Sun & Rebec, 2005) or stress-induced (Capriles et al. 2003) reinstatement, while DA infusion alone into the prelimbic cortex produced reinstatement of cocaine-seeking (McFarland & Kalivas, 2001). However, while the prelimbic cortex...
has been implicated in cue-induced reinstatement (McLaughlin & See, 2003), the role of prefrontal DA receptor modulation of cue-induced relapse remains unknown.

It has recently been reported that the neurocircuitry underlying reinstatement of heroin-seeking showed overlapping, yet distinct differences with the neurocircuitry for reinstatement of cocaine-seeking (Rogers et al. 2008). The prelimbic cortex was one of the key brain regions necessary to maintain reinstatement produced by either heroin-associated cues or heroin priming. While opiates, including heroin, act primarily via stimulation of the µ-opioid receptor, heroin activation of µ-opioid receptors indirectly stimulates DA release. Dopaminergic mechanisms presumably play a key role in relapse in opiate addiction, probably via DA D₁ receptors mediate prefrontal cortex neuronal excitability important for cognitive and motivational functions (Vijayraghavan et al. 2007). Thus, the present study examined the impact of acute DA D₁ receptor blockade in the prelimbic cortex on heroin-seeking triggered by heroin-paired cues or heroin-priming injections.

Methods

Subjects

Male Sprague–Dawley rats (n=31, initial weight 250–275 g; Charles River, USA) were individually housed on a 12-h reversed light/dark cycle (lights on 18:00 hours). Animals were given water ad libitum and maintained on 25 g standard rat chow per day during chronic heroin self-administration and then placed on ad-libitum feeding for the remainder of the experiment. Rats were habituated to handling and allowed to adapt for a minimum of 4 d prior to the start of the experiment. Housing and care of the rats were carried out in accordance with the ‘Guide for the Care and Use of Laboratory Rats’ (Institute of Laboratory Animal Resources on Life Sciences, National Research Council) and procedures were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina.

Heroin self-administration

Procedures for heroin self-administration have been previously described in detail (Rogers et al. 2008). Self-administration occurred in standard operant chambers linked to a data collection programme (Med Associates Inc., USA). Rats underwent initial lever training on a fixed ratio 1 (FR1) schedule of food pellet reinforcement with an active or inactive lever available during a 15-h overnight training session. Two days after lever training, rats were implanted with jugular catheters and intracranial guide cannulae. In brief, animals were anesthetized (ketamine and xylazine, 66 and 1.33 mg/kg; equithesin, 0.5 ml/kg i.p.) and catheters were implanted into the right jugular vein and secured with sutures. Immediately following catheter surgery, animals were placed into a stereotaxic frame and bilateral stainless-steel guide cannulae (26-gauge) were inserted into the medial prefrontal cortex at the following coordinates: +3.0 A/P, ±0.7 M/L, −1.0 D/V. Catheters were flushed once daily for 4 d after surgery with an antibiotic solution of cefazolin (100 mg/ml; Schein Pharmaceuticals, USA) and heparinized saline (70 U/ml; Elkins-Sinn, USA). For the duration of the experiment, each subject received 0.1 ml heparinized saline (10 U/ml) prior to self-administration and cefazolin +70 U/ml heparinized saline following each session. To verify catheter patency, rats occasionally received a 0.12 ml infusion of methohexital sodium (10.0 mg/ml i.v.; Eli Lilly and Co., USA), a short-acting barbiturate that produces a rapid loss of muscle tone.

Five to seven days after surgery, rats began self-administration of heroin (diacetylmorphine HCl, National Institute on Drug Abuse, USA) along a FR1 schedule in daily 3-h sessions at an initial dose of 50 µg/50 µl per infusion for 2 d, followed by 10–12 d of self-administration at a dose of 25 µg/50 µl per infusion. The houselight signalled the initiation of the session and remained illuminated throughout the entire session for all experimental phases. Active lever responses resulted in a 2-s activation of the infusion pump and a 5-s presentation of a conditioned stimulus (CS) complex, which consisted of a white cue-light and a tone (78 dB, 4.5 kHz). Following each infusion, responding on the active lever had no consequences during a 20-s time-out period. Inactive lever presses had no consequences, but were recorded. After the last day of self-administration, rats experienced daily 3-h extinction sessions, in which responses on either the active or inactive lever were recorded, but resulted in no programmed consequences (i.e. no infusion and no CS presentation). Animals continued under extinction conditions until they reached a criterion of a minimum of 10 d and ≤25 lever presses per session for two consecutive days.

Reinstatement testing

Following extinction, rats underwent six reinstatement tests, using a counterbalanced, within-subjects design, with a minimum of 2 d of extinction between...
has been previously used in many studies of intra-cranial drug injections. Injection cannulae (33-gauge) were inserted to a depth of 2 mm below the tip of the guide cannulae (prelimbic cortex) just prior to placement into the chamber. Injection cannulae were connected to 10 µl syringes (Hamilton Co., USA) mounted on an infusion pump (Harvard Apparatus, USA). SCH 23390 hydrochloride (Sigma-Aldrich, USA) or PBS vehicle (pH 7.0 for both) were infused at a volume of 0.5 µl/side over a 2-min time-period. This volume has been previously used in many studies of intracranial drug effects in the prefrontal cortex, including SCH 23390 (Capriles et al. 2003; Duvauchelle et al. 1998). Injection cannulae were left in place for 1 min prior to and after the infusion.

During the 3-h cue reinstatement tests, each active lever press resulted in the CS presentation in the absence of heroin reinforcement. For heroin-primed reinstatement, a single, non-contingent dose of heroin (0.25 mg/kg s.c.) was administered immediately prior to the 3-h session, during which lever responses had no programmed consequences. After completion of all testing, the rats were anaesthetized with equithesin and transectionally perfused with PBS and 10% formaldehyde solution. The brains were dissected and stored in 10% formaldehyde solution prior to sectioning in order to verify cannulae placements.

Locomotor activity

In order to assess the specificity of SCH 23390 effects, locomotor responses were measured in a novel environment after SCH 23390 infusion in a subset of animals from the low-dose cohort after all reinstatement testing was completed. Animals (n = 5 per group) were infused with vehicle or SCH 23390 at the highest dose of 2.0 µg/side immediately prior to being placed in a Plexiglas open-field apparatus. Each chamber was equipped with a Digiscan monitor (Omnitech Electronics, USA) containing 16 photobeams (eight on each horizontal axis) that tabulated total distance (cm) travelled. Beam breaks were detected by a Digiscan analyser and recorded by DigiPro software (version 1.4).

Data analysis

Reinstatement of responding from extinction levels and the effects of SCH 23390 on cue-induced and heroin-primed reinstatement were analysed using one-way analysis of variance (ANOVA), followed by pairwise comparisons with the Student–Newman–Keuls test. Locomotor activity was analysed with a two-way ANOVA (group × time). Analyses were considered statistically significant at p < 0.05. All data are presented as mean ± S.E.M.

Results

No differences were found in responding between the two experimental cohorts for heroin self-administration, extinction responding, or response to intracranial vehicle infusions. Responding during the last 2 d of heroin self-administration on the active lever was 69.32 ± 12.77, while responding on the inactive (non drug-paired) lever was 3.55 ± 0.79. The number of heroin infusions for the last 2 d of self-administration was 22.74 ± 1.82, which resulted in an average of 1.64 ± 0.13 mg/kg per session when adjusted for body weight.

Figure 1 shows lever responding for extinction and during cue-induced and heroin-primed reinstatement of drug-seeking after intracranial infusions of vehicle or SCH 23390. Significant group differences were found for cue-induced reinstatement for both the low-dose (F(3,60) = 4.77, p < 0.01) and high-dose (F(3,56) = 6.59, p < 0.001) cohorts (Fig. 1a, b). Compared to extinction, responding on the previously heroin-paired lever in the presence of cues significantly reinstated drug-seeking following vehicle infusion at a magnitude similar to that previously found for animals with a history of heroin self-administration (Rogers et al. 2008). SCH 23390 at doses ranging from 0.1 to 2.0 blocked the increase in responding over baseline extinction. When compared to responding after vehicle infusion, post-hoc analyses for vehicle vs. SCH 23390 showed significant differences at both the 0.2 and 2.0 doses (p < 0.05). For heroin-primed reinstatement, significant group differences were found for both the low-dose (F(3,58) = 5.65, p < 0.005) and high-dose (F(3,56) = 8.02, p < 0.001) cohorts (Fig. 1c, d). Responding on the previously heroin-paired lever after a priming injection of heroin produced robust drug-seeking as previously reported (Rogers et al. 2008). While the lower doses of SCH 23390 had no significant effects, the two higher doses (0.2 and 2.0) blocked heroin-primed reinstatement (p < 0.05). Comparison of vehicle vs. SCH 23390 infusions showed significant differences...
from vehicle at both the 0.2 and 2.0 doses \((p < 0.05)\). In contrast to responding on the previously heroin-paired (active) lever, inactive lever responding showed no significant differences between groups for either cohort, except for a slight, but significant, reduction during cue-induced reinstatement following the 2.0 \(\mu\)g dose of SCH 23390 \((p < 0.05)\).

In order to further assess possible non-specific effects of DA \(D_1\) receptor blockade, the impact of SCH 23390 on general locomotor activity in response to a novel environment was examined. Upon exposure to the novel environment, animals with either vehicle or SCH 23390 (2.0 \(\mu\)g/side) infusions in the prelimbic cortex exhibited robust locomotor activity that decreased over the 1-h test session (Fig. 2). A significant main effect for time was found \((F_{11,88} = 36.37, p < 0.001)\), but no significant effect of group \((F_{1,8} = 0.01, p = 0.98)\), or a group \(\times\) time interaction \((F_{11,88} = 0.83, p = 0.61)\), thus indicating no effect of the highest dose of SCH 23390 on general locomotor activity.

**Discussion**

The present results demonstrate that both heroin-paired cue and heroin-primed reinstatement of heroin-seeking depend upon intact prelimbic cortex DA \(D_1\) receptor function. This DA \(D_1\) receptor dependency is particularly noteworthy, in that doses of SCH 23390 that produced significant attenuation of reinstatement were relatively low, with profound reduction of both forms of reinstatement at doses \(\geq 0.2 \mu\)g/side, and significant attenuation of cue-induced reinstatement, even at 0.1 \(\mu\)g/side. Prior studies with intracranial cortical infusions of SCH 23390 have not gone below

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**Fig. 1.** Responses (mean \(\pm\) S.E.M.) on the previously heroin-associated active (■) and non-heroin-associated inactive (□) levers at the end of extinction (Ext) trials (mean of the last 2 d) and following vehicle or SCH 23390 (0.02–2.0 \(\mu\)g/side) infusions into the prelimbic cortex immediately prior to testing. Lever responses are indicated for conditioned-cued reinstatement after (a) low and (b) high doses and for heroin-primed reinstatement after (c) low and (d) high doses. Significant differences are noted for reinstatement over extinction levels (Student–Newman–Keuls test, * \(p < 0.05\)), and between vehicle and SCH 23390 (Student–Newman–Keuls test, † * \(p < 0.05\)).
et al. found that D₁ receptor function is critical for reward-motivated attentional processing (Granon et al. 2000). Thus, heightened D₁ receptor tone is probably required to maintain sufficient activation of the cortical circuitry that drives reinstatement of heroin-seeking. During heroin self-administration, activation of μ-opioid receptors disinhibits GABA neurons in the ventral tegmental area, consequently increasing DA release in the prefrontal cortex (Kelley et al. 1980; Noel & Gratton, 1995). These prolonged actions of heroin on prefrontal DA function not only alter D₁ receptor activity, but probably play a critical role in the associative learning of cue–drug interactions that can trigger drug desire during abstinence. Although there are no published reports of increased cortical DA measured under relapse conditions, the current data suggest that such an increase occurs under conditions of cue- or drug-activated relapse. D₁ receptor antagonism in the prefrontal cortex during reinstatement probably works downstream of midbrain μ-receptor activity, via blockade on cortical glutamatergic neurons (Wirkner et al. 2004; Young & Yang, 2005). However, a recent report suggests that co-localized μ-opioid and DA D₁ receptors in the cortex can form hetero-oligomers (Juhasz et al. 2008), which may provide an additional point of modulation by D₁ receptor blockade in subjects with a history of chronic heroin intake.

The prefrontal cortex primarily projects to the nucleus accumbens core, as well as several limbic regions (Voorn et al. 2004). Cocaine-seeking during relapse has been shown to depend upon a prefrontal cortical-striatal glutamatergic pathway (Kalivas & Volkow, 2005), and this pathway has recently been demonstrated to be activated during reinstatement of heroin-seeking (LaLumiere & Kalivas, 2008). DA D₁ receptors in the prefrontal cortex probably play a critical role in modulating these corticostriatal projections for multiple stimuli (cues, drugs, stress) across different classes of abused drugs. In addition, prefrontal DA D₁ receptors may also play a role in drug-seeking, as reported for cocaine-primed reinstatement (Sun & Rebec, 2005). Thus, future exploration of prefrontal cortex DA receptor-mediated drug-seeking will provide new insights for developing anti-relapse pharmacotherapy for addiction.

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Statement of Interest
None.

References


