

## Orexin/hypocretin is necessary for context-driven cocaine-seeking

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### ABSTRACT

Orexin/hypocretin signaling at the orexin 1 receptor (OX<sub>1</sub>R) has recently been implicated in addiction and relapse. We examined the role of the orexin system in cocaine-seeking elicited by a drug-associated context following abstinence or extinction from chronic cocaine self-administration. Male Sprague–Dawley rats self-administered cocaine in 2-h sessions for 10 days, followed by extinction training or extended abstinence in the home cage. The OX<sub>1</sub>R antagonist SB-334867 (SB; 10, 20, or 30 mg/kg, i.p.) was administered prior to re-exposure to the cocaine self-administration environment. We found that pretreatment with SB significantly attenuated cocaine-seeking when rats were placed back into the self-administration environment following either 1 day or 2 weeks of abstinence (no extinction), or following extinction of cocaine-seeking in an alternative environment (distinct from the training environment). These results indicate that orexin signaling at OX<sub>1</sub>R is critical for conditioned cocaine-seeking elicited by a drug-associated context, following either extinction or abstinence.

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### 1. Introduction

Drug addiction is characterized by a persistent vulnerability to relapse to drug use after days or even years of abstinence (O'Brien, 1997). In cocaine addicts, craving and subsequent relapse can be triggered by cues associated with drug use (Sinha et al., 2000; Wallace, 1989). Similarly, in rodent models of addiction, an extinguished cocaine-seeking response can be reinstated by discrete drug-paired cues (e.g., tone and light) previously paired with drug infusions (Meil and See, 1996), or by contextual cues previously associated with drug availability (Crombag et al., 2002; Fuchs et al., 2005). For context-induced reinstatement, animals self-administer drug in the presence of a set of contextual cues (visual, auditory, olfactory, and tactile) and then undergo extinction of drug-responding in an alternative environment. Subsequent re-exposure to the original self-administration environment causes reinstatement of the drug-seeking response (termed an ABA renewal procedure) (Bouton and Bolles, 1979).

Recent animal models have also focused on relapse following abstinence, which may represent a habitual, compulsive type of drug-seeking (Fuchs et al., 2006). Abstinence models of relapse may more directly reflect human addiction because subjects undergo forced abstinence following chronic self-administration, without explicit extinction of drug-seeking behavior in the drug-associated context. In contrast, extinction is a form of inhibitory

learning that reduces cocaine-seeking in the absence of drug reward, and may reverse some of the neuroadaptations caused by chronic cocaine use (Self et al., 2004). As such, drug-seeking following extinction or abstinence is associated with distinct neurocircuitry (Fuchs et al., 2006).

The novel neurotransmitter orexin (also known as hypocretin) has recently been implicated in a variety of addiction processes (Borgland et al., 2006; Georgescu et al., 2003; Harris et al., 2005; Narita et al., 2006). Orexin A and B are neuropeptides made exclusively in neurons in hypothalamus (de Lecea et al., 1998; Sakurai et al., 1998), and act at two receptors, OX<sub>1</sub>R and OX<sub>2</sub>R, which are widely distributed throughout the central nervous system (Marcus et al., 2001). Orexin has been extensively implicated in the maintenance of arousal states and the sleep disorder narcolepsy with cataplexy (Siegel, 2004); these functions have been primarily associated with signaling at OX<sub>2</sub>R (Dugovic et al., 2009; Lin et al., 1999; Willie et al., 2003).

Blockade of orexin signaling at OX<sub>1</sub>R, via the selective antagonist SB-334867 (SB), has been shown to attenuate cue- and stress-induced reinstatement of cocaine- and ethanol-seeking (Boutrel et al., 2005; Lawrence et al., 2006; Richards et al., 2008; Smith et al., in press). Additionally, orexin neurons are stimulated (as indexed by Fos expression) by contextual stimuli associated with cocaine, morphine, ethanol, or food reward (Dayas et al., 2008; Harris and Aston-Jones, 2006; Harris et al., 2005), indicating that the orexin system may be involved in drug-seeking triggered by associative contexts. To test the role of the orexin system in context-elicited cocaine-seeking, we administered SB prior to drug-seeking following 1 day or 2 weeks of abstinence from chronic cocaine

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self-administration, or prior to context-induced reinstatement of extinguished drug-seeking using an ABA design. A portion of these results have been previously published in abstract form (Smith and Aston-Jones, 2008; Tahsili-Fahadan and Aston-Jones, 2009).

## 2. Methods

### 2.1. Animals

Naïve male Sprague–Dawley rats (initial weight 250–300 g; Charles River, Raleigh, NC) were single- or pair-housed in a temperature- and humidity-controlled animal facility at MUSC (AAALAC-accredited; NCCR C06 grant RR015455) under a reversed 12-h light/dark cycle (lights off at 6 a.m.), with ad libitum access to food and water. All experiments were approved by the Institutional Animal Care and Use Committee at MUSC and conducted according to specifications of the National Institutes of Health as outlined in the Guide for the Care and Use of Laboratory Animals.

### 2.2. Intravenous catheter surgery

Following acclimation to the animal facility, rats were implanted with chronic indwelling intravenous catheters while under ketamine/xylazine anesthesia (+equithesin in some cases). A non-steroidal anti-inflammatory was administered as an analgesic prior to surgery. The catheters were constructed of silastic tubing (Dow Corning, Midland, MI) connected to a modified guide cannula (C313G-5UP-SPC12, Plastics One, Roanoke, VA), which was mounted on ProLite polypropylene monofilament mesh (Atrium, Hudson, NH) using Ortho-Jet acrylic (Lang Dental, Wheeling, IL); a small silicone bubble was placed 3.8 cm from the end of the silastic tubing. Briefly, the free end of silastic tubing was inserted into (and secured to) the right jugular vein, while the other end passed subcutaneously over the shoulder to the cannula which was mounted on the back and exited via a biopsy hole. Beginning 3 days after surgery, catheters were flushed once daily with 0.1 ml each of the antibiotic cefazolin (100 mg/ml) and heparin (100 U/ml). For each self-administration session, catheters were flushed with 0.1 ml saline to ensure patency prior to attachment to the cocaine infusion line (PE-50 tubing) and spring tether in the self-administration chamber, and flushed with 0.1 ml each of cefazolin and heparin following the session. Self-administration sessions began after one week of recovery from surgery.

### 2.3. Drugs

Cocaine HCl (NIDA, Research Triangle Park, NC) was dissolved in 0.9% sterile saline. The OX<sub>1</sub>R antagonist SB-334867 (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl urea hydrochloride; generously donated by Eli Lilly, Indianapolis, IN) was suspended in 2% DMSO and 10% 2-hydroxypropyl- $\beta$ -cyclodextrin (Sigma, St. Louis, MO) in sterile water; 10, 20, or 30 mg/kg was given intraperitoneally (i.p., 4 ml/kg, pH 6–7) 30min prior to test sessions in all experiments.

### 2.4. Cocaine self-administration

Self-administration sessions were carried out in operant chambers housed in sound-attenuating cubicles and controlled via a MED-PC IV program (Med Associates, St. Albans, VT). Rats learned to lever-press for intravenous cocaine (fixed ratio-1; 0.2 mg/50  $\mu$ l infusion via motorized pump; 20-s time-out after each infusion) in 2-h daily sessions. Presses on an inactive lever had no programmed consequences. Rats were given 10 self-administration sessions in which they earned  $\geq 10$  infusions.

### 2.5. Experiment 1: cocaine-seeking following 1 day of abstinence

Cocaine infusions during self-administration were paired with discrete tone + light cues (78 dB, 2900 Hz; white stimulus light above the active lever). The red house light (on the wall opposite the levers) was turned off during cocaine infusions and time-outs. Following self-administration, rats experienced 7 daily extinction sessions, during which presses on either lever had no consequences (no drug or cues). Thirty min prior to the first extinction session only, animals were treated with SB (10, 20, or 30 mg/kg) or vehicle.

### 2.6. Experiment 2: cocaine-seeking following 2 weeks of abstinence

For these animals, cocaine infusions during self-administration were given in the absence of discrete cues. Following self-administration, animals were given a 2-week abstinence period in their home cages; during the first week animals remained in the housing room, and during the second week animals were transported to the self-administration testing room, but remained in their home cages. Rats then experienced a single extinction session (relapse test), during which presses on either lever had no consequences (no drug or cues). Thirty min prior to the extinction session, animals were treated with SB (10, 20, or 30 mg/kg) or vehicle.

### 2.7. Experiment 3: context-induced reinstatement of cocaine-seeking following extinction

Context-induced reinstatement was carried out using an ABA design (Crombag and Shaham, 2002), in which two separate environments were created in the operant chambers using a combination of different visual, auditory, olfactory, and tactile cues. Context A consisted of flashing light + tone cues (78 dB, 2900 Hz; white stimulus light above the active lever; 2 s on/2 s off), lemon odor, and a grid floor, whereas context B consisted of continuous red house light, vanilla odor, and a tile floor. Animals were randomly assigned to self-administer cocaine in either context A or B. Rats then experienced daily extinction sessions in the alternative context (B for A animals, A for B animals), during which presses on either lever had no consequences (no drug or cues), until they met the criteria of two consecutive sessions with  $< 25$  active lever presses (minimum of 7 sessions prior to the first reinstatement test; minimum of 2 sessions between subsequent reinstatement tests). Two 2-h context-induced reinstatement tests were conducted during which animals were returned to their original self-administration context. Presses on either lever had no consequences. Thirty min prior to reinstatement sessions, animals were treated with different doses of SB (10, 20, or 30 mg/kg) or vehicle in a counterbalanced design.

Reinstatement data from 3 rats (one in each of the 20 mg/kg SB, 30 mg/kg SB, and vehicle groups) was excluded from analysis ( $> 2.5$  standard deviations different from group mean for active lever).

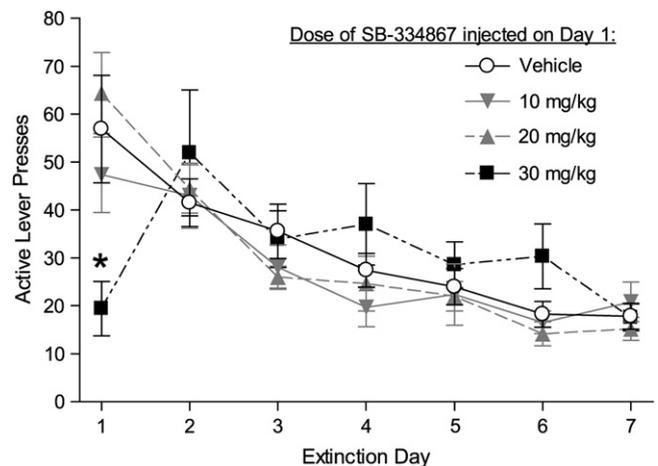
### 2.8. Statistical analyses

All results are represented as mean  $\pm$  SEM. Data from different experiments were analyzed using ANOVAs (one-way or mixed-model), with test session or time as a repeated-measure when appropriate. If a significant  $F$  value was obtained for ANOVA, post-hoc analyses were performed using Tukey–Kramer's multiple comparison tests.  $p$ -values less than 0.05 were considered as significant.

## 3. Results

### 3.1. Experiment 1: effects of SB on cocaine-seeking following 1 day of abstinence

Fig. 1 shows the mean number of active lever presses during the first 7 extinction sessions following 1 day of abstinence from chronic cocaine self-administration. Animals were pretreated with SB at 10 mg/kg ( $n = 11$ ), 20 mg/kg ( $n = 13$ ), 30 mg/kg ( $n = 12$ ), or vehicle ( $n = 12$ ) 30min prior to the first extinction session only. Mixed-model ANOVA for active lever pressing across all 7 extinction sessions revealed no significant effect for group ( $F_{(3,44)} = 0.16$ ;  $p = 0.92$ ), but a significant overall effect for session ( $F_{(6,264)} = 21.95$ ;  $p < 0.0001$ ), indicating that animals showed a significant decrease



**Fig. 1.** Attenuation of cocaine-seeking after 1 day of abstinence by OX<sub>1</sub>R antagonist SB-334867. Rats were pretreated with SB (i.p.) at 10 mg/kg ( $n = 11$ ), 20 mg/kg ( $n = 13$ ), 30 mg/kg ( $n = 12$ ), or vehicle ( $n = 12$ ) 30min prior to the first extinction session only. Mean ( $\pm$ SEM) number of presses on the active lever in 2-h sessions in the self-administration chamber is shown. Responding during the first extinction session was significantly different for the group pretreated with 30 mg/kg SB as compared to vehicle (\* $p < 0.05$ ).

in active lever responding across extinction sessions. There was also a significant interaction between group and session ( $F_{(18,264)} = 3.67$ ;  $p < 0.0001$ ). One-way ANOVAs for each extinction session revealed significant differences between groups on the first extinction session ( $F_{(3,44)} = 5.37$ ;  $p < 0.005$ ) and sixth extinction session ( $F_{(3,44)} = 3.22$ ;  $p < 0.05$ ). On the first extinction session, the 30 mg/kg SB group was significantly different from the 20 mg/kg SB ( $p < 0.005$ ) and vehicle groups ( $p = 0.018$ ). On the sixth extinction session, groups receiving 20 and 30 mg/kg SB were significantly different from each other ( $p < 0.05$ ). These data indicate that SB pretreatment attenuated active lever responding on the first extinction session, but had no lasting effect on extinction responding in subsequent sessions.

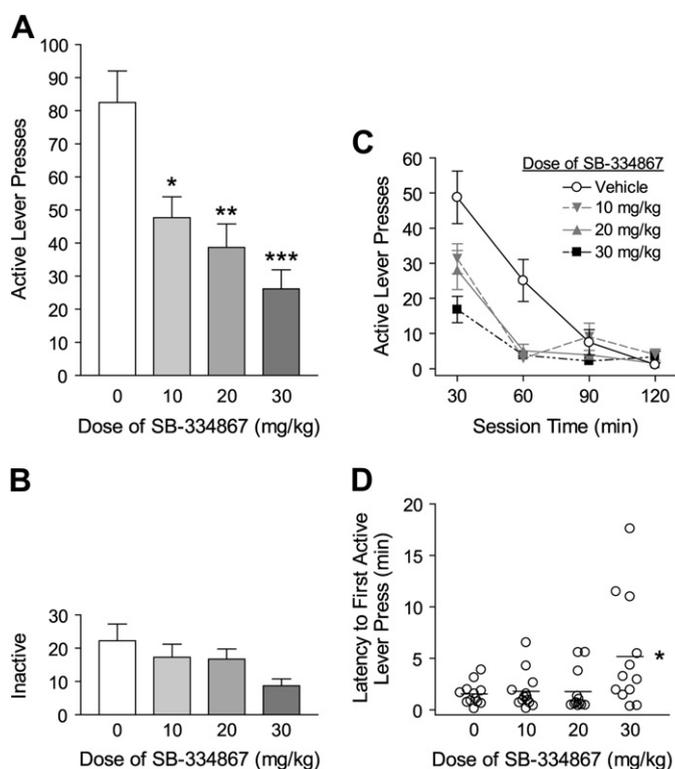
Mixed-model ANOVA for inactive lever pressing across all 7 extinction sessions showed no significant effect for group, session, or an interaction between group and session. One-way ANOVAs for each extinction session revealed a significant difference between groups on the first extinction session only ( $F_{(3,44)} = 3.11$ ;  $p < 0.05$ ). On the first extinction session, groups receiving 30 mg/kg SB and vehicle were significantly different ( $p < 0.05$ ).

Self-administration data were analyzed to assess differences in baseline responding between groups. No significant differences were observed between groups for cocaine intake or active lever responding during the last two self-administration sessions. On the last two self-administration days, the across-group ( $n = 48$  total) mean for cocaine infusions was 39.0 ( $\pm 1.8$ ) and 39.6 ( $\pm 1.7$ ) per session, and active lever presses was 52.0 ( $\pm 4.4$ ) and 51.6 ( $\pm 3.3$ ) per session.

### 3.2. Experiment 2: effects of SB on cocaine-seeking following 2 weeks of abstinence

Fig. 2 shows the mean number of active (Fig. 2A) and inactive (Fig. 2B) lever presses during the first extinction session following 2 weeks of abstinence from chronic cocaine self-administration. Animals were pretreated with either SB (10, 20, or 30 mg/kg) or vehicle 30min prior to the session. Mean number of active lever presses per 30-min time bin (Fig. 2C) and latency to the first active lever press for individual animals (Fig. 2D) are also shown. One-way ANOVA across the four groups (Fig. 2A) showed a significant effect for SB pretreatment on active lever responding ( $F_{(3,45)} = 10.77$ ;  $p < 0.0001$ ), and post-hoc analyses revealed that all groups receiving SB pretreatment were significantly different from the group receiving vehicle. Specifically, groups receiving 10 mg/kg ( $n = 13$ ;  $p < 0.01$ ), 20 mg/kg ( $n = 12$ ;  $p < 0.001$ ), or 30 mg/kg ( $n = 12$ ;  $p < 0.0005$ ) SB were significantly different from the vehicle group ( $n = 12$ ). Mixed-model ANOVA for active lever responding in 30-min bins across the four groups (Fig. 2C) showed significant effects for SB pretreatment ( $F_{(3,45)} = 10.80$ ;  $p < 0.0001$ ), session time ( $F_{(3,135)} = 57.19$ ;  $p < 0.0001$ ), and interaction between pretreatment and time ( $F_{(9,135)} = 4.26$ ;  $p < 0.0001$ ). Post-hoc analyses revealed that at the 30-min time point, active lever presses for 20 mg/kg and 30 mg/kg SB groups were significantly different from vehicle group ( $p < 0.05$  and 0.0005, respectively), while at the 60-min time point, 10, 20, and 30 mg/kg SB groups were significantly different from vehicle group ( $p < 0.01$ , 0.05, and 0.05, respectively). One-way ANOVA across the four groups (Fig. 2D) showed a significant effect for SB pretreatment on the latency to first active lever press ( $F_{(3,45)} = 4.01$ ;  $p = 0.013$ ). Post-hoc analyses revealed that pretreatment with 30 mg/kg SB was significantly different from vehicle, 10 mg/kg SB, and 20 mg/kg SB ( $p < 0.05$ ).

Similar analyses for inactive lever responding (Fig. 2B) revealed no significant effect for SB pretreatment on inactive lever responding ( $F_{(3,45)} = 2.27$ ;  $p = 0.09$ ).



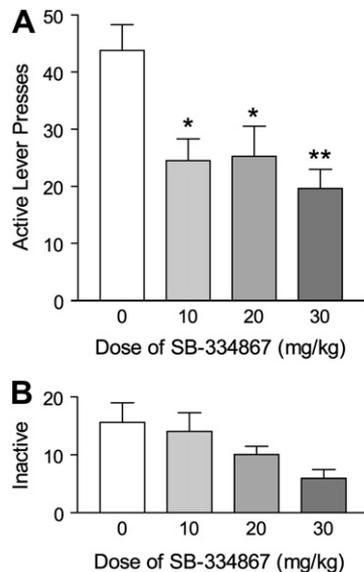
**Fig. 2.** Attenuation of cocaine-seeking by OX<sub>1</sub>R antagonist SB-334867 after 2 weeks of abstinence. Following cocaine self-administration and 2 weeks of abstinence in the home cage (no extinction), rats were pretreated with either SB (10, 20, or 30 mg/kg, i.p.) or vehicle 30min prior to an unreinforced extinction session. Mean ( $\pm$ SEM) number of presses on the active (A) and inactive (B) levers in 2-h sessions in the self-administration chamber is shown. Active lever responding was significantly attenuated for groups pretreated with SB at 10 mg/kg ( $n = 13$ ), 20 mg/kg ( $n = 12$ ), or 30 mg/kg ( $n = 12$ ), as compared to vehicle ( $n = 12$ ) ( $*p < 0.01$ ,  $**p < 0.001$ ,  $***p < 0.0005$ ). Inactive lever responding was not significantly affected by SB pretreatment. Mean ( $\pm$ SEM) number of active lever presses per 30-min time bin (C) and latency to the first active lever press for individual animals (D; means are represented by horizontal line segments) are also shown. Active lever responding was significantly affected by SB pretreatment ( $p < 0.0001$ ) at 30- and 60-min time points. Latency to first press the active lever was significantly different following pretreatment with 30 mg/kg SB as compared to all other groups ( $*p < 0.05$ ).

Self-administration data were analyzed to assess differences in baseline responding between groups. No significant differences were observed between groups for cocaine intake or active lever responding during the last two self-administration sessions. On the last two self-administration days, the across-group ( $n = 49$  total) mean for cocaine infusions was 28.2 ( $\pm 2.3$ ) and 29.4 ( $\pm 1.7$ ) per session, and active lever presses was 51.8 ( $\pm 7.4$ ) and 56.4 ( $\pm 7.5$ ) per session.

### 3.3. Experiment 3: effects of SB on context-induced reinstatement of cocaine-seeking following extinction

As shown in Fig. 3A, SB pretreatment had a significant effect on active lever responding during context-induced reinstatement (one-way ANOVA,  $F_{(3,69)} = 5.66$ ;  $p = 0.0016$ ). Further analyses revealed that active lever presses were significantly different from the vehicle-treated group ( $n = 18$ ) in groups pretreated with SB 10 mg/kg ( $n = 19$ ;  $p < 0.05$ ), 20 mg/kg ( $n = 20$ ;  $p < 0.05$ ), and 30 mg/kg ( $n = 16$ ;  $p < 0.01$ ). SB pretreatment sessions (10, 20, and 30 mg/kg) were not significantly different from each other.

Mixed-model ANOVA for context-induced reinstatement following SB or vehicle pretreatment as compared to the prior extinction session revealed significant effects for reinstatement



**Fig. 3.** Attenuation of context-induced reinstatement of cocaine-seeking by OX<sub>1</sub>R antagonist SB-334867. Animals were trained to self-administer cocaine in a distinct context and then given extinction training in an alternate environment. Rats were pretreated with SB (10, 20, or 30 mg/kg, i.p.) or vehicle 30min prior to re-exposure to the original self-administration context. Mean ( $\pm$ SEM) number of presses on the active and inactive lever in a 2-h reinstatement session in the self-administration chamber is shown. A, Active lever responding during context-induced reinstatement of extinguished cocaine-seeking was significantly attenuated by SB at 10 mg/kg ( $n = 19$ ), 20 mg/kg ( $n = 20$ ), and 30 mg/kg ( $n = 16$ ) as compared to vehicle ( $n = 18$ ) (\* $p < 0.05$ , \*\* $p < 0.01$ ). B, Inactive lever responding was not significantly affected by SB pretreatment.

( $F_{(1,69)} = 59.30$ ;  $p < 0.0001$ ), pretreatment ( $F_{(3,69)} = 5.43$ ;  $p < 0.005$ ), and interaction between reinstatement and pretreatment ( $F_{(3,69)} = 4.73$ ;  $p < 0.005$ ). Post-hoc analyses indicated significant reinstatement following pretreatment with vehicle ( $p = 0.0001$ ) or 20 mg/kg SB ( $p < 0.05$ ), but no significant reinstatement effect following pretreatment with 10 or 30 mg/kg SB. As compared to the prior extinction session, reinstatement sessions following pretreatment with 10, 20, or 30 mg/kg SB were significantly different from reinstatement following vehicle pretreatment ( $p < 0.005$ , 0.005, and 0.0005, respectively), but reinstatement sessions following SB pretreatment were not significantly different from each other.

One-way ANOVA across the four doses showed no significant difference for inactive lever responding between SB and vehicle sessions (Fig. 3B;  $F_{(3,69)} = 2.63$ ;  $p = 0.057$ ). Mixed-model ANOVA for context-induced reinstatement following SB or vehicle pretreatment as compared to the prior extinction session revealed a significant effect for reinstatement ( $F_{(1,69)} = 19.41$ ;  $p < 0.0001$ ), but no significant effect for pretreatment ( $F_{(3,69)} = 2.19$ ;  $p = 0.098$ ).

Self-administration data were analyzed to assess baseline responding between groups receiving different SB dosing. No significant differences were observed between groups for cocaine intake or active lever responding during the last two self-administration sessions, or for active lever responding during the first 7 days of extinction. On the last two self-administration days, the across-group ( $n = 73$  total) mean for cocaine infusions was 33.4 ( $\pm 1.4$ ) and 36.5 ( $\pm 1.8$ ) per session, and active lever presses was 50.4 ( $\pm 3.6$ ) and 61.4 ( $\pm 5.0$ ) per session. During extinction, repeated-measures ANOVA revealed a significant main effect for extinction session ( $F_{(6,390)} = 18.79$ ;  $p < 0.0001$ ), indicating that animals showed a significant decrease in active lever responding across extinction sessions. The mean for active lever presses on days 1 and 7 of extinction was 40.9 ( $\pm 2.8$ ) and 14.2 ( $\pm 1.4$ ).

#### 4. Discussion

The current studies show that orexin signaling at OX<sub>1</sub>R is involved in context-elicited cocaine-seeking following abstinence or extinction from cocaine self-administration. The OX<sub>1</sub>R antagonist SB-334867 attenuated cocaine-seeking following cocaine abstinence for 1 day (Fig. 1) or 2 weeks (Fig. 2). In rats given only 1 day of abstinence, 30 mg/kg (and not 10 or 20 mg/kg) SB reduced initial extinction responding to levels seen during late extinction. On subsequent extinction days without 30 mg/kg SB pretreatment, these animals showed a rebound of responding and extinction levels similar to vehicle-treated animals. In rats given 2 weeks of abstinence, SB significantly attenuated responding at all doses tested (10, 20 and 30 mg/kg). Finally, SB (10, 20, and 30 mg/kg) also attenuated context-induced reinstatement of extinguished cocaine-seeking in an ABA contextual design (Fig. 3). Distinct neural pathways are involved in drug-seeking following extinction versus abstinence (Fuchs et al., 2006), as well as reinstatement elicited by cues versus context (Fuchs et al., 2005), so results from these experiments suggest that signaling at OX<sub>1</sub>R plays a general role in conditioned cocaine-seeking and is most likely interacting with neurocircuitry common to all of these types of cocaine-seeking.

Previous studies also found that orexin transmission at OX<sub>1</sub>R is involved in unreinforced drug-seeking. SB blocked cue- and stress-induced reinstatement of extinguished drug-seeking for cocaine and ethanol in the self-administration paradigm (Boutrel et al., 2005; Lawrence et al., 2006; Richards et al., 2008; Smith et al., in press). Orexin neurons showed Fos activation following drug-seeking in an environment previously associated with morphine, cocaine, or ethanol (Dayas et al., 2008; Harris and Aston-Jones, 2006; Harris et al., 2005). In contrast to this consistent role of orexin in conditioned drug-seeking, orexin appears to play a much more variable role in drug reinforcement. Orexin signaling was not necessary for self-administration of cocaine (Smith et al., in press), methamphetamines (Boutrel, 2008), or sucrose (Richards et al., 2008), but was involved in self-administration of ethanol (Lawrence et al., 2006; Richards et al., 2008) and drug-stimulus conditioning for morphine (Harris et al., 2007; Narita et al., 2006).

Results from the current experiments, along with a growing body of literature, support the hypothesis that antagonism of signaling at OX<sub>1</sub>R does not cause reductions in wakefulness or operant responding. OX<sub>1</sub>R antagonists have been shown to have no significant effect on sleep-wake states, whereas OX<sub>2</sub>R antagonists significantly decrease latency to non-REM sleep and cause increases in sleep time (Dugovic et al., 2009; Smith et al., 2003). This corresponds with studies that have associated dysfunction of signaling at OX<sub>2</sub>R but not OX<sub>1</sub>R with the sleep disorder narcolepsy (Lin et al., 1999; Willie et al., 2003). Here, we found that SB had no significant effects on inactive lever pressing following abstinence or extinction. Although responding on this lever is not necessarily a reflection of general arousal, this suggests that attenuation of cocaine-seeking by SB is not due to sedation or decreased locomotion. Additionally, as discussed above, SB had no effect on self-administration responding for certain types of drug and food rewards.

Importantly, the current results show that orexin is critical for cocaine-seeking following either extinction or abstinence. Previous studies exploring the role of orexin in relapse have focused primarily on a reinstatement paradigm following extinction. However, abstinence models are thought to more directly reflect relapse in humans because addicts rarely undergo explicit extinction of drug-associated stimuli during drug abstinence. Drug-seeking following both extinction and abstinence is dependent upon dorsolateral caudate-putamen (dlCPu) (Fuchs et al., 2006).

However, cocaine-seeking following abstinence was not attenuated by inactivation of dorsal prefrontal cortex (dPFC) or basolateral amygdala (BLA), as was context- and cue-induced reinstatement (Fuchs et al., 2006, 2005; McLaughlin and See, 2003). This may reflect a difference of prefrontal cortical and striatal involvement in cued versus habitual, compulsive responding (Everitt and Robbins, 2005; See et al., 2007). The levels of responding we observed here following 2 weeks of abstinence were higher than following extinction, indicating an 'incubation' effect, as previously observed (Grimm et al., 2001; Tran-Nguyen et al., 1998). Lower doses of SB (10 and 20 mg/kg) which had no effect on cocaine-seeking after 1 day of abstinence (Fig. 1) caused significant attenuation of cocaine-seeking after 2 weeks of abstinence (Fig. 2), which may indicate that orexin is involved in the incubation of responding that is observed following extended periods of abstinence, or in the habitual nature of cocaine-seeking following abstinence.

Following extinction of cocaine-seeking, SB significantly reduced reinstatement induced by both cues (Smith et al., in press) and context (Fig. 3). Therefore, despite differences in the stimulus properties for cues and contexts (stimulus complexity, response-contingent versus passive presentation (Fuchs et al., 2005)), orexin is involved in the ability of each to elicit cocaine-seeking. Both cue- and context-induced reinstatement of cocaine-seeking are dependent upon dPFC, BLA, nucleus accumbens (NAc) core, dlCPu, and dopaminergic transmission (Crombag et al., 2002; Fuchs et al., 2006, 2005, 2004, 2008; McLaughlin and See, 2003; Meil and See, 1997; See et al., 2001). However, only context-induced reinstatement involves NAc shell and dorsal hippocampus (Fuchs et al., 2005, 2008; Rogers and See, 2007). Context-induced reinstatement of cocaine-seeking was also found to be associated with Fos activation in lateral hypothalamus, but only in non-orexinergic neurons (Hamlin et al., 2008). This is in contrast to the current finding that orexin signaling is involved in context-induced reinstatement of cocaine-seeking, and indicates that context-induced Fos induction in LH may not fully represent the role of orexin in this type of reinstatement.

Overall, these studies show that orexin is involved in cocaine-seeking elicited by cues and contexts, whether following extinction or abstinence from cocaine self-administration. These findings indicate that orexin is necessary for conditioned reinforcement or conditioned motivation for cocaine in the absence of drug, and that orexin is acting within neural pathways that are generally involved in conditioned cocaine-seeking. Future studies are warranted to investigate the local site of action for SB, which may help determine the underlying mechanisms of relapse. Taken with previous studies, the current results indicate that orexin signaling at OX<sub>1</sub>R may be an important therapeutic target for future addiction treatments.

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