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Contribution of a Mesocorticolimbic Subcircuit to Drug Context-Induced Reinstatement of Cocaine-Seeking Behavior in Rats

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Cocaine-seeking behavior triggered by drug-paired environmental context exposure is dependent on orbitofrontal cortex (OFC)–basolateral amygdala (BLA) interactions. Here, we present evidence supporting the hypothesis that dopaminergic input from the ventral tegmental area (VTA) to the OFC critically regulates these interactions. In experiment 1, we employed site-specific pharmacological manipulations to show that dopamine D1-like receptor stimulation in the OFC is required for drug context-induced reinstatement of cocaine-seeking behavior following extinction training in an alternate context. Intra-OFC pretreatment with the dopamine D1-like receptor antagonist, SCH23390, dose-dependently attenuated cocaine-seeking behavior in an anatomically selective manner, without altering motor performance. Furthermore, the effects of SCH23390 could be surmounted by co-administration of a sub-threshold dose of the D1-like receptor agonist, SKF81297. In experiment 2, we examined effects of D1-like receptor antagonism in the OFC on OFC-BLA interactions using a functional disconnection manipulation. Unilateral SCH23390 administration into the OFC plus GABA agonist-induced neural inactivation of the contralateral or ipsilateral BLA disrupted drug context-induced cocaine-seeking behavior relative to vehicle, while independent unilateral manipulations of these brain regions were without effect. Finally, in experiment 3, we used fluorescent retrograde tracers to demonstrate that the VTA, but not the substantia nigra, sends dense intra- and interhemispheric projections to the OFC, which in turn has reciprocal bi-hemispheric connections with the BLA. These findings support that dopaminergic input from the VTA, via dopamine D1-like receptor stimulation in the OFC, is required for OFC–BLA functional interactions. Thus, a VTA–OFC–BLA neural circuit promotes drug context-induced motivated behavior.

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INTRODUCTION

The lateral orbitofrontal cortex (OFC) and basolateral amygdala (BLA) are integral components of the mesocorticolimbic neural circuitry that regulates the ability of cocaine-paired discrete or contextual cues to trigger powerful drug craving and drug-seeking behaviors. In abstinent cocaine users, exposure to cocaine-paired environmental stimuli elicits neural activation in the OFC and BLA and concomitant increase in cocaine craving (Childress *et al*, 1999; Duncan *et al*, 2007; Grant *et al*, 1996; London *et al*, 1999). In laboratory animals, OFC or BLA functional inactivation inhibits drug context-induced cocaine-seeking behavior (Fuchs *et al*, 2005; Lasseter *et al*, 2009). Moreover, the OFC and BLA display functional interdependence in the control of drug context-induced cocaine-seeking behavior such that interhemispheric or intrahemispheric disconnection of the OFC and BLA attenuates this behavior (Lasseter *et al*, 2011). However, the larger neural circuitry within which the OFC and BLA interact to promote drug context-induced goal-directed behaviors remains to be investigated.

Emerging evidence suggests that dopaminergic neurotransmission in the OFC may be necessary for a drug-paired context to produce cocaine-seeking behavior. The ventral tegmental area (VTA) sends dopaminergic projections to the OFC (Berger *et al*, 1991; Dunnett and Robbins, 1992; Frankle *et al*, 2006; Geisler *et al*, 2007; Sesack and Grace, 2010). Furthermore, dopaminergic neurotransmission in the frontal cortices critically regulates higher-order cognitive function, including reward-related processing by the OFC that likely facilitates cocaine-seeking behavior (Cetin *et al*, 2004; Dalley *et al*, 2004; Kheramin *et al*, 2004; Ragozzino *et al*, 1999; Ward *et al*, 2009; Winter *et al*, 2009). Systemic dopamine D1-like receptor antagonism impairs drug context-induced cocaine-seeking behavior (Crombag

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et al, 2002). However, to date, no study has ascertained whether dopaminergic neurotransmission in the OFC is critical for drug context-induced cocaine-seeking behavior.

Based on the above findings, we hypothesized that input from the VTA to the OFC—via dopamine D1-like receptor stimulation—regulates interactions between the OFC and BLA that promote the motivational effects of cocaine-paired environmental stimuli on drug-seeking behavior. To test this hypothesis, in experiment 1, we examined the effects of bilateral dopamine D1-like receptor antagonism in the OFC and in the adjacent primary/secondary motor cortex (M1/2, anatomical control region) on drug context-induced cocaine-seeking behavior and on indices of instrumental and general motor performance. We confirmed the receptor specificity of the effects on cocaine-seeking behavior by co-administering a dopamine D1-like receptor agonist. In experiment 2, we evaluated the effects of VTA–OFC–BLA functional disconnection and control manipulations on drug context-induced cocaine seeking. Finally, in experiment 3, we used retrograde fluorescent tracers to verify that the requisite anatomical connections exist to form a putative VTA–OFC–BLA circuit.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (250–300 g; Charles River, Wilmington, MA, USA) were housed individually in a climate-controlled vivarium on reversed light-dark cycle. Rats received 20–25 g of rat chow per day with water available *ad libitum*. Animal housing and treatment protocols followed the Guide for the Care and Use of Laboratory Animals (National Research Council, USA, 2011).

Food Training and Surgery

In experiments 1 and 2, rats ($N=90$) were trained to lever press under a fixed-ratio 1 (FR-1) food reinforcement schedule during a single overnight session in order to facilitate subsequent drug self-administration training (Fuchs *et al*, 2007). The operant conditioning chambers and levers used during food training were different from those used later for drug self-administration and extinction training. Forty-eight hours later, surgical anesthesia was induced using ketamine hydrochloride and xylazine (66.6 and 1.33 mg/kg, i.p., respectively) with additional dosing applied, as needed, in order to maintain full anesthesia throughout the surgery. Rats were surgically implanted with intravenous jugular catheters and 26-gauge stainless steel guide cannulae (plastics one) aimed bilaterally or unilaterally at the OFC ($+3.5$ mm AP, $+/-3.0$ mm ML, -3.4 mm DV, from bregma), M1/2 ($+3.5$ mm AP, $+/-3.0$ mm ML, -2.4 mm DV, from bregma), or BLA (-2.7 mm AP, $+/-5.2$ mm ML, -6.7 mm DV, from bregma).

Cocaine Self-Administration and Extinction Training

After surgical recovery, rats were trained to press one (active) lever under an FR-1 schedule of cocaine reinforcement (cocaine hydrochloride, 0.15 mg/0.05 ml infusion, i.v.; NIDA, Research Triangle Park, NC) with a 20 s timeout

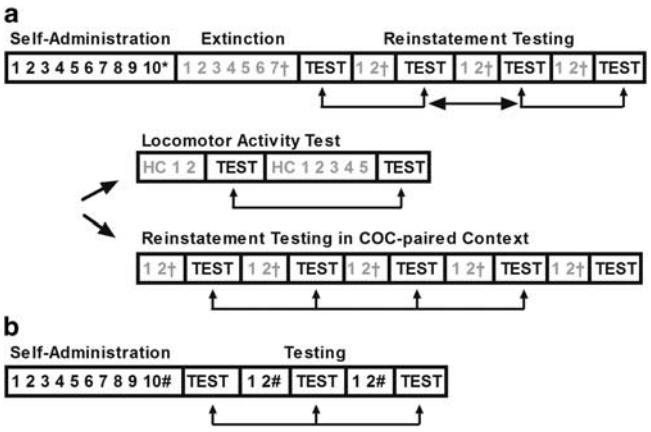

Figure 1 Schematic representation of (a) the timeline for the drug context-induced reinstatement experiments and (b) for the food-reinforced instrumental control experiment. In the reinstatement experiments (a), rats had to reach acquisition (*Represents $\geqslant$10 cocaine infusions per session for minimum 10 sessions) and extinction (†Represents $\leqslant$25 active lever presses per session for two consecutive sessions) criteria before drug context-induced reinstatement testing. After the fourth drug context-induced reinstatement test session, in one subgroup of rats, photobeam interruptions were assessed in a novel context. Before each locomotor activity test, the rats remained in their home cages (HC). In the other subgroup of rats, reinstatement of extinguished cocaine-seeking behavior was assessed in the previously cocaine-paired context following agonist administration (tests 1–4) and following cocaine or saline priming (test 5). In the food-reinforced instrumental control experiment, rats had to reach a stability criterion ($^{\#}$Represents $<$10% variability in food-reinforced active lever presses on two consecutive sessions) before testing. In each experiment, the order of drug pretreatments (vehicle; VEH first, agonist and/or antagonist dose first) and context exposure (cocaine-paired context first, extinction context first) were counterbalanced, when appropriate, across tests sessions as indicated by the arrows.

period (see experimental timeline in Figure 1). Responses on a second (inactive) lever were recorded but had no scheduled consequences. Training occurred during 2 h sessions in operant conditioning chambers configured to form one of two distinct contexts (contexts 1 and 2, described in Supplementary Methods). Rats that failed to obtain $\geqslant$10 cocaine infusions/session on $\geqslant$10 sessions were eliminated from the experiment. The remaining rats received daily 2 h extinction training sessions in the alternate context (context 1 or 2). During the sessions, lever responses were recorded but had no scheduled consequences. Before session 4, rats were adapted to the intracranial microinfusion procedure, as described previously (Lasseter *et al*, 2011). Rats that reached an extinction criterion ($\geqslant$7 sessions with $\leqslant$25 active lever responses/session during the last two sessions) were used for subsequent reinstatement testing.

Reinstatement Testing

Lever pressing was assessed in the cocaine-paired and extinction contexts during 1 h test sessions. Immediately before testing, rats received intracranial (0.5 μl/hemisphere) or systemic pretreatment with one dose of an antagonist and/or agonist or vehicle (VEH), as described below. Intracranial microinfusions were delivered via 33 Ga stainless steel injection cannulae (2 mm extension past the guide cannula; plastics one) over 2 min. The order of the two

pretreatment doses and testing contexts was counterbalanced across test sessions based on cocaine history (see experimental timeline in Figure 1a). Between test sessions, rats received additional daily extinction training sessions until they re-obtained the extinction criterion.

In experiment 1, we evaluated whether D1-like receptor stimulation was required in the OFC or M1/2 for drug context-induced cocaine seeking. Rats received bilateral pretreatment with phosphate-buffered saline VEH or the dopamine D1-like receptor antagonist, SCH23390, into the OFC (0.02 or 0.2 μg, based on Capriles *et al*, 2003; $n = 8$–10/group) or M1/2 (0.2 μg, $n = 9$). To assess the receptor specificity of SCH23390 effects, additional rats received bilateral intra-OFC pretreatment with SCH23390 (0.2 μg) in combination with 0.1% dimethyl sulfoxide (DMSO) VEH or the D1-like agonist, SKF81297 (0.1 or 0.3 μg, $n = 7$/group). After the fourth test session, responding in this second cohort of rats was extinguished in the cocaine-paired context. We then assessed whether intra-OFC SKF81297 (0.1, 0.3 or 1.0 μg, $n = 14$) was sufficient to reinstate extinguished lever responding in the previously cocaine-paired context relative to VEH, using a within-subjects design. Finally, we examined whether the rats retained the capacity for reinstatement after repeated testing by examining whether i.p. cocaine priming (10 mg/kg, $n = 7$/group) reinstated lever responding relative to VEH, using a between-subjects design.

In experiment 2, we examined whether D1-like receptor stimulation in the OFC controls intrahemispheric and interhemispheric interactions between the OFC and BLA that mediate drug context-induced cocaine-seeking behavior. To bilaterally disrupt intrahemispheric neural communication (Figure 3a), rats ($n = 12$) received unilateral SCH23390 (0.2 μg, based on experiment 1) pretreatment into the OFC *plus* unilateral $GABA_B/GABA_A$ receptor agonist cocktail, baclofen + muscimol (BM; 106.8/5.7 ng, respectively; based on the experiment by Lasseter *et al* (2011)), pretreatment into the contralateral BLA. To bilaterally disrupt interhemispheric communication, additional rats ($n = 10$) received the same pretreatments ipsilaterally. The same rats were also tested following contralateral or ipsilateral VEH pretreatment. Additional control groups were tested following unilateral SCH23390 or VEH pretreatment into the OFC ($n = 11$) or unilateral BM or VEH pretreatment into the BLA ($n = 7$). Based on our previous finding that both intra- and interhemispheric OFC-BLA interactions control drug context-induced cocaine seeking (Lasseter *et al*, 2011), functional interdependence within the VTA–OFC–BLA circuit was predicted to manifest as a superadditive effect following contralateral or ipsilateral manipulation relative to the effects of separate, unilateral manipulation of each target brain region.

General and Instrumental Motor Behavior

Intracranial manipulations can produce motor deficits that impair instrumental performance. Hence, the effects of intra-OFC SCH23390 or VEH pretreatment on general locomotor activity (that is, photobeam interruptions) were assessed in a novel context during a 1-h test session, using the rats in experiment 1 ($n = 8$–10/group). The effects of the same manipulations on food-reinforced instrumental behavior were assessed in experimentally naïve rats ($n = 9$). These procedures and the experimental timeline are described in Supplementary Methods and Figure 1a and b.

Histology and Data Analysis

In experiments 1 and 2, rats were overdosed using ketamine/xylazine (66.6 and 1.3 mg/kg i.v. or 199.8 and 3.9 mg/kg i.p., respectively, depending on catheter patency). The brains were dissected out and stored in 10% formaldehyde solution. Coronal sections (75 μm) were cut on a vibratome. Cannula placements were examined on cresyl violet-stained (Kodak, Rochester, NY, USA) sections using light microscopy and mapped onto schematics from the rat brain atlas of Paxinos and Watson (1997). Behavioral data were analyzed for rats with correct cannula placement(s) using analysis of variance (ANOVAs) or *t*-test, where appropriate, as described in Supplementary Methods. Significant ANOVA main and interaction effects were further investigated using Tukey *post-hoc* tests, when appropriate. α was set at 0.05.

Infusion and Quantification of Fluorescent Retrograde Tracers

In experiment 3, we qualitatively compared the relative density of monosynaptic projections from the VTA and substantia nigra (SN) with the OFC and between the BLA and OFC. Rats were anesthetized as described above. Red Retrobeads (1:4 dilution, 0.5 μl, Lumafluor, Durham, NC) and Green Retrobeads (undiluted) were infused unilaterally into the left or right BLA ($n = 4$; −2.7 mm AP, +/−5.2 mm ML, −8.7 mm DV, from bregma) and OFC ($n = 4$; +3.5 mm AP, +/−3.0 mm ML, −5.4 mm DV, from bregma), respectively, using standard stereotaxic procedures. Microinfusions were delivered over 5 min with the 33 Ga infusion needle left in place for 10 min post infusion. Fourteen days later, rats were overdosed, as described above, and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde. The brains were dissected out, post-fixed, then cryoprotected in 30% sucrose–0.1% sodium azide solution. Coronal sections (30 μm) were cut on a freezing microtome. Images were captured at ×20 magnification using a fluorescence microscope. Retrogradely labeled cell bodies were counted bilaterally on representative images in 0.64 mm^2 sampling areas using NIH Image J.

RESULTS

Behavioral History

Cannula placements were verified in the target brain regions (Supplementary Figure S1). There were no group differences in cocaine intake (mean ± SEM, 11.09 ± 0.62 mg/kg per session) or in lever responding during the self-administration or extinction phases (see Supplementary Results and Supplementary Table S1).

Reinstatement Testing

In each experiment, re-exposure to the cocaine-paired context resulted in robust active lever pressing following

VEH pretreatment, independent of test order (extinction or cocaine-paired context first), treatment order, or the pretreatment administered on the other test day ($F<2.74$, $P>0.13$). Therefore, VEH data were collapsed across these variables. Inactive lever responding was low in all experiments and was not altered by the treatment manipulations (all treatment main and interaction effects, $F<5.69$, $P>0.054$; Supplementary Figure S2 and S3).

Intra-OFC SCH23390 Pretreatment Dose-Dependently Impairs Cocaine Seeking

At test, bilateral infusions of SCH23390 into the OFC altered drug context-induced reinstatement in a dose- and context-dependent manner (Figure 2b, all main and interaction effects, $F_{(1-2,\ 20-35)}=8.17$–63.87, $P=0.0001$–0.001). The 0.2 μg, but not the 0.02 μg, dose of SCH23390 attenuated active lever responding in the cocaine-paired context (Tukey test, $P<0.01$) relative to VEH. Consequently, there was no difference in active lever responding in the two contexts (Tukey test, $P>0.05$). SCH23390 did not alter responding in the extinction context relative to VEH. Additional time-course analysis of responding in the cocaine-paired context indicated that responding declined across time (time main effect, $F_{(2,16)}=13.78$, $P=0.0001$; 20 min interval 1 > 2–3, Tukey test, $P<0.05$), and the 0.2 μg dose of SCH23390 attenuated responding independent of time (Figure 2c, treatment main effect, $F_{(1,8)}=5.13$, $P=0.05$; treatment by time interaction effect, $F_{(2,16)}=1.98$, $P=0.17$).

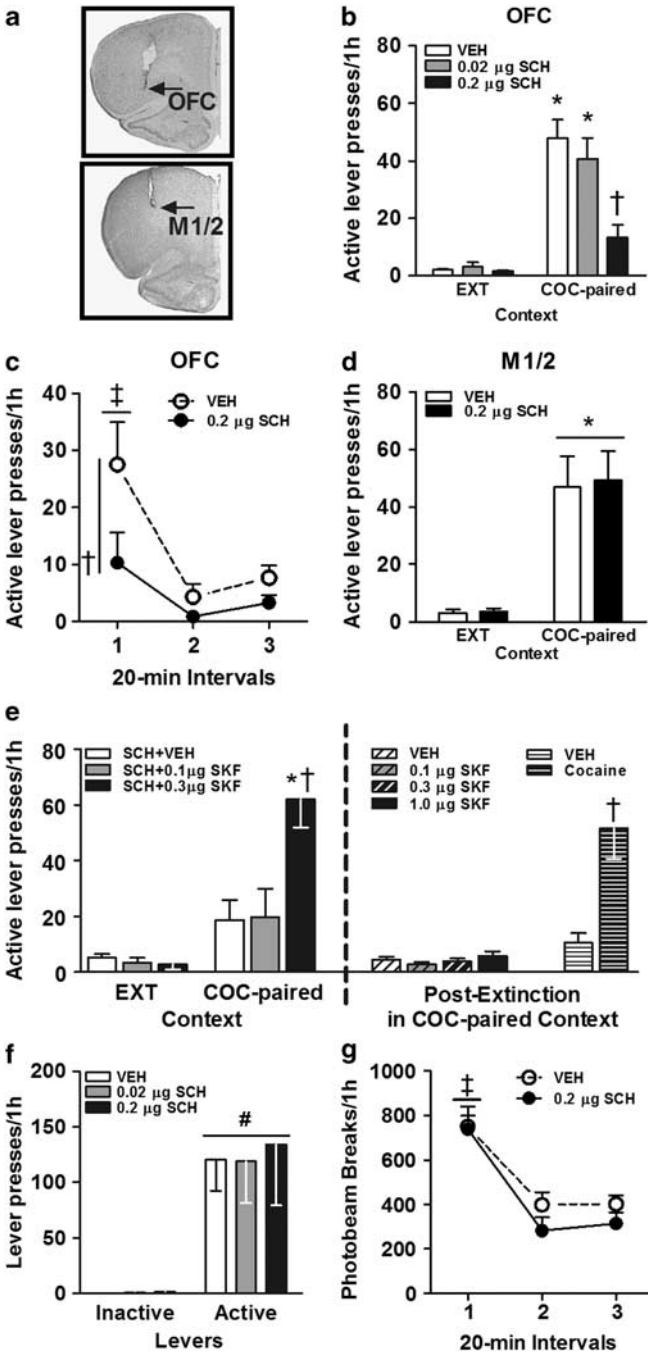

Figure 2 Dopamine D1-like receptor stimulation in the OFC, but not the M1/2, is necessary for drug context-induced cocaine-seeking behavior. The arrows on the photomicrographs in (a) identify the most ventral point of the injection cannula tracts on representative cresyl violet-stained brain sections (see Supplementary Figure S1 for placement distribution). Panels b–e depict non-reinforced active lever responses (mean/1 h ± SEM) during testing in the extinction context (EXT) and/or the previously cocaine-paired context (COC-paired) following bilateral infusion of phosphate-buffered saline vehicle (VEH) or SCH23390 into the OFC (b and c), $n=8$–10/group) or M1/2 (d), $n=9$) or following co-administration of SCH23390 (0.2 μg/0.5 μl per hemisphere) with 0.1% DMSO VEH or SKF81297 into the OFC (e left, $n=7$/group). Panel e right shows the reinstatement of extinguished active lever responses (mean/1 h ± SEM) following intra-OFC VEH or SKF81297 ($n=14$/group) or following intraperitoneal saline VEH or cocaine (10 mg/kg, $n=7$/group) priming. Panel f depicts food-reinforced active and inactive lever responses (mean/1 h ± SEM) following bilateral infusion of VEH or SCH23390 into the OFC ($n=9$). Panel g depicts photobeam breaks (mean/1 h ± SEM) triggered by the movement of subjects in a novel context during a 1-h locomotor activity test, following bilateral infusion of VEH or SCH23390 into the OFC ($n=8$–10/group). *Represent significant difference relative to responding in the extinction context. (b and e) Analysis of variance (ANOVA) context simple main effect, Tukey test, $P<0.05$; (d) ANOVA context main effect, $P<0.05$). †Represent significant difference relative to VEH (b and e): ANOVA treatment simple main effect, Tukey test, $P<0.05$; (c): ANOVA treatment main effect, $P=0.05$). #Represents significant difference relative to the inactive lever (d): ANOVA lever main effect, $P<0.01$). ‡Represents significant difference relative to all other time points (c and e): ANOVA time simple main effect, interval 1 > 2–3, $P<0.05$).

Intra-M1/2 SCH23390 Pretreatment Does not Alter Cocaine Seeking

Bilateral intra-M1/2 infusion of the behaviorally active (0.2 μg) dose of SCH23390 did not alter active lever responding in either context relative to VEH (Figure 2d, treatment main and interaction effects, $F<1.26$; $P>0.26$). Therefore, following VEH or SCH23390 pretreatment, active lever responding was greater in the cocaine-paired context than in the extinction context (context main effect, $F_{(1,8)}=42.18$, $P=0.0001$).

Intra-OFC SCH23390 Effect is Surmountable by SKF81297

The effect of intra-OFC SCH23390 (0.2 μg) pretreatment on active lever responding was dose-dependently impaired by SKF81297 co-administration (Figure 2e left; all treatment

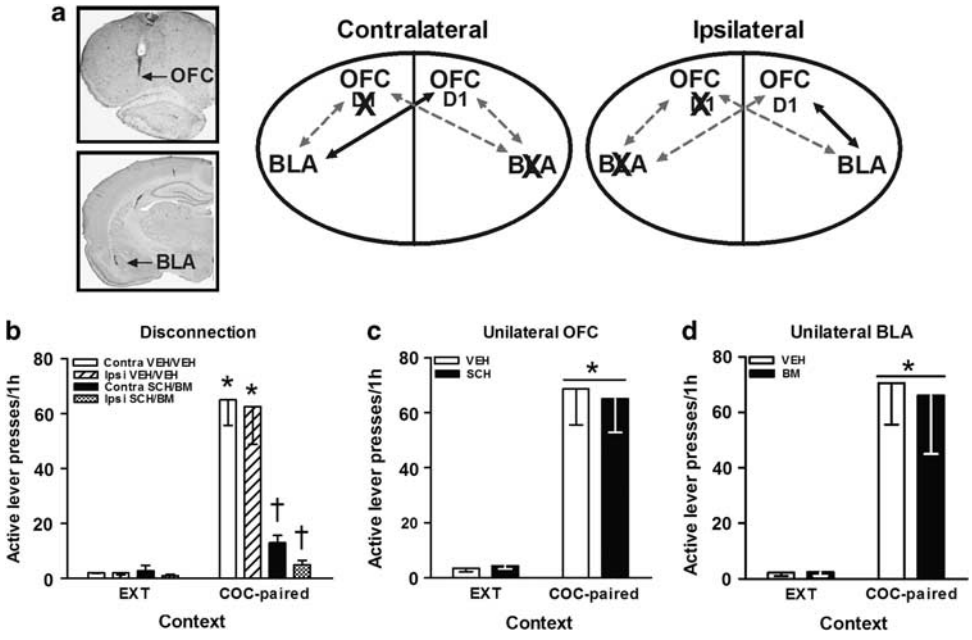

Figure 3 Dopamine D1-like receptor stimulation in the orbitofrontal cortex (OFC) is required for intrahemispheric and interhemispheric interactions between the OFC and basolateral amygdale (BLA) in the control of drug context-induced cocaine-seeking behavior. In panel a, arrows identify the most ventral point of the injection cannula tracts on photomicrographs of representative cresyl violet-stained brain sections (see Supplementary Figure S1 for placement distribution) and illustrate the effects of the functional disconnection manipulation on information flow within the putative circuit as depicted on a diagram (solid arrows, intact flow; segmented arrows, disrupted flow). Panels b–d show non-reinforced active lever responses (mean/1 h ± SEM) during testing in the extinction context (EXT context) and the previously cocaine-paired context (COC-paired context). Immediately before testing, rats in the disconnection experiment received infusions of SCH23390 (0.2 μg/0.5 μl/hemisphere) unilaterally into the OFC paired with baclofen + muscimol (BM; 106.8/5.7 ng, 0.5 μl/hemisphere) into the contralateral or ipsilateral BLA ($n = 12$ and 10/group, respectively) or vehicle (VEH) into the contralateral or ipsilateral OFC and BLA (b). Rats in the unilateral control experiments received infusions of 0.2 μg of SCH23390 or VEH into the OFC (c), $n = 11$) or 106.8/5.7 ng BM or VEH into the BLA (d), $n = 7$). *Represents significant difference relative to responding in the extinction context. (b) Analysis of variance (ANOVA) context simple main effect, Tukey test, $P < 0.01$; (c and d) ANOVA context main effect, $P < 0.05$). †Represents significant difference relative to VEH pretreatment. (b) ANOVA treatment simple main effect, Tukey test, $P < 0.01$).

main and interaction effects, $F_{(1-2,25)} = 4.91–31.62$, $P = 0.001–0.016$). Co-administration of the 0.3 μg, but not the 0.1 μg, dose of SKF81297 with SCH23390 increased active lever responding in the cocaine-paired context relative to the extinction context (Tukey test, $P < 0.01$) and relative to SCH23390 alone (Tukey test, $P < 0.01$), without altering responding in the extinction context.

Importantly, bilateral intra-OFC pretreatment with SKF81297 was insufficient to reinstate active lever responding following extinction in the cocaine-paired context (Figure 2e right), relative to VEH ($F_{(3,\ 39)} = 1.21$, $P = 0.32$). Conversely, cocaine (10 mg/kg, i.p.) priming was sufficient to increase active lever responding (Figure 2e right; $t_{(13)} = -2.97$, $P = 0.01$), relative to VEH, demonstrating the continued capacity of the groups for reinstatement.

Intra-OFC SCH23390 Effects do not Reflect Motor Impairment

Intra-OFC SCH23390 did not alter food-reinforced instrumental motor behavior (Figure 2f) or general locomotor activity (Figure 2g), relative to VEH pretreatment. Active lever responding was significantly greater than inactive lever responding during the food-reinforced test sessions (lever main effect, $F_{(1,17)} = 17.32$, $P = 0.004$) independent of SCH23390 (0.02 or 0.2 μg) pretreatment (treatment main and interaction effects, $Fs < 0.06$, $P > 0.69$). The number of photobeam breaks during the 1 h locomotor activity test session decreased across time (time main effect, $F_{(2,20)} = 28.29$, $P = 0.001$; 20 min interval 1 > 2–3; Tukey test, $P < 0.01$) independent of SCH23390 (0.2 μg) pretreatment (treatment main and interaction effects, $Fs < 1.09$, $P > 0.32$).

VTA–OFC–BLA Functional Disconnection has Superadditive Effect on Cocaine Seeking

Intra-OFC SCH23390 + intra-BLA BM pretreatment impaired active lever responding relative to VEH + VEH pretreatment in a context-specific manner following either contralateral or ipsilateral administration (treatment and context main and interaction effects, $F_{(1,20)} = 43.23–74.10$, $P = 0.0001$; surgery condition main and interaction effects, $F \leqslant 0.52$, $P \geqslant 0.48$; Figure 3b). Following ipsilateral or contralateral VEH + VEH pretreatment, active lever responding was greater in the cocaine-paired context than in the extinction context (Tukey tests, $P < 0.01$). Ipsilateral or contralateral SCH23390 + BM pretreatment attenuated active lever responding relative to VEH + VEH pretreatment in the cocaine-paired context (Tukey tests, $P < 0.01$) without altering responding in the extinction context. Therefore, following SCH23390 + BM pretreatment, there was no difference in active lever responding in the two contexts (Tukey tests, $P > 0.05$). The effect of SCH23390 + BM pretreatment was independent of the particular hemisphere in which SCH23390 was administered into the OFC

($t_{(20)} = 0.79$, $P = 0.44$) or BM was administered into the BLA ($t_{(20)} = 0.16$, $P = 0.88$).

Unlike functional disconnection, unilateral intra-OFC SCH23390 (Figure 3c, treatment main and interaction effects, $F < 0.07$, $P > 0.79$) or intra-BLA BM (Figure 3d, treatment main and interaction effects, $F < 0.06$, $P > 0.81$) pretreatment failed to alter active lever responding in either context relative to VEH. Thus, active lever responding was greater in the cocaine-paired context than in the extinction context independent of treatment (context main effect, OFC: $F_{(1,10)} = 31.02$, $P < 0.001$; BLA: $F_{(1,6)} = 17.72$, $P = 0.006$).

Distribution of Retrogradely Labeled Cell Bodies

Retrograde tracing data illustrating select projections between the elements of the putative VTA–OFC–BLA circuit are shown in Figure 4. Retrogradely labeled cell bodies were detected in the ipsilateral and contralateral VTA (Figure 4a) and BLA (Figure 4c), but not in the SN (Figure 4b), following unilateral Green Retrobeads microinfusion into the OFC. In addition, retrogradely labeled cell bodies were detected in the ipsilateral and contralateral OFC (Figure 4d) following unilateral Red Retrobeads microinfusion into the BLA.

DISCUSSION

Findings in the current study provide the first evidence that a VTA–OFC–BLA neural circuit regulates the ability of a drug-paired environmental context to elicit cocaine-seeking behavior. We examined this circuitry using a three-step approach. In experiment 1, we demonstrated that dopamine D1-like receptor stimulation in the OFC is required for drug context-induced cocaine-seeking behavior. In experiment 2, we further showed that dopamine D1-like receptor stimulation in the OFC is critical for interhemispheric or intrahemispheric communication between the OFC and BLA that controls this behavior. Finally, in experiment 3, we confirmed that the VTA, but not the SN, sends dense bilateral projections to the OFC. In addition, the OFC has bilateral and reciprocal connections with the BLA. Taken together, these findings support the hypothesis that input from the VTA—via the stimulation of dopamine D1-like receptors in the OFC—gates communication between the OFC and BLA that promotes the reinstatement of drug context-induced cocaine-seeking behavior.

Dopamine D1-like receptor stimulation in the OFC critically regulates the motivational effects of drug-paired contextual stimuli on cocaine-seeking behavior. The effect of SCH23390 did not reflect non-specific motor deficits, given that this manipulation failed to alter inactive lever responding in either context (Supplementary Figure S2), food-reinforced instrumental performance (Figure 2f) or general locomotor activity in a novel context (Figure 2g). It is unlikely that SCH23390 impaired context discrimination as it did not enhance active lever responding in the extinction context (Figure 2b). Similarly, it is unlikely that SCH23390 facilitated extinction learning given that it did not increase the rate of decline in active lever responding during the test session in the cocaine context (Figure 2c). Furthermore, the effect of SCH23390 was anatomically specific to the OFC in that bilateral infusions of SCH23390

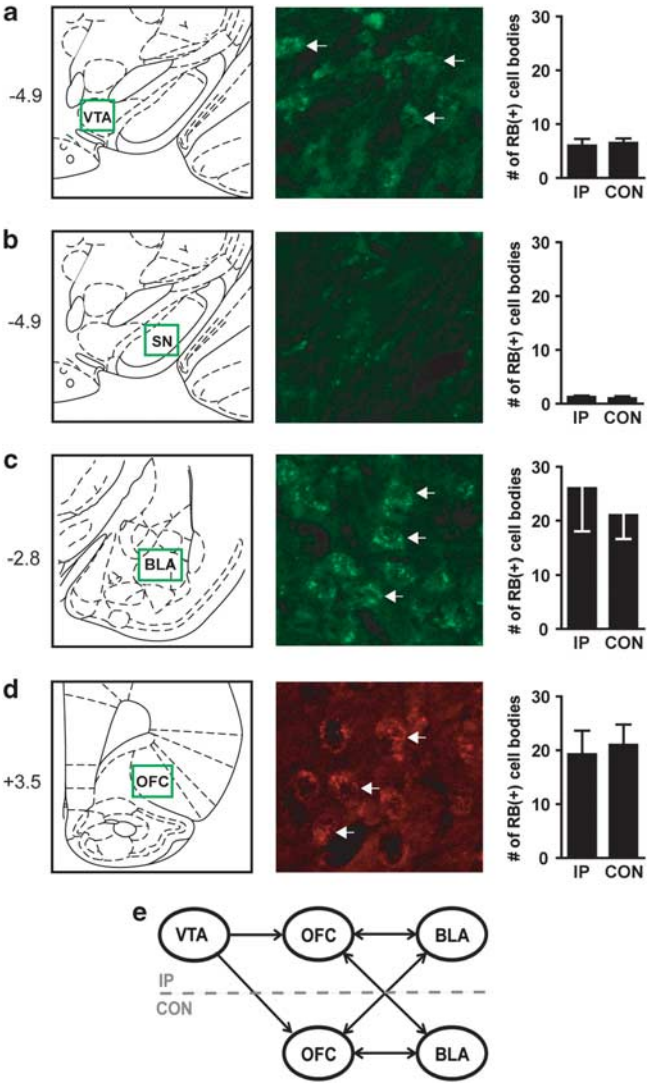

Figure 4 Anatomical connections within a VTA–OFC–BLA (VTA, ventral tegmental area; OFC, orbitofrontal cortex; BLA, basolateral amygdala) neural circuit as indicated by retrograde tracing (a–d) and summarized on a diagram (e). Red and Green Retrobeads were infused into the OFC or BLA (see Supplementary Figure S1 for placement distribution) and retrogradely labeled cell bodies were quantified bilaterally in the areas identified on the schematics adapted from the rat brain atlas of Paxinos and Watson (1997). Numbers to the left of the schematics indicate the distance from bregma in millimeters. The arrows on the photomicrographs identify representative retrogradely labeled cell bodies (× 20 magnification). The graphs depict the total number of retrobead-positive cell bodies (RB(+); mean/0.64 mm^2 sampling area ± SEM) in the ipsilateral (IP) and contralateral (CON) VTA (a), substantia nigra (SN) (b), and BLA (c) following unilateral Green Retrobeads microinfusion into the left or right OFC ($n = 4$) as well as in the ipsilateral and contralateral OFC (d) following unilateral Red Retrobeads microinfusion into the left or right BLA ($n = 4$).

into the dorsally adjacent M1/2 region did not alter cocaine seeking (Figure 2d) similar to other reward-related motor behaviors in previous studies (Hosp *et al*, 2011; Luft and Schwarz, 2009; Molina-Luna *et al*, 2009). Although SCH23390 has been routinely used to ascertain the functional contribution of dopamine D1-like receptors, it also acts as a serotonin 5-HT_{2C} receptor agonist (Kalkman *et al*, 1998; Rupniak *et al*, 1986), and systemic 5-HT_{2C} receptor stimulation attenuates explicit cue- and context-induced

cocaine-seeking behaviors (Neisewander and Acosta, 2007; Fletcher *et al*, 2008). Therefore, it is significant that the effect of SCH23390 in the OFC could be overcome by co-administration of the D1-like receptor agonist, SKF81297 (Figure 2e left), at a dose that alone failed to reinstate cocaine-seeking behavior in rats that could exhibit reinstatement in response to cocaine priming (Figure 2e right). Together, these results indicate that D1-like receptor stimulation is necessary, but not sufficient, for the reinstatement of cocaine-seeking behavior, similar to instrumental responding maintained by conditioned reinforcement (Winstanley *et al*, 2006). Conditioned stimuli elicit prolonged increases in dopamine neurotransmission (Garris *et al*, 1993; Sesack *et al*, 1998), which may permit the OFC to maintain the internal motivational representation of cocaine-paired contextual stimuli by facilitating glutamatergic inputs to the OFC (Cepeda *et al*, 1998a; Lapish *et al*, 2006; Schultz, 2002; Seamans *et al*, 2003). Furthermore, this phenomenon likely involves glutamatergic afferents from the BLA (Fuchs *et al*, 2005, 2004; Lasseter *et al*, 2009, 2011). Interestingly, the training protocol used in the present study fails to elicit significant context-induced reinstatement of food-seeking behavior, as shown previously (Xie *et al*, 2010). Thus, contextual conditioning with natural reinforcers may be less robust due to overshadowing by explicit food-related/conditioned stimuli or qualitatively different due to background conditioning, relative to contextual conditioning with intravenous drug reinforcers.

Given that the VTA is the primary source of dopamine to the OFC (Figure 4a; Berger *et al*, 1991), unilateral SCH23390-induced D1-like receptor antagonism in the OFC in combination with BM-induced functional inactivation of the contralateral or ipsilateral BLA could be used to disconnect and thereby probe the putative VTA–OFC–BLA circuit in the present study (Figure 3a). Contralateral or ipsilateral intra-OFC SCH23390 + intra-BLA BM pretreatment attenuated drug context-induced cocaine-seeking behavior relative to VEH in a context- and lever-specific manner (Figure 3b and Supplementary Figure S3). These findings expand upon our previous study (Lasseter *et al*, 2011), in which BM-induced functional disconnection of the OFC and BLA inhibited drug context-induced reinstatement of cocaine-seeking behavior without altering food-reinforced instrumental responding or general motor activity. These findings also add to a larger literature that indicates that the OFC and BLA are critical for explicit cue- and drug context-induced cocaine-seeking behaviors (Atkins *et al*, 2008; Crombag *et al*, 2008; Fuchs *et al*, 2005, 2004, 2008; Grimm and See, 2000; Hamlin *et al*, 2008; Kantak *et al*, 2002; Lasseter *et al*, 2009, 2011; McLaughlin and See, 2003; Neisewander *et al*, 2000; See *et al*, 2001; Zavala *et al*, 2008).

Ipsilateral and contralateral OFC-BLA manipulations similarly impaired drug context-induced cocaine seeking (Figure 3b), suggesting that interactions within the putative VTA–OFC–BLA circuit are complex. Unilateral manipulation of the OFC (Figure 3c) or BLA alone (Figure 3d) failed to alter drug context-induced cocaine-seeking behavior, consistent with previous reports (Everitt *et al*, 1991; Fuchs *et al*, 2007; Saddoris *et al*, 2005). Hence, the robust attenuation in cocaine seeking following ipsilateral or contralateral OFC-BLA manipulation was superadditive compared with the effects of independent, unilateral manipulations of the OFC plus BLA. A superadditive effect following ipsilateral manipulation may occur when functional impairment requires disruption of neuronal function beyond a theoretical threshold and indicate independent contribution by the brain regions. More likely, dopamine from the VTA regulates interhemispheric and intrahemispheric communication between the OFC and BLA via D1-like receptor stimulation in the OFC. This interpretation is supported by anatomical evidence from previous studies (Berger *et al*, 1991; Dunnett and Robbins, 1992; Frankle *et al*, 2006; Reynolds *et al*, 2006; Sesack and Grace, 2010; Watabe-Uchida *et al*, 2012) and from our retrograde tracing experiment indicating that the VTA (Figure 4a), but not the SN (Figure 4b), projects to the OFC. Moreover, the OFC and BLA share direct, reciprocal intrahemispheric and interhemispheric connections (Figure 4c and d; Carmichael and Price, 1995a; Ghashghaei and Barbas, 2002; Krettek and Price, 1977; McDonald, 1991) as well as indirect connections relayed through the mediodorsal thalamus (Cavada *et al*, 2000; Demeter *et al*, 1990; Ghashghaei and Barbas, 2002; Macey *et al*, 2003; Miyashita *et al*, 2007). Therefore, we propose that an intricate, intra- and interhemispheric VTA–OFC–BLA neural circuit controls drug context-induced motivation for cocaine.

Notably, the disconnection procedure does not permit one to identify the exact sequence of information processing within a neural circuit. Future research will be necessary in order to parse out the precise microcircuits by which the VTA, OFC and BLA interact to promote drug context-induced cocaine seeking. For instance, although dopaminergic input from the VTA to the OFC controls neuronal activity (Aou *et al*, 1983), back-projections from the OFC to the VTA may also regulate dopaminergic input to the OFC itself and to the BLA (Takahashi *et al*, 2009). In particular, input from the VTA to the BLA may be important for processing the motivational effects of cocaine-paired contextual stimuli, given that dopamine D1-like receptor antagonism in the BLA prevents explicit drug-paired cues from eliciting cocaine-seeking behavior (Alleweireldt *et al*, 2005; Berglind *et al*, 2006; Mashhoon *et al*, 2009; See *et al*, 2001).

Importantly, the VTA–OFC–BLA neural circuit is likely a component of a larger neural system. Within this system, the OFC and BLA are well-positioned to integrate context-relevant multi-modal sensory input from sensory cortices with reward-related information from other mesocorticolimbic brain regions in order to guide goal-directed behaviors (Brog *et al*, 1993; Carmichael and Price, 1995b; Fuchs *et al*, 2008; McDannald *et al*, 2004; Price, 1986; Sah *et al*, 2003; Xie *et al*, 2010). For instance, in addition to dopaminergic input from the VTA to the OFC and BLA, glutamatergic inputs from the OFC and BLA and dopaminergic input from the VTA converge on dendritic spines of nucleus accumbens (NAc) medium spiny neurons (Kelley *et al*, 1982; Bouyer *et al*, 1984; Haber *et al*, 1995). The NAc may then gate the throughput of information to the limbic loop of the basal ganglia that promotes the expression of goal-directed behavior (Cepeda *et al*, 1993; Cepeda and Levine, 1998b; Di Ciano and Everitt, 2004; Kiyatkin and Rebec, 1996). In concert with this, unilateral inactivation of the BLA paired with dopamine D1-like receptor antagonism in the NAc reduces behavioral responding to sucrose-predictive cues (Ambroggi *et al*, 2008), and optogenetic

inhibition of glutamatergic projections from the BLA to the NAc prevents cue-maintained sucrose consumption (Stuber *et al*, 2011).

In conclusion, results from the current study suggest that a mesocorticolimbic subcircuit exists in which dopaminergic input from the VTA to the OFC, via dopamine D1-like receptor stimulation, regulates intra- and interhemispheric interactions between the OFC and BLA that promote drug context-induced cocaine seeking. From an addiction treatment perspective, it will be important to systematically identify the brain regions with which the newly characterized VTA–OFC–BLA circuit interacts. Complementing this approach, future studies will need to characterize putative drug-induced and experience-based neuroadaptations within the circuitry that enhance cue reactivity and the propensity for cue-induced relapse in substance abusers.

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