INVOLVEMENT OF A MESOCORTICOLIMBIC SUBCIRCUIT IN THE REINSTATEMENT OF DRUG CONTEXT-INDUCED COCAINE-SEEKING BEHAVIOR IN RATS

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ABSTRACT

HEATHER C. LASSETER: Involvement of a mesocorticolimbic subcircuit in the reinstatement of drug context-induced cocaine-seeking behavior in rats
(Under the direction of Rita A. Fuchs Lokensgard)

The orbitofrontal cortex (OFC) and basolateral amygdala (BLA) control the ability of cocaine-paired environmental contexts to elicit relapse in addicts and cocaine-seeking behavior in laboratory animals. Whether these brain regions interact within a single neural circuitry or work independently to control this behavior remains to be ascertained. Given that extensive anatomical connections exist between the OFC and BLA, it was postulated that serial information processing occurs between these brain regions. To test this hypothesis, Experiment 1 utilized a functional disconnection procedure to disrupt communication between the OFC and BLA. Rats received microinfusions of the $GABA_{A/B}$ agonists, baclofen+muscimol (BM) or vehicle (VEH) unilaterally into the OFC plus the contralateral or ipsilateral BLA immediately before tests for cocaine-seeking behavior (responding on a previously cocaine-paired lever) in the cocaine-paired context or an alternate context (extinction context). Exposure to the previously cocaine-paired context, but not the extinction context, reinstated extinguished cocaine-seeking behavior. BM treatment in the OFC plus the contralateral or ipsilateral BLA attenuated this behavior relative to VEH, suggesting that inter- and intra-hemispheric interactions between the OFC and BLA are critical for drug context-induced motivation for cocaine. Next, Experiment 2 evaluated whether dopamine D1 receptor stimulation in the OFC contributed to drug context-induced cocaine seeking. The dopamine D1-like receptor

antagonist, SCH23390, or VEH was administered bilaterally into the OFC before testing. Intra-OFC SCH23390 treatment dose-dependently attenuated drug context-induced cocaine seeking relative to VEH, implicating dopamine D1 receptors in drug context-induced motivation for cocaine. The ventral tegmental area (VTA) provides the sole source of dopamine to the OFC. Therefore, Experiment 3 assessed whether dopamine input from the VTA to the OFC critically regulates interactions between the OFC and BLA. SCH23390 or VEH was administered unilaterally into the OFC plus BM or VEH into the contralateral or ipsilateral BLA before testing. The SCH23390/BM manipulation attenuated drug context-induced cocaine seeking relative to VEH. Together, these findings indicate that the VTA regulates both interhemispheric and intrahemispheric interactions between the OFC and BLA via the stimulation of dopamine D1 receptors in the OFC and that this newly characterized VTA-BLA-OFC neural circuit promotes drug context-induced motivation for cocaine.

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5-HT2c	serotonin receptor 2c
ANOVA	analysis of variance
AP	anterior-posterior
BLA	basolateral amygdala
BM	baclofen+muscimol
COC CTX	cocaine-paired context
CS	conditioned stimuli
DA	dopamine
DH	dorsal hippocampus
dlCPu	dorsal lateral caudate putamen
DV	dorsal-ventral
EXT CTX	extinction context
FR1	fixed ratio 1
GABA	gamma-aminobutyric acid
i.p.	intraperitoneal
i.v.	intravenous
LH	lateral hypothalamus
LS	lateral septum
MC	primary and secondary motor cortices
MDT	mediodorsal thalamus
ML	medial-lateral
NA	nucleus accumbens

NMDA	N-methyl-D-aspartic acid
OFC	orbitofrontal cortex
PFC	prefrontal cortex
SEM	standard error of the mean
VEH	vehicle
VH	ventral hippocampus
VTA	ventral tegmental area

CHAPTER 1
GENERAL INTRODUCTION

Background and Significance of the Problem

Cocaine addiction remains a prominent public health and social issue in the United States, with approximately 22.3 million people classified as having substance abuse or dependence problems according to the 2007 National Survey on Drug Use and Health (NIDA). Although dependence on alcohol accounted for the vast majority of such problems, cocaine represented the second most abused illicit drug, with 1.6 million individuals classified as abusing or being dependent on cocaine. In this same year, 808,000 individuals – including both current and former cocaine users – reported receiving treatment for cocaine abuse and dependence from hospitals, rehabilitation centers, and mental health centers. However, only 2.4 million out of the 6.9 million people specified as needing treatment for illicit drug use actually obtained medical or social support from such specialty treatment facilities (Substance Abuse and Mental Health Service Administration 2009). In addition to the adverse health consequences of cocaine abuse, cocaine addiction affects non-users by contributing to the high economic cost of addiction incurred by the United States each year, a figure approaching $193 billion dollars (National Drug Intelligence Center 2011).

Cocaine addiction manifests as a chronic relapsing disorder characterized by recurrent periods of drug use and abstinence from drug taking. As such, the successful treatment of cocaine addiction is severely impeded by high propensity for relapse

observed in former drug users, even after individuals have completed detoxification and rehabilitation programs. Remarkably, relapsing persists even when individuals experience diminished drug-induced euphoria, are faced with adverse consequences (i.e., health risks, incarceration, and family problems), or express a desire to cease drug-taking activities (Volkow and Fowler, 2000). Chronic drug users typically develop an inability to control drug seeking, which becomes compulsive or impulsive in nature (American Psychiatric Association, 1994). Evidence suggests that the transition from recreational drug use to drug addiction may be related to either neural sensitivity predisposing one to drug addiction or neural plasticity resulting from prolonged drug exposure and/or drug-related learning experiences (Franklin et al., 2002; Volkow et al., 2002).

Importantly, re-exposure to environmental contexts in which cocaine use previously occurred can produce powerful drug craving and seeking, thereby potentiating relapse in abstinent drug users (O'Brien et al., 1992; Childress et al., 1993; Foltin and Haney, 2000; Rohsenow et al., 2000; Franklin et al., 2009) and facilitating the reinstatement of drug-seeking behaviors in cocaine-experienced laboratory animals (Alleweireldt et al., 2001; Crombag et al., 2002; Crombag and Shaham, 2002; Fuchs et al., 2005; Fuchs et al., 2008a; Lasseter et al., 2010). Extensive research has indicated that drug context-induced relapse to cocaine seeking is regulated by a mesocorticolimbic neural circuitry. Therefore, from an addiction-treatment perspective, it is important to understand how interactions between elements of this neurocircuitry regulate relapse behaviors.

Animal Models of Cocaine Addiction and Relapse

The development of preclinical animal models of addiction has been informed by the understanding that relapse to drug taking is facilitated by a form of Pavlovian conditioning in cocaine users. Over the course of chronic drug use, both discrete, response-contingent cues (i.e. drug-taking paraphernalia) and contextual stimuli (i.e. environments in which drug use typically occurred) are repeatedly paired with the rewarding effects of the drug. These cues can subsequently acquire conditioned rewarding, conditioned reinforcing, and/or incentive motivational properties through associative learning properties that are based on the temporal relationship and contingency between the presentation of these stimuli and the delivery of the primary reward. In laboratory animals, c*onditioned rewarding* and *conditioned reinforcing* properties are demonstrated, respectively, by either an attraction to the drug-paired stimuli (i.e. approach or orienting towards the stimulus) or the ability of stimulus presentations to maintain instrumental responding (Newlin, 1992). Conversely, *incentive motivation* for drug is thought to manifest as an increase in instrumental responding in order to obtain drug, even when the primary reinforcer is not delivered (Markou et al., 1993). Because re-exposure to drug-associated environmental contexts can precipitate relapse in humans by eliciting powerful drug-craving and drug-seeking behaviors, several *in vivo* animal models have been developed to assess the neural mechanisms by which conditioned stimuli elicit cocaine-seeking behavior.

One of the most widely-used paradigms for studying relapse to cocaine-seeking behaviors in animals is the extinction-reinstatement model of addiction. In this model, subjects are trained to perform an instrumental response (i.e. lever responding) for

intravenous cocaine reinforcement in an operant conditioning chamber where drug infusions are either explicitly paired with the presentation of a response-contingent conditioned stimulus (CS) or they are administered while the animal is passively exposed to a distinct environmental context (drug-paired context). As a result, these stimuli acquire the ability to elicit cocaine-seeking behavior even in the absence of drug reinforcement (Fuchs et al. 2005; Crombag et al. 2008). After animals reach an arbitrary acquisition criterion, responding is extinguished either in the absence of the response-contingent CS or in an environmental context (extinction context) that is distinctly different from the drug-paired context. During extinction training, instrumental responses are not reinforced with cocaine infusions, and this typically results in a rapid decline in lever responding. Once responding has declined to a preset, arbitrary extinction criterion, animals are given reinstatement test sessions. These test sessions consist of re-exposure to either the cocaine-paired context or the response-contingent CS in the absence of cocaine reinforcement. During the test, the increase, or *reinstatement*, of responding is thought to reflect the ability of the context or CS to produce motivation to seek cocaine.

Evaluation of the Contextual Extinction-Reinstatement Model

Both the CS-based and context-based extinction-reinstatement models have excellent translational value for examining the neural underpinnings of human drug addiction. These paradigms possess strong face and predictive validity as models of cue-induced drug relapse because, similar to humans, the animals control cocaine self-administration instead of receiving passive drug exposure. However, the context-based

extinction-reinstatement model confers distinct advantages over the CS-based extinction-reinstatement model. First, in the contextual model, subjects are passively exposed to the cocaine-paired contextual cues, which results in uniform cue exposure across subjects during training and reinstatement testing. Second, passive cue presentation mirrors the human condition in that relapse to drug taking is typically precipitated by inadvertent exposure to cocaine-paired cues. Third, responding during the reinstatement test session provides an index of drug context-induced *incentive motivation* for the drug rather than *conditioned reinforcement* (i.e. where responding for cue presentation is the end goal) given the lack of an explicit, response-contingent cue (Fuchs et al., 2008b). However, it is difficult to ascertain whether the context acts as an *occasion setter* that predicts the reinforcement of instrumental responses or as a weakly associated Pavlovian CS (Crombag et al., 2008).

Despite its high face and predictive validity, the context-based extinction-reinstatement model possesses some limitations. Higher rates of attrition are observed during self-administration training in the context-based model relative to the CS-based paradigm, which may reflect that response acquisition is demanding in the absence of a response-contingent CS. Furthermore, the drug-paired context must be highly multi-modal in order to achieve a level of salience sufficient to elicit robust responding during the reinstatement test session and to permit repeated testing using a within-subjects design. As a result, experimental findings may not be readily generalized to other types of drug-paired cues. Extinction training may also reduce the face validity of this and other reinstatement models given that humans seldom undergo explicit extinction training prior to drug relapse (Katz and Higgins, 2003). Furthermore, different neural substrates

may underlie drug seeking following extinction training versus drug-free abstinence periods given that extinction training is an active learning process that induces neuroplasticity (Self and Nestler 1998; Self et al. 2004). However, it should be noted that some extinction experience may be accrued by abstinent drug users whenever cocaine is not available despite the presence of drug-related conditioned stimuli. Moreover, extinction training is necessary to isolate the influence of the cocaine-paired context on instrumental responding and disambiguate it from other factors that contribute to drug seeking, such as stress. Thus, while there are some limitations to the extinction-reinstatement model, it remains a powerful tool for exploring the neurobiological mechanisms of cue-induced drug relapse, a research endeavor that may prove critical for developing effective anti-relapse pharmacotherapies.

Anatomical Connectivity of the OFC and BLA

The OFC and BLA represent integral parts of the mesocorticolimbic neural circuitry that directs context-induced cocaine-seeking behavior in animal models of drug relapse (Neisewander et al., 2000; Kantak et al., 2002; Fuchs et al., 2005; Crombag et al., 2008; Fuchs et al., 2008b; Zavala et al., 2008; Lasseter et al., 2009; Mashhoon et al., 2010). Both the OFC and BLA receive integrated multi-sensory input from higher level sensory cortices and share direct and indirect (i.e. via the thalamus) outputs to several key elements of the neural circuitry proposed to mediate drug context-induced cocaine-seeking behavior, including the nucleus accumbens (NA), dorsal hippocampus (DH), dorsal medial prefrontal cortex (PFC), lateral hypothalamus (LH), ventral hippocampus (VH), and ventral tegmental area (VTA) (Christie et al., 1987; McDonald, 1991; Ray and

Price, 1992; Brog et al., 1993; Haber et al., 1995; O'Donnell and Grace, 1995; Groenewegen et al., 1996; Pikkarainen et al., 1999; Bossert et al., 2004; Fuchs et al., 2005; Bossert et al., 2007). Most notably, the OFC and BLA share robust reciprocal projections, which provide for significant information sharing between these brain regions (Kretek and Price, 1977). Based on this pattern of anatomical connectivity, both the OFC and BLA are well-positioned to integrate and process information from sensory regions in order to generate outcome expectancies that guide behavioral responses, including drug context-induced cocaine seeking (Price, 1986; Carmichael and Price, 1995b; Carmichael and Price, 1995a; McDannald et al., 2004). In addition, dopaminergic projections from the VTA to areas of the mesocorticolimbic neural circuitry may contribute to the ability of drug-paired cues to elicit drug-seeking behavior (Kiyatkin et al., 1993; Martin-Fardon et al., 2000). In particular, the VTA sends dense, topographically organized projections to cortical layers V and VI of the OFC that contain the densest population of dopamine D1-like receptors (Berger et al., 1991; Dunnett and Robbins, 1992; Frankle et al., 2006; Reynolds et al., 2006; Sesack and Grace, 2010). Thus, intra-OFC dopamine signaling may regulate interactions between the OFC and BLA that direct drug context-induced cocaine seeking.

Involvement of the OFC and BLA in Drug Addiction

Extensive evidence suggests that both the OFC and BLA regulate drug context-induced cocaine-seeking behavior by playing an acute role in monitoring the motivational salience of drug-paired conditioned cues. In human cocaine users, exposure to cocaine-paired stimuli elicits enhances neural activation of the OFC and BLA, which has been

positively correlated with self-reports of cocaine craving (Grant et al., 1996; Childress et al., 1999; London et al., 1999; Duncan et al., 2007). Similarly, in cocaine-experienced rats, re-exposure to a cocaine-paired context elicits enhanced expression of the activity-dependent immediate-early genes (IEGs) *c-fos, zif-268, BDNF*, and *arc* in the OFC and BLA, relative to IEG expression observed in saline-yoked controls exposed to a saline-paired context or cocaine-experienced rats exposed to a non-drug paired context (Neisewander et al., 2000; Hamlin et al., 2008; Hearing et al., 2008b; Hearing et al., 2008a). Furthermore, temporary bilateral functional inactivation of the OFC or BLA prevents drug-paired conditioned cues or environmental contexts from eliciting the reinstatement of cocaine-seeking behavior (See et al., 2001a; Kantak et al., 2002; McLaughlin and See, 2003; See et al., 2003; Fuchs et al., 2004; Fuchs et al., 2005; Lasseter et al., 2009).

In human cocaine users, addictive behaviors may either prompt or be facilitated by structural, physiological, and functional abnormalities in the frontal cortex. Cocaine users typically display abnormalities in frontal cortical regions, including decreased gray matter density in the OFC and anterior cingulate as well as diminished baseline blood glucose metabolism in the frontal cortex, which can be proportional to drug use (Volkow et al., 1991; Volkow and Fowler, 2000; Bolla et al., 2003b; Matochik et al., 2003a). Consistent with the idea that hypofrontality either predisposes one to cocaine addiction or results from chronic cocaine use, OFC damage in drug-naïve individuals produces behavioral impairments similar to those observed in cocaine addicts, including maladaptive decision-making, impulsive behavior, and perseveration of non-rewarding responses (Bechara et al., 1994; O'Doherty et al., 2001). Indeed, long-term damage to

either the OFC or BLA produces compulsive drug context-induced cocaine-seeking behaviors in animal models of drug relapse. Prolonged loss of output from the OFC caused by fiber-sparing NMDA lesions enhances drug context-induced cocaine-seeking behavior (Lasseter et al., 2009). Similarly, BLA lesions increase motivation for cocaine following exposure to non-contingent presentations of a previously cocaine-paired CS and produce enduring deficits in response inhibition under extinction conditions (Fuchs and See, 2002). In summary, the OFC and BLA critically contribute to maintaining and updating the motivational representation of drug-paired environmental stimuli. Therefore, exploring how the OFC and BLA interact to regulate drug craving and drug seeking is important for enhancing our understanding of relapse behaviors.

Interactions Between the OFC and BLA in Reward-related Behaviors

Converging lines of evidence suggest that interactions between the OFC and BLA may be critical for mediating goal-directed behaviors. As noted above, the OFC and BLA share dense, topographically-organized intrahemispheric and interhemispheric anatomical projections (Krettek and Price, 1977a; McDonald, 1991; Carmichael and Price, 1995a; Ghashghaei and Barbas, 2002). In addition to monosynaptic connections, information between the OFC and BLA can also be relayed through the mediodorsal thalamus (MDT), which provides an anatomical substrate for extensive functional interactions between the OFC and BLA (Demeter et al., 1990; Cavada et al., 2000; Ghashghaei and Barbas, 2002; Macey et al., 2003; Miyashita et al., 2007). Interestingly, amygdalocortical and amygdalothalamic pathways to the OFC involve distinct subpopulations of neurons within the OFC and BLA, indicating that these parallel

pathways may convey functionally distinct information (McDonald, 1991; Macey et al., 2003; Miyashita et al., 2007).

In accordance with this anatomical evidence, behavioral studies indicate that interactions between the OFC and are critical for processing the incentive motivational properties of reward-predictive cues and then using this information to guide behavioral responding. Monkeys with unilateral lesions of the OFC plus the contralateral BLA, which functionally disconnect these brain regions, are unable to update their behavioral responses when the motivational value of reward-predictive cues changes (Baxter et al., 2000; Izquierdo et al., 2004). Similarly, crossed neural inactivation of the OFC plus the contralateral BLA in rats produces inflexible behavioral responding on an odor reversal task (Churchwell et al., 2009). While electrophysiological studies have confirmed that intrahemispheric interactions between the OFC and BLA promote behavioral flexibility during reward reversal tasks, putative interhemispheric interactions have not been similarly explored (Saddoris et al., 2005). Interestingly, however, contralateral OFC plus BLA lesions - which disrupt intrahemispheric interactions between these brain regions - only transiently disrupt performance on a reinforcer devaluation task, which contrasts with the enduring behavioral deficits observed followed either bilateral OFC or BLA lesions (Baxter et al., 2000; Izquierdo and Murray, 2010). Thus, at least in the reinforcer devaluation task, recovery of function may occur following the permanent disruption of intrahemispheric interactions, perhaps through the strengthening of intact interhemispheric functional connectivity between these brain regions, which underscores the functional importance of both pathways. However, whether the OFC and BLA

exhibit functional interdependence with respect to drug context-induced cocaine-seeking behavior has yet to be determined.

Contribution of Mesocorticolimbic Dopaminergic Neurotransmission to Reward-related Behaviors

The mesocorticolimbic dopamine system is comprised of dopamine neurons whose cell bodies are located in the VTA of the midbrain (Oades and Halliday, 1987). These dopamine neurons project to various brain regions that contribute to drug context-induced cocaine-seeking behaviors, including the PFC, NA, BLA, and DH (Oades and Halliday, 1987; Sesack and Pickel, 1990; Frankle et al., 2006). Importantly, the VTA provides the sole source of dopamine to the OFC (Berger et al., 1991; Dunnett and Robbins, 1992; Frankle et al., 2006; Reynolds et al., 2006; Sesack and Grace, 2010). Dopamine neurotransmission in the OFC critically contributes to higher executive functions, including working memory, decision-making, behavioral flexibility, and reward-related processing (Ragozzino et al., 1999; Cetin et al., 2004; Dalley et al., 2004; Kheramin et al., 2004; Walker et al., 2009; Winter et al., 2009). As a result, blocking dopamine D1-like receptor stimulation in the OFC produces behavioral impairments, including inflexible responding during changing reward contingencies (Winter et al., 2009), reduced sensitivity for conditioned reinforcement on a progressive ratio schedule of food reinforcement (Cetin et al., 2004), and inability to update the motivational significance of reward-related stimuli under reversal conditions (Calaminus and Hauber, 2008). Similarly, disrupting communication between the VTA and the OFC prevents animals

from updating the value of a CS during a Pavlovian over-expectation task (Takahishi et al., 2010).

Dopamine receptor stimulation in the OFC and elsewhere may be necessary for a cocaine-paired context to elicit motivation for cocaine. Consistent with this, exposure to drug-paired conditioned stimuli reliably enhances dopamine release in terminal regions of the mesocorticolimbic dopamine system concomitant with the expression of drug-seeking behaviors in animals (Di Chiara and Imperato, 1988; Kiyatkin et al., 1993; Weiss et al., 2000; Di Ciano et al., 2001; Phillips et al., 2003; Schiffer et al., 2009). Furthermore, dopamine D1-like receptor antagonism in the PFC, BLA, and NA impairs CS-induced drug seeking (Alleweireldt et al., 2005; Sun and Rebec, 2005; Berglind et al., 2006; Schmidt and Pierce, 2006; Mashhoon et al., 2009; Fricks-Gleason and Marshall, 2010). Similarly, dysregulation of the prefrontal cortical dopamine system may contribute to the compulsive and impulsive drug-seeking behaviors observed in former cocaine addicts. For instance, abstinent cocaine users exhibit significant decreases in dopamine D2 receptor availability and dopamine release in the ventral striatum, and these abnormalities are positively correlated with hypoactivity in the OFC (London et al., 2000; Volkow and Fowler, 2000; Volkow et al., 2002; Volkow et al., 2009). In contrast, administering methylphenidate to cocaine addicts to increase extrasynaptic dopamine elicits hyperactivity in the OFC, the degree of which is positively correlated with cocaine-craving (Volkow et al., 1999; 2005). Preclinical studies further suggest that dopamine neurotransmission in the OFC contributes to some forms of cocaine-seeking behavior given that dopamine D1-like receptor antagonism in the OFC attenuates stress-induced cocaine-seeking behavior without altering responding maintained by sucrose

reinforcement (Capriles et al., 2003). While systemic blockade of dopamine D1-like receptors has been shown to impair drug context-induced cocaine seeking (Caggiula et al., 2001; Crombag et al., 2002; Liu and Weiss, 2002; Bossert et al., 2009), no study to date has established the precise contribution of dopamine D1-like receptor stimulation in the OFC to drug context-induced motivation for cocaine.

Overview of the Experiments

The overarching goal for the experiments in this dissertation was to enhance our understanding of the mesocorticolimbic neural circuitry that contributes to drug context-induced cocaine seeking, specifically by exploring whether dopaminergic input from the VTA to the OFC, via the stimulation of dopamine D1-like receptors, critically regulates putative OFC-BLA interactions that control this behavior. The experiments assessed drug context-induced incentive motivation for cocaine using the context-based extinction-reinstatement model of addiction (Fuchs et al., 2008a). To this end, rats were trained to self-administer cocaine in a distinct environmental context followed by extinction training in a different context. Context-induced motivation for cocaine was then assessed as drug seeking (i.e. non-reinforced lever presses) in the cocaine-paired context and in the extinction context in the absence of cocaine. Experiments in Chapter 2 assessed whether the OFC and BLA exhibit functional interactions in the control of drug context-induced cocaine seeking or, alternatively, whether these brain regions regulate cocaine seeking independently via parallel circuitries. Based on our recent findings (Lasseter et al., 2010), experiments in Chapter 3 assessed whether dopamine D1-like receptor stimulation in the OFC critically contributes to drug context-induced cocaine-

seeking behavior. Finally, experiments in Chapter 4 explored whether dopaminergic input from the VTA to the OFC, via dopamine D1 receptor signaling, critically regulates both intra- and interhemispheric interactions between the OFC and BLA that promote cocaine seeking following exposure to drug paired environmental stimuli.

CHAPTER 2

INTERACTIONS BETWEEN THE ORBITOFRONTAL CORTEX AND BASOLATERAL AMYGDALA IN THE REGULATION OF DRUG CONTEXT-INDUCED REINSTATEMENT OF COCAINE-SEEKING BEHAVIOR

INTRODUCTION

Extensive evidence suggests that the lateral orbitofrontal cortex (OFC) and basolateral amygdala (BLA) regulate drug context-induced cocaine-seeking behavior. In cocaine users, exposure to cocaine-paired stimuli elicits enhanced neural activity in the OFC and BLA and this is positively correlated with self-reports of cocaine craving (Grant et al., 1996; Childress et al., 1999; London et al., 1999; Duncan et al., 2007). Similarly, in cocaine-experienced rats, re-exposure to drug-paired contexts elicits neural activation in the OFC and BLA concomitant with drug-seeking behavior (Neisewander et al., 2000; Hamlin et al., 2008; Hearing et al., 2008b; Hearing et al., 2008a). Moreover, the functional integrity of the OFC and BLA is necessary for drug context-induced reinstatement of cocaine seeking (See et al., 2001a; Kantak et al., 2002; McLaughlin and See, 2003; See et al., 2003; Fuchs et al., 2004; Fuchs et al., 2005; Lasseter et al., 2009).

The OFC and BLA may be part of a serial neural circuit such that sequential information processing by these brain regions critically contributes to drug context-induced cocaine-seeking behaviors. Converging lines of evidence suggest that functional interactions between the OFC and BLA are necessary for a variety of goal-directed behaviors (Schoenbaum et al., 1998; Baxter et al., 2000; Schoenbaum et al., 2000;

Saddoris et al., 2005; Stalnaker et al., 2007a; Churchwell et al., 2009). In fact, it has been postulated that maladaptive drug-seeking behaviors may reflect cocaine-induced neurophysiological abnormalities in an orbitofrontal-amygdala circuit (Stalnaker et al., 2007b). However, no study to date has investigated whether the OFC and BLA functionally interact to direct drug context-induced cocaine seeking or, alternatively, control this behavior independently, via parallel circuitries.

Experiment 1 employed a functional disconnection procedure to explore whether the OFC and BLA exhibit sequential information processing to regulate drug context-induced reinstatement of cocaine-seeking behavior. Given that dense intra- and inter-hemispheric connections exist between the OFC and BLA (Cavada et al., 2000; Ghashghaei and Barbas, 2002), the functional significance of interactions by both ipsilateral and contralateral projections was investigated. To bilaterally disrupt intrahemispheric neural communication between the OFC and BLA, baclofen+muscimol (BM) – a GABA agonist cocktail that suppresses neural activity in cell bodies without affecting fibers of passage (Martin and Ghez, 1999) – was infused unilaterally into the OFC plus into the contralateral BLA immediately before assessing drug context-induced cocaine-seeking behavior. To bilaterally disrupt interhemispheric communication between the OFC and BLA, additional groups received BM infusions unilaterally into the OFC plus the ipsilateral BLA. Because unilateral manipulation of either brain region may alter drug context-induced cocaine seeking, functional interdependence between the OFC and BLA was predicted to manifest as a superadditive effect following either the contralateral or ipsilateral manipulation relative to the sum of effects observed following separate, unilateral manipulations of each brain region. Our laboratory has previously

verified that unilateral BLA inactivation fails to impair drug context-induced cocaine seeking (Fuchs et al., 2007). Thus, to test for a superadditive effect, a separate control group received unilateral manipulations of the OFC. Given the pattern of neural connectivity between the OFC and BLA described above, we predicted that both the intrahemispheric and interhemispheric manipulation would produce a greater impairment in drug context-induced cocaine seeking relative to unilateral functional inactivation of the OFC, but that such manipulations would not alter general motor activity or food-reinforced instrumental behavior.

METHODS

Animals

Male Sprague-Dawley rats (n = 53; 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* (Institute of Laboratory Animal Resources on Life Sciences, 1996) and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Food training

To expedite cocaine self-administration training, all rats (n=53) were trained to lever press on under a continuous schedule of food reinforcement (45 mg pellets; Noyes, Lancaster, NH, USA) in sound-attenuated operant conditioning chambers (26 x 27 x 27 cm high; Coulbourn Instruments, Allentown, PA, USA) during a 16-h overnight session.

Active lever responses resulted in the delivery of one food pellet only; inactive lever responses had no programmed consequences. During food training, contextual stimuli subsequently used for cocaine conditioning were not present.

Surgery

Forty-eight h after food training, all rats were fully anesthetized using ketamine hydrochloride and xylazine (66.6 and 1.33 mg/kg, i.p., respectively). Chronic indwelling jugular catheters were constructed in house and were surgically implanted into the right jugular vein of a subset of rats (n=35), as described previously (Fuchs et al., 2007). All rats (n=53) were stereotaxically implanted with stainless-steel guide cannulae (26 gauge; Plastics One) aimed dorsal to the left or right BLA (-2.7 mm AP, +/-5.2 mm ML, -6.7 mm DV, relative to bregma) and/or the left or right OFC (+3.5 mm AP, +/-3.0 mm ML, -3.4 DV) using standard procedures. Stainless steel screws and cranioplastic cement secured the guide cannulae to the skull. Stylets (Plastics One) and Tygon caps sealed the guide cannulae and catheter, respectively. To extend catheter patency, the catheters were flushed daily with an antibiotic solution of cefazolin (10.0 mg/ml; Schein Pharmaceuticals, Albuquerque, NM, USA) and heparinized saline (70 U/ml; Baxter Health Care Corp, Deerfield, IL, USA), as described previously (Fuchs et al., 2007). Rats were given a 5-day post-operative recovery period before the start of the experiment. Catheter patency was evaluated periodically using propofol (1mg/0.1ml, i.v. Eli Abbott Lab, North Chicago, IL, USA), which produces a rapid loss of muscle tone only when administered intravenously.

Cocaine self-administration training

Cocaine self-administration and extinction training sessions were conducted in operant conditioning chambers configured to one of two unique environmental contexts (**Table 1**: Context A, Context B) that differed along visual, auditory, tactile, and olfactory modalities, as described previously (Fuchs et al., 2005; Fuchs et al., 2007; Fuchs et al., 2008a). Rats had no exposure to these contextual stimuli prior to cocaine self-administration training; these stimuli were presented throughout each session independent of responding.

The rats were randomly assigned to receive daily 2h cocaine self-administration training sessions in Context A or B during their dark cycle (**Table 2**). Context A consisted of a continuous red house light (0.4 fc brightness) on the wall opposite the levers, an intermittent pure tone (80 dB, 1 kHz, 2 sec on, 2 sec off), a pine-scented air freshener strip (4.5 x 2 cm, Car Freshener Corp, Watertown, NY, USA), and wire mesh flooring (26 X 27 cm). Context B consisted of an intermittent white stimulus light above the left lever (1.2 fc brightness, 2 sec on, 4 sec off), a continuous pure tone (75 dB, 2.5 kHz), a vanilla-scented air freshener strip (4.5 x 2 cm, Sopus Products, Moorpark, CA, USA), and ceramic tile bisecting the chamber (19 cm X 27 cm). Rats had no exposure to these contextual stimuli prior to cocaine self-administration training and these stimuli were presented throughout each cocaine self-administration training session independent of responding.

Responses on one (active) lever were reinforced under an FR1 schedule of cocaine reinforcement (cocaine hydrochloride; 0.15 mg/infusion, equivalent to ~0.50 mg/kg/infusion; i.v; NIDA, Research Triangle Park, NC, USA). A 20s time-out period

followed each 2s infusion during which lever responses were recorded, but had no programmed consequences. Responses on the other (inactive) lever were recorded but had no programmed consequences. Training continued until the rats obtained ≥ 10 cocaine infusions/session on at least 10 training days (i.e., acquisition criterion).

Table 1. Contextual Stimuli During Training

	Stimulus Components			
CTX	**Visual**	**Auditory**	**Olfactory**	**Tactile**
A	Red house light	Intermittent tone (80 dB, 1 kHz/2s on, 2 s off)	Pine car air freshener strip	Wire grid floor (26 x 27 cm)
B	Intermittent white cue light above inactive lever	Continuous tone (2.5 kHz)	Vanilla car air freshener strip	Angled tile wall Metal bar floor (19 x 27 cm)

Table 2. Context Counterbalancing During Training

Self-administration		Extinction		Test Days
Context A (cocaine-paired)	→	Context B	→	Context A or B
Context B (cocaine-paired)	→	Context A	→	Context B or A

Extinction Training

After meeting the acquisition criterion, rats received daily 2h extinction training sessions in the context (Context A→B or B→A) that distinctly differed from the cocaine self-administration training context. Lever presses were recorded, but had no programmed consequences. Immediately prior to the behavioral session on extinction day 4, rats were acclimated to the intracranial infusion procedure. To this end, injection cannulae (33 Ga, Plastics One) were inserted into the rats' guide cannulae to a depth 2 mm below the tip of the guide cannulae and were left in place for 4 minutes. No drug was infused. Extinction training consisted of a minimum of 7 sessions plus additional sessions, as needed, until

the rats reached the extinction criterion (≤ 25 active lever presses/session during two consecutive sessions).

Reinstatement Testing

After the rats reached the extinction criterion, cocaine seeking was assessed in the cocaine-paired and extinction contexts during four test sessions (**Fig 2.1**). Immediately prior to testing, rats received microinfusions of the $GABA_B/GABA_A$ agonist cocktail baclofen+muscimol (BM; 1.0/0.1 mM; 0.5 µl/hemisphere, respectively; pH ~7.0) phosphate buffered saline vehicle (VEH; 0.5 µl/hemisphere) either (a) unilaterally into the OFC plus contralateral BLA, (b) unilaterally into the OFC plus ipsilateral BLA, or (c) unilaterally into the OFC alone. This BM dose was selected based on previous findings that administration of this dose into the OFC or BLA impairs drug context-induced cocaine-seeking behavior in a brain-region specific manner (Fuchs et al., 2007; Lasseter et al., 2009). The infusions were delivered over 2 min, and the injection cannulae were left in place for 1 min before and 1 min after the infusion. During the test sessions, lever responding had no programmed consequences. Session length was 1 h to allow for repeated testing without significant extinction learning. Both the order of testing in the two contexts and the order of intracranial treatments (BM, VEH) were counterbalanced based on mean active lever responding during the last three self-administration training days. Subjects received additional extinction sessions in the extinction context between test sessions until they re-obtained the extinction criterion (see above).

Locomotor activity and food-reinforced instrumental behavior

Intracranial manipulations can produce motor deficits that impair instrumental performance during the reinstatement tests. Hence, the effects of BM and VEH treatment on general locomotor activity and food-reinforced instrumental behavior were examined.

Locomotor Activity

Locomotor activity was assessed during two 1h test sessions held 5 days apart, starting 48 h after the last reinstatement test session. Locomotor activity was measured in novel Plexiglas chambers (42 x 20 x 20 cm) equipped with an array of eight photodetectors and light sources. Prior to testing, rats received intracranial microinfusions of BM or VEH using the infusion procedures and treatment order applied in the reinstatement experiment. A computerized activity system (San Diego Instruments, San Diego, CA) recorded the number of consecutive photobeams interrupted by rats moving in the activity chamber.

Food-Reinforced Instrumental Responding

Food-reinforced lever pressing behavior was assessed in experimentally naïve rats (n=18). After overnight food training and stereotaxic surgery (described above), the rats received additional daily 2h food self-administration training sessions in Context 1 or 2 until responding stabilized (i.e., $\leq$ 20% variability in active lever responding across two consecutive sessions), using previously described methods (Xie *et al*., 2010). After the stability criterion was reached, two 1-h test sessions were conducted. Immediately before the test sessions, rats received BM or VEH infusions into the OFC and the ipsilateral or contralateral BLA using the infusion procedure described above. The order of

intracranial treatments was counterbalanced across the two test sessions based on mean active lever responding during the last two training sessions. During the training and test sessions, active lever presses were reinforced with food pellets (45 mg, Purina) under an FR1 schedule with a 20s timeout period. Inactive lever presses were recorded, but had no programmed consequences. Between the test sessions, rats received a minimum of two additional food self-administration training sessions to re-establish baseline responding.

Histology

After the last experimental session, rats were overdosed using ketamine hydrochloride and 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, stored in 10% formaldehyde solution, and then sectioned at a thickness of 75 µm using a vibratome. The sections were stained using cresyl violet (Kodak, Rochester, NY, USA). Cannula placements were verified using light microscopy and were mapped onto schematics from the rat brain atlas (Paxinos and Watson, 1997).

Data analysis

Only data from rats with correct cannula placements were included in the data analysis. Potential pre-existing differences between the treatment groups in (a) lever responses and cocaine intake during the last three days of self-administration training, (b) lever responses during the first seven days of extinction training, and (c) the number of days needed to reach the extinction criterion were analyzed using mixed factors ANOVAs with surgery condition (contralateral, ipsilateral, unilateral) and subsequent treatment

order (BM first, VEH first) as between-subjects factors and time (day) as the within-subjects factor, where appropriate. The effects of BM and VEH infusions on (a) lever responses during the contextual reinstatement test sessions, (b) the number of photobeam breaks during the locomotor activity tests, and (c) food-reinforced instrumental responding were assessed using mixed factors ANOVAs with surgery condition (ipsilateral, contralateral) as the between-subjects factor and treatment (BM, VEH), testing context (cocaine-paired context, extinction context), time (20-min intervals), and/or lever (active, inactive) as within-subjects factors, when appropriate. Because the variables OFC hemisphere (left, right) and BLA hemisphere (left, right) are not orthogonal, the hemispheric laterality of significant effects was analyzed separately for the contralateral, ipsilateral, and unilateral surgery groups using planned t-tests. Significant main and interaction effects were investigated using simple main effects tests or Tukey *post hoc* tests. Alpha was set at 0.05.

RESULTS

Histology

Photomicrographs of representative cannula placements as well as schematics of the distribution of cannula placements are provided in **Fig 2.3**. The target brain regions were defined as the lateral and ventrolateral subregions of the OFC and the basolateral and lateral nuclei of the BLA. High power microscopy confirmed that there was no evidence of abnormal tissue damage (i.e., extensive cell loss or gliosis) at the infusion sites. Data from rats with misplaced cannulae (n=9) were excluded from data analysis. For the remaining cocaine-trained rats (n=29), the most ventral point of the cannula tract was

correctly located within the target brain regions of the contralateral (right OFC/left BLA, n=6; left OFC/right BLA, n=4), ipsilateral (right OFC/BLA, n=6; left OFC/BLA, n=4), and unilateral groups (right OFC, n=5; left OFC, n=4). For the remaining food-trained rats (n=15), the cannula tract was correctly located within the target brain regions of the contralateral (right OFC/left BLA, n=4; left OFC/right BLA, n=4) and ipsilateral groups (right OFC/BLA, n=4; left OFC/BLA, n=3).

Self-Administration Responding

The groups with cannulae aimed at the contralateral or ipsilateral OFC plus BLA or unilaterally at the OFC exhibited stable active lever responding for cocaine reinforcement over the last three days of self-administration training, with a within-subjects variability of <10% in daily cocaine intake. There was no difference between these groups in active lever responding (all day and surgery type main effects and interactions, all $Fs \leq 1.02$, $p \geq 0.41$) or inactive lever responding (all day and surgery type main and interaction effects, all $Fs \leq 1.14$, $p \geq 0.34$) during the last three days of self-administration training. Collapsed across groups, the mean active and inactive lever responding ± SEM was 42.92 ± 3.13 and 4.42 ± 1.72, respectively, while the mean cocaine intake ± SEM was approximately 11.55 ± 0.83 mg/kg per session (23.10 ± 1.65 infusions) (data not shown). Separate ANOVAs further indicated that there were no pre-existing differences in either lever responding or cocaine intake during the last three self-administration training days as a function of surgery condition (contralateral, ipsilateral, unilateral) or subsequent treatment order (BM first, VEH first) (all treatment order main effects and interactions, all $Fs \leq 2.39$, $p \geq 0.14$), or hemispheric laterality (left, right; all $ts \leq 1.61$, $p \geq 0.12$).

Extinction Responding

Upon removal of cocaine reinforcement during extinction training, active and inactive lever responding gradually declined (active lever: day main effect, $F_{(6, 156)} = 24.58$, $p = 0.0001$; day 1 > day 2-7, Tukey test, $p < 0.01$; inactive lever: day main effect, $F_{(6, 156)} = 10.10$, $p = 0.0001$; day 1 > day 2-7, Tukey test, $p < 0.05$). There was no difference between the contralateral OFC/BLA-cannulated, ipsilateral OFC/BLA -cannulated, and unilateral OFC-cannulated groups in active or inactive lever responding during the first seven days of extinction training (all surgery type main effects and surgery type X day interactions, all $Fs \leq 2.83$, $p \geq 0.08$). Separate ANOVAs indicated that there were no differences in active lever responding as a function of subsequent treatment order for either the contralateral OFC/BLA- or ipsilateral OFC/BLA-cannulated groups (all treatment order main effects and treatment order X day interactions, all $Fs \leq 0.69$, $p \geq 0.25$). The unilateral OFC-cannulated group that subsequently received VEH exhibited more active lever pressing than the group that subsequently received BM (treatment order X day interaction, $F_{(6,42)} = 0.41$, $p = 0.003$; treatment order main effect, $F_{(1,7)} = 5.60$, $p = 0.050$) on the first day of extinction training (VEH > BM day 1; Tukey test, $p < 0.05$), after which no group differences were observed. There was no difference between groups of similar surgery condition in inactive lever responding based on subsequent treatment order (all treatment order main effects and treatment order X day interactions, all $Fs \leq 1.78$, $p \geq 0.11$). There was also no difference in the mean number of days ± SEM required to reach the extinction criterion (7.45 ± 1.61) as a function of surgery condition (contralateral, ipsilateral, unilateral; $F_{(2,26)} = 0.22$, $p = 0.81$) or subsequent treatment order

(all ts<1.90, $p > 0.10$). Similarly, there was no difference between the groups in the mean number of days ± SEM needed to re-obtain the extinction criterion between reinstatement test sessions (2.1 ± 0.06). Hence, it is unlikely that pre-existing differences accounted for group differences in reinstatement responding during the subsequent test sessions.

Effects of OFC-BLA functional inactivation on drug context-induced reinstatement of cocaine-seeking behavior

Following VEH pretreatment, the contralateral and ipsilateral OFC/BLA-cannulated groups exhibited an increase in non-reinforced active lever responding upon exposure to the previously cocaine-paired context relative to responding in the extinction context (**Fig 2.4A-B**; context main effect, $F_{(1,18)} = 50.04$, $p = 0.0001$). BM pretreatment impaired active lever responding relative to VEH pretreatment in a context-specific manner following administration into either the contralateral OFC/BLA or the ipsilateral OFC/BLA (treatment X context interaction, $F_{(1,18)} = 33.51$, $p = 0.0001$; treatment main effect, $F_{(1,18)} = 24.49$, $p = 0.0001$; only statistically significant effects are reported). Specifically, independent of surgery condition, BM pretreatment attenuated active lever responding relative to VEH pretreatment in the cocaine-paired context (Tukey test, $p < 0.001$), but did not alter responding in the extinction context. As a result, following BM pretreatment, there were no differences between responding in the cocaine-paired context and the extinction context. The effect of BM pretreatment on active lever responding in the cocaine-paired context was independent of the particular hemisphere into which BM

was administered for either the OFC ($t_{(18)} = 0.29$, $p = 0.78$) or the BLA ($t_{(18)} = 0.66$, $p = 0.52$)

Following VEH pretreatment, the contralateral and ipsilateral OFC/BLA-cannulated groups exhibited a slight increase in inactive lever responding upon exposure to the previously cocaine-paired context relative to responding in the extinction context (**Fig 2.4D-E;** context main effect, $F_{(1,18)} = 8.49$, $p = 0.009$). BM pretreatment impaired inactive lever responding relative to VEH pretreatment in a context-specific manner following administration into either the contralateral OFC/BLA or the ipsilateral OFC/BLA (treatment X context interaction, $F_{(1,18)} = 10.08$, $p = 0.005$; treatment main effect, $F_{(1,18)} = 10.83$, $p = 0.004$; only statistically significant effects are reported). Specifically, independent of surgery condition, BM pretreatment attenuated inactive lever responding relative to VEH pretreatment in the cocaine-paired context (Tukey test, $p < 0.05$) but not in the extinction context. The effect of BM pretreatment on inactive lever responding in the cocaine-paired context was independent of the particular hemisphere into which BM was administered for either the OFC ($t_{(18)} = 0.64$, $p = 0.53$) or the BLA ($t_{(18)} = 0.29$, $p = 0.77$).

Effects of unilateral OFC functional inactivation on drug context-induced reinstatement of cocaine-seeking behavior

During the reinstatement test sessions, the unilateral OFC-cannulated group exhibited an increase in non-reinforced active lever responding in the previously cocaine-paired context relative to responding in the extinction context following VEH pretreatment (**Fig 2.4C**; context main effect, $F_{(1,8)} = 34.56$, $p = 0.001$). BM pretreatment administered

unilaterally into the OFC did not alter active lever responding relative to VEH pretreatment in either context (treatment X context interaction, $F_{(1,8)} = 0.07$, $p = 0.80$; treatment main effect, $F_{(1,8)} = 0.01$, $p = 0.91$). Exposure to the cocaine-paired context did not alter responding on the inactive lever relative to responding in the extinction context (**Fig 2.4F**; context, $F_{(1,8)} = 2.07$, $p = 0.19$), and BM pretreatment administered unilaterally into the OFC did not alter inactive lever responding relative to VEH pretreatment in either context (treatment X context interaction, $F_{(1,8)} = 1.43$, $p = 0.266$; treatment main effect, $F_{(1,8)} = 2.49$, $p = 0.15$).

Locomotor Activity

BM pretreatment failed to alter locomotor activity relative to VEH pretreatment in the contralateral OFC/BLA-cannulated, ipsilateral OFC/BLA-cannulated, and unilateral OFC-cannulated groups (**Fig 2.5A-C**). In all groups, the number of photobeam breaks decreased at a similar rate over the three 20-min intervals of the locomotor test session as the groups habituated to the novel context (all time main effects, all $Fs \geq 45.77$, $p = 0.0001$; interval 1 > intervals 2-3; Tukey test, $p < 0.01$). In addition, BM pretreatment did not alter the number of photobeam breaks relative to VEH pretreatment (all treatment main effects and interactions, all $Fs \leq 0.11$, $p \geq 0.07$).

Food-reinforced Instrumental Behavior

BM pretreatment failed to alter food-reinforced instrumental performance relative to VEH pretreatment in the contralateral or ipsilateral OFC/BLA-cannulated groups (**Fig 2.6A-B**). Independent of surgery condition and treatment, all groups exhibited more

active lever responding than inactive lever responding (lever main effect, $F_{(1,13)} = 110.33$, $p = 0.0001$). Furthermore, BM pretreatment administered unilaterally into the OFC plus the contralateral or ipsilateral BLA did not alter food-reinforced responding alone or as a function of surgery condition or lever (treatment main effect and all treatment interactions, all Fs < 0.46, $p > 0.51$).

DISCUSSION

Experiments in Chapter 1 explored putative functionally significant interactions between the OFC and BLA in drug context-induced cocaine-seeking behavior. To this end, the effects of unilateral functional inactivation of the BLA plus the contralateral or ipsilateral OFC were assessed on the expression of cocaine seeking elicited by re-exposure to a drug-paired environmental context. Contralateral or ipsilateral administration of BM into the OFC plus BLA produced a profound attenuation of the reinstatement of drug context-induced cocaine seeking (**Fig 2.4A-B**). While some drug context-induced cocaine-seeking behavior was also recorded on the inactive lever (**Fig 2.4D-E**), this phenomenon is often observed when behavioral conditioning occurs in the absence of an explicit cocaine-paired conditioned stimulus (Fuchs et al., 2007; Fuchs et al., 2009; Lasseter et al., 2010). Furthermore, this alternate form of cocaine-seeking behavior was also impaired by BM treatment. Importantly, BM-induced decreases in drug context-induced cocaine seeking were unlikely to reflect non-specific deficits in instrumental motor performance given that functional inactivation of the contralateral or ipsilateral OFC plus BLA did not alter active lever responding in the extinction context (**Fig 2.4A-B**), general motor activity in a novel context (**Fig 2.5A-C**), or food-reinforced

instrumental behavior (**Fig 2.6A-B**). In addition, previous findings from our lab and from other investigators have demonstrated that bilateral functional inactivation of the OFC and BLA fails to alter cocaine-primed reinstatement of cocaine seeking (Lasseter and Fuchs, unpublished observation; Grimm and See, 2000). Overall, these findings indicate that neural activity in both the OFC and BLA is necessary for using the memory or motivational significance of cocaine-associated environmental stimuli to guide goal-directed behavior. Such results are consistent with previous research indicating that the OFC and BLA are integral parts of the mesocorticolimbic neural circuitry known to direct cue and context-induced cocaine-seeking behavior in the reinstatement and renewal animal models of drug relapse (Grimm and See, 2000; Neisewander et al., 2000; See et al., 2001a; Kantak et al., 2002; McLaughlin and See, 2003; Fuchs et al., 2004; Fuchs et al., 2005; Atkins et al., 2008; Crombag et al., 2008; Fuchs et al., 2008a; Hamlin et al., 2008; Zavala et al., 2008; Lasseter et al., 2009). Moreover, the present study significantly extends this line of research by suggesting that the OFC and BLA co-regulate drug context-induced cocaine seeking via sequential information processing or by providing necessary input to a common downstream target within a neural circuit.

When interpreting the finding that ipsilateral and contralateral OFC plus BLA neural inactivation produced similar impairment in cocaine seeking, it is important to note that rats exhibited robust drug context-induced cocaine-seeking behavior following unilateral functional inactivation of the OFC (**Fig 2.4C**) or BLA (Fuchs et al., 2007). Our findings are consistent with previous studies demonstrating that unilateral OFC or BLA manipulations are insufficient to disrupt the acquisition of reversal learning (Saddoris et al., 2005) or the expression of conditioned appetitive behaviors, including drug context-

induced cocaine seeking and sucrose-conditioned place preference (Everitt et al., 1991; Fuchs et al., 2007), even though these manipulations are capable of disrupting some forms of conditioned learning and reward processing (LaBar and LeDoux, 1996; Izquierdo et al., 2004; Markham et al., 2010). Thus, one possible interpretation of the current findings is that the ipsilateral and contralateral OFC plus BLA manipulations crossed the threshold of neural inactivation sufficient to disrupt drug context-induced cocaine seeking independent of functional connectivity between the OFC and BLA. However, given that unilateral functional inactivation of either the OFC or BLA failed to alter the motivational significance of the cocaine-paired environmental context, it is unlikely that additive effects of these manipulations accounted for the robust effects of both the contralateral and ipsilateral OFC plus BLA inactivation observed in the current study, even if we are dealing with a nonlinear system.

A more likely possibility is that functionally significant *interactions* between the OFC and BLA may be necessary for the control of drug context-induced cocaine-seeking behavior. Given that the magnitude of impairment in context-induced cocaine seeking was similar following ipsilateral and contralateral neural inactivation of the OFC and BLA, the ability of the cocaine-paired context to elicit cocaine seeking may rely equally on the functional integrity of intrahemispheric and interhemispheric connections between the OFC and BLA, which were bilaterally disrupted by the contralateral and ipsilateral BM manipulations, respectively. This explanation is supported by considerable anatomical evidence indicating that the OFC and BLA share dense reciprocal intra- and interhemispheric projections that are topographically organized (Krettek and Price, 1977a; McDonald, 1991; Carmichael and Price, 1995a; Ghashghaei and Barbas, 2002).

Additional connections between the OFC and BLA are relayed through the MDT, providing an anatomical substrate for extensive functional interactions between the OFC and BLA (Demeter et al., 1990; Cavada et al., 2000; Ghashghaei and Barbas, 2002; Macey et al., 2003; Miyashita et al., 2007). Interestingly, amygdalocortical and amygdalothalamic pathways to the OFC involve distinct subpopulations of neurons within the OFC and BLA, indicating that these parallel pathways may convey functionally distinct information between the OFC and the BLA (McDonald, 1991; Macey et al., 2003; Miyashita et al., 2007).

The explanation that communication between the OFC and BLA subserves drug-seeking behaviors is further supported by evidence that functional interdependence exists between these brain regions in the regulation of various goal-directed behaviors. Highlighting the importance of intrahemispheric communication between the OFC and BLA, previous studies have demonstrated that contralateral – although not ipsilateral – OFC and BLA neural inactivation disrupts performance on an odor reward-reversal task (Churchwell et al., 2009), while contralateral OFC plus BLA lesions disrupt affective processing as evidenced by attenuated reinforcer devaluation effects and impaired object reversal learning (Baxter et al., 2000; Izquierdo et al., 2004). Furthermore, electrophysiological studies have confirmed that intrahemispheric interactions between the OFC and BLA promote behavioral flexibility on an odor discrimination task, although putative interhemispheric interactions have not been similarly explored (Saddoris et al., 2005). Interestingly, however, contralateral OFC and BLA lesions only transiently disrupt performance on a reinforcer devaluation task in contrast to the enduring behavioral deficits produced by bilateral OFC or BLA lesions (Izquierdo et al.,

2004; Izquierdo and Murray, 2007; Izquierdo and Murray, 2010). Thus, at least in the reinforcer devaluation task, recovery of function may occur after permanently disrupting intrahemispheric interactions between the OFC and BLA due to the strengthening of interhemispheric functional connectivity between brain regions that had been left intact, which underscores the importance of both pathways.

In conclusion, results from the current study provide important evidence that interactions between the OFC and BLA are necessary for the expression of drug context-induced motivation for cocaine. This form of cocaine-seeking behavior may depend on intrahemispheric and interhemispheric information processing by the OFC and BLA via direct reciprocal anatomical projections or via the convergence of requisite information from both of these brain regions onto a third brain region within the circuitry. As noted above, one particular region the OFC and BLA may interact with to direct cocaine-seeking behavior is the MDT given that the MDT makes similar contributions to conditioned behaviors as the OFC and BLA (Aggleton and Mishkin, 1983; Gaffan and Murray, 1990; Corbit et al., 2003). Moreover, a crossed-disconnection procedure indicated that the OFC and BLA interact with the MDT in the regulation of reward-based decision making (Izquierdo and Murray, 2010). In addition, the OFC and BLA send afferents to several elements of the neural circuitry that mediate context-induced reinstatement of drug-seeking behavior, including the nucleus accumbens (NA), DH, PFC, and VTA (Christie et al., 1987; McDonald, 1991; Ray and Price, 1992; Brog et al., 1993; Haber et al., 1995; O'Donnell and Grace, 1995; Groenewegen et al., 1996; Pikkarainen et al., 1999; Bossert et al., 2004; Fuchs et al., 2005; Bossert et al., 2007). In particular, our laboratory has demonstrated that interactions between the BLA and DH as

well as between the BLA and PFC, are necessary for drug context-induced cocaine-seeking behavior (Fuchs et al., 2007). Furthermore, interactions between the BLA and NA may be necessary for this behavior given that communication between these brain regions promotes responding for sucrose- and cocaine-paired conditioned stimuli (Schoenbaum et al., 2002; Ambroggi et al., 2008; Di Ciano, 2008) and is critical for the expression of sucrose-conditioned place preference (Everitt et al., 1991). Finally, dopamine input from the VTA may regulate OFC-BLA interactions in context-induced cocaine-seeking behavior given that dopamine D1-like receptor antagonism in either the OFC or BLA is sufficient to impair the acquisition and expression of cue-induced cocaine seeking and decreases the break point under a progressive ratio schedule for food reinforcement, respectively (See et al., 2001a; Cetin et al., 2004; Berglind et al., 2006). Because the OFC and BLA exert important control over the motivational aspects of drug-paired environmental stimuli, further explication of the larger neural circuitry within which they interact to direct drug-seeking behavior may provide insight into the prevention of environmentally-induced drug relapse.

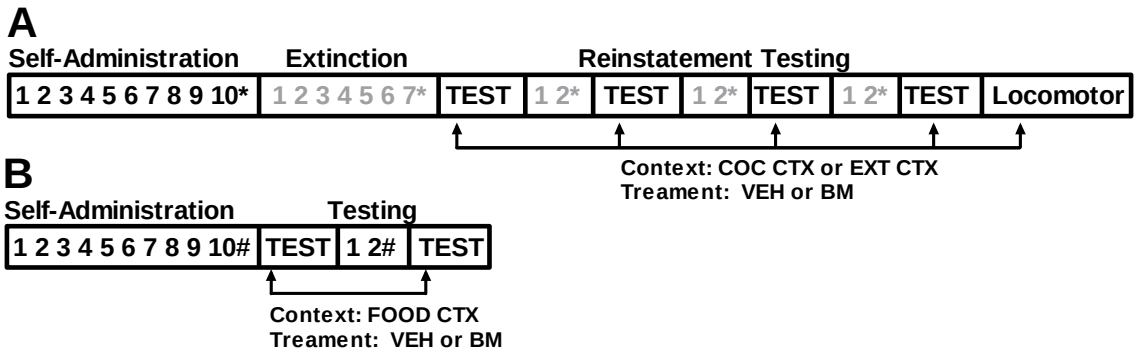


Fig 2.1 Schematic representation of the timeline for the drug context-induced reinstatement experiments (***A***) and the food-maintained instrumental control experiments (***B***). Arrows on the schematics identify sessions in which VEH or BM was administered immediately prior to testing. The order of drug treatment (BM, VEH) and the order of exposure to the two testing contexts during reinstatement testing (cocaine-paired context, COC CTX; extinction context, EXT CTX) were counterbalanced, where appropriate. *Asterisks* indicate that the rats had to reach an acquisition criterion (≥ 10 infusions per session for minimum 10 sessions) to complete self-administration training and had to satisfy our extinction criterion (≤ 25 active lever presses per session for two consecutive sessions) before each test session. *Pound signs* indicate that rats had to reach a stability criterion (≤ 10% variability in active lever presses for two consecutive sessions) before each test session.

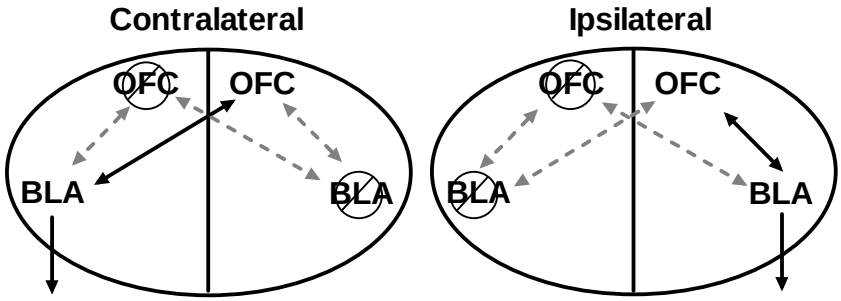


Fig 2.2 Schematic representation of the functional disconnection procedure. Following the contralateral manipulation, putative intrahemispheric processing by the OFC and BLA was expected to be disrupted bilaterally, while interhemispheric processing by the OFC and BLA was expected to be spared in one hemisphere. Following the ipsilateral manipulation, putative interhemispheric processing was expected to be disrupted bilaterally, while intrahemispheric processing by the OFC and BLA was expected to be spared in one hemisphere. Solid lines represent communication that is preserved between two intact brain regions. Dotted lines represent communication that is transiently disrupted following neural inactivation of the BLA and OFC.

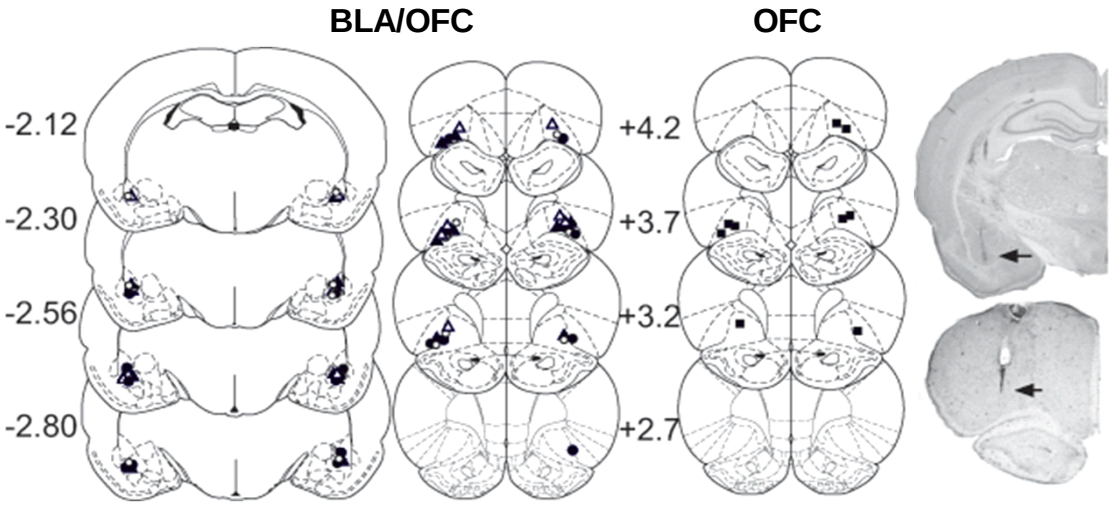


Fig 2.3 Schematic and photographic representation of injection cannula placements. The *arrows* on the photomicrographs identify the most ventral point of the infusion cannula tracts on representative cresyl violet-stained brain sections. The symbols on the schematics (Paxinos and Watson, 1997) represent the most ventral point of the infusion cannula tracts for rats that received unilateral microinfusions into the OFC plus the contralateral BLA (cocaine self-administration: *closed circles*, food-maintained responding: *open circles*) or the ipsilateral BLA (cocaine self-administration: *filled triangles*, food-maintained responding: *open triangles*), or a unilateral microinfusion into the OFC alone (*filled squares*). Numbers indicate the distance from bregma in millimeters.

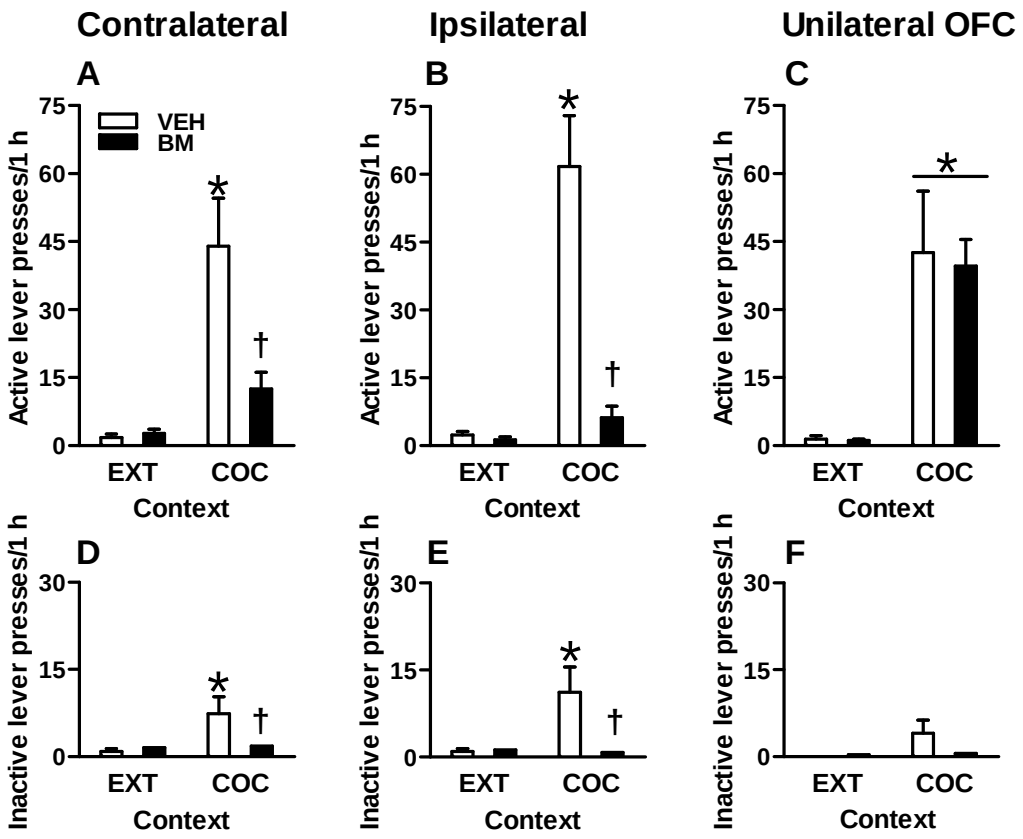


Fig 2.4 Bilateral inhibition of intrahemispheric or interhemispheric connections between the OFC and BLA similarly attenuates drug context-induced reinstatement of cocaine-seeking behavior. The panels depict non-reinforced active and inactive lever responses (mean/1h ± SEM) during testing in the extinction context (EXT context) and the previously cocaine-paired context (COC context). Immediately before testing, VEH or BM was infused unilaterally into the OFC plus the contralateral BLA (***A,D***) or the ipsilateral BLA (***B,E***), or unilaterally into the OFC alone (***C,F***). *Asterisks* represent significant difference relative to responding in the extinction context (***A-B, D-E:*** ANOVA context simple main effect, $p < 0.05$; ***C:*** ANOVA context main effect, $p < 0.05$). *Daggers* represent significant difference relative to VEH pretreatment (***A-B, D-E:*** ANOVA treatment simple main effect, $p < 0.05$).

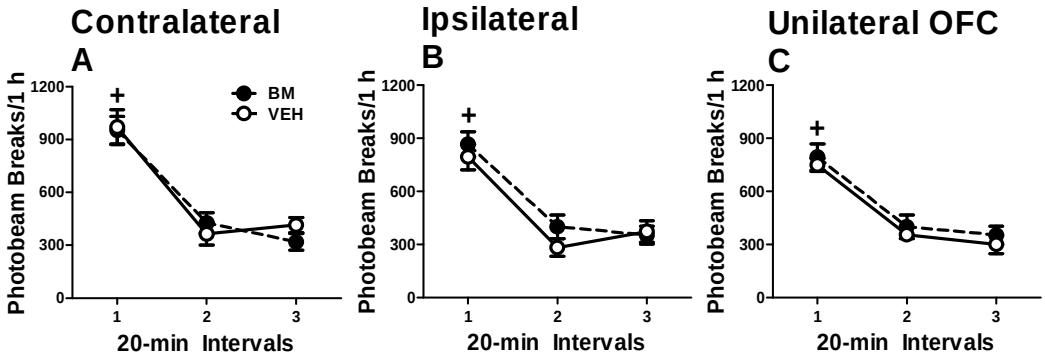


Fig 2.5 Bilateral inhibition of intrahemispheric or interhemispheric connections between the BLA and OFC does not alter general motor activity. Photobeam breaks (mean/1h ± SEM) were triggered by the movement of subjects in a novel context during a 1-h locomotor activity test. Immediately before testing, VEH or BM was infused unilaterally into the OFC plus the contralateral BLA (***A***) or the ipsilateral BLA (***B***), or unilaterally into the OFC alone (***C***). *Plus signs* represent significant difference relative to all other time points (***A-C:*** ANOVA time simple main effect, interval 1> intervals 2-3, $p < 0.05$).

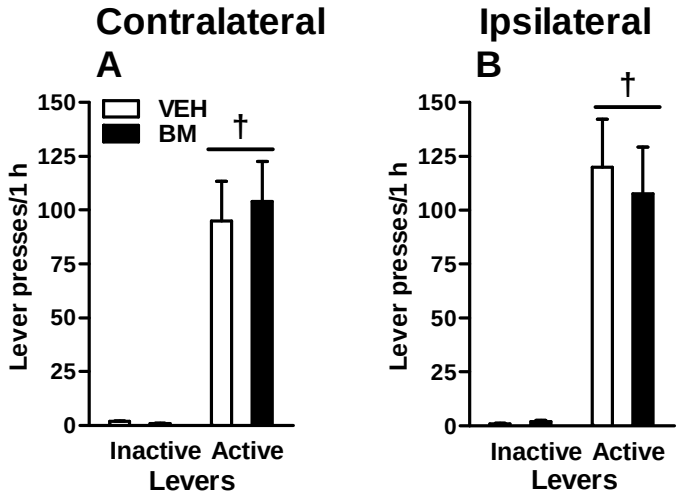


Fig 2.6 Bilateral inhibition of intrahemispheric or interhemispheric connections between the BLA and OFC fails to alter food-reinforced instrumental responding. The panels depict active and inactive lever responses (mean/1h + SEM) during testing in the food self-administration context. Immediately before testing, BM or VEH was infused unilaterally into the OFC plus the contralateral BLA (***A***) or the ipsilateral BLA (***B***). BM treatment did not alter food reinforced active or inactive lever responding relative to VEH treatment. *Daggers* represent significant difference relative to responding on the inactive lever (***A-B***, ANOVA lever main effect, active > inactive, $p<0.05$).

CHAPTER 3

DOPAMINE D1-LIKE RECEPTOR SIMULATION IN THE ORBITOFRONTAL CORTEX IS CRITICAL FOR DRUG CONTEXT-INDUCED COCAINE-SEEKING BEHAVIOR

INTRODUCTION

Extensive evidence suggests that the lateral OFC represents an integral part of the mesocorticolimbic neural circuitry that regulates the reinstatement of drug context-induced cocaine-seeking behaviors. Exposure to cocaine-paired conditioned stimuli elicits hyperactivity in the OFC of former cocaine users concomitant with increases in cocaine craving (Grant et al., 1996; Childress et al., 1999; London et al., 1999; Duncan et al., 2007). Similarly, cocaine-experienced rats exhibit enhanced neural activation in the OFC following exposure to a cocaine-paired context relative to exposure to an non-drug paired context (Neisewander et al., 2000; Hamlin et al., 2008; Hearing et al., 2008b; Hearing et al., 2008a). While prolonged loss of output from the OFC actually enhances drug context-induced cocaine-seeking behaviors, neural inactivation of the OFC prevents drug-paired cues or contexts from eliciting cocaine-seeking (Fuchs et al., 2004; Lasseter et al., 2009). Results from experiments in Chapter 2 established that interactions between the OFC and BLA regulate the motivational effects of a drug-paired context on conditioned behavior (Lasseter et al., 2011). However, the neuropharmacological mechanisms within the OFC that contribute to this phenomenon remain poorly understood.

Evidence suggests that dopaminergic neurotransmission in the OFC may be necessary for a drug-paired context to produce cocaine-seeking behavior. The VTA sends dense dopaminergic projections to regions of the OFC that are rich in dopamine D1 receptors (Berger et al., 1991; Dunnett and Robbins, 1992; Frankle et al., 2006; Reynolds et al., 2006; Sesack and Grace, 2010). In fact, D1 receptors are significantly more abundant than D2 receptors in the frontal cortices (Boyson et al. 1986; Dawson et al. 1986; Lidow et al. 1989), highlighting their importance in regulating neural activity. Furthermore, dopaminergic neurotransmission in the frontal cortices critically contributes to higher cognitive functions that likely facilitate cocaine-seeking behavior, including reward-related processing by the OFC (Ragozzino et al., 1999; Cetin et al., 2004; Dalley et al., 2004; Kheramin et al., 2004; Ward et al., 2009; Winter et al., 2009). Hence, blocking dopamine D1-like receptors in the OFC produces behavioral impairments, including inflexible responding during changing reward contingencies (Winter et al., 2009), reduced sensitivity for conditioned reinforcement on a progressive ratio schedule of food reinforcement (Cetin et al., 2004), and inability to update the motivational significance of reward-related stimuli under reversal conditions (Calaminus and Hauber, 2008). In particular, dopamine neurotransmission via the stimulation of dopamine D1 receptor populations in the OFC may contribute to some drug-seeking behaviors given that dopamine D1-like receptor antagonism in the OFC attenuates stress-induced cocaine-seeking behavior (Capriles et al., 2003). However, while systemic blockade of dopamine D1-like receptors impairs context-induced drug-seeking behaviors (Caggiula et al., 2001; Crombag et al., 2002; Liu and Weiss, 2002; Bossert et al., 2009), studies have not attempted to identify the specific dopamine receptor population that is involved.

Moreover, no study to date has assessed whether dopaminergic neurotransmission in the OFC is critical for a cocaine-paired context to elicit cocaine-seeking behavior.

Thus, experiments in Chapter 3 were designed to evaluate the hypothesis that dopamine regulates drug context-induced cocaine seeking via the stimulation of dopamine D1 receptors in the OFC. To this end, rats received bilateral microinfusions of the highly selective dopamine D1-like receptor antagonist SCH23390 or VEH into the OFC immediately before re-exposure to a cocaine-paired or non-cocaine-paired context. Rats in an anatomical control experiment received similar treatment into the primary and secondary motor cortices (MC, anatomical control brain region) that are dorsally adjacent to the OFC. To discriminate between impairments in motivation versus motor performance, we also assessed the effects of intra-OFC SCH23390 treatment on locomotor behavior and on food-reinforced instrumental behavior. Overall, we predicted that SCH23390 treatment administered into the OFC, but not the MC, would dose-dependently attenuate drug context-induced cocaine seeking relative to VEH treatment without altering motor performance in the control experiments.

METHODS

Animals

Male Sprague-Dawley rats (n = 27, 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 Rats* (Institute of Laboratory Animal Resources on Life Sciences, 1996) and were

approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill

Food training

To expedite cocaine self-administration training, all rats (n = 27) were trained to lever press under a continuous schedule of food reinforcement (45 mg pellets; Noyes, Lancaster, NH, USA) in sound-attenuated operant conditioning chambers (26 x 27 x 27 cm high; Coulbourn Instruments, Allentown, PA, USA) during a 16-h overnight session. Two levers were present in the chamber, located on either side of a wall-mounted food tray. Each response on one lever (active lever) resulted in the delivery of one food pellet only; responses on the other lever (inactive lever) had no programmed consequences. During food training, contextual stimuli subsequently used for cocaine conditioning were not present.

Surgery

Forty-eight h after food training, all rats were fully anesthetized using ketamine hydrochloride and xylazine (66.6 and 1.33 mg/kg, i.p., respectively). Chronic indwelling jugular catheters were constructed in house and were surgically implanted into the right jugular vein of a subset of rats (n= 19), as described previously (Fuchs et al., 2007). All rats were stereotaxically implanted with stainless-steel guide cannulae (26 gauge; Plastics One) aimed bilaterally at the OFC (+3.5 mm AP, +/-3.0 mm ML, -3.4 DV) or the MC anatomical control region (+3.5 mm AP, +/-3.0 mm ML, -1.4 DV) using standard procedures. Stainless steel screws and cranioplastic cement secured the guide cannulae to

the skull. Stylets (Plastics One) and Tygon caps sealed the guide cannulae and catheter, respectively. To extend catheter patency, the catheters were flushed daily with an antibiotic solution of cefazolin (10.0 mg/ml; Schein Pharmaceuticals, Albuquerque, NM, USA) and heparinized saline (70 U/ml; Baxter Health Care Corp, Deerfield, IL, USA), as described previously (Fuchs et al., 2007). Rats were given a 5-day post-operative recovery period before the start of the experiment. Catheter patency was evaluated periodically using propofol (1mg/0.1ml, i.v. Eli Abbott Lab, North Chicago, IL, USA), which produces a rapid loss of muscle tone only when administered intravenously.

Cocaine self-administration training

The rats were randomly assigned to receive daily 2h cocaine self-administration training sessions during their dark cycle. Training occurred in operant conditioning chambers configured to one of two unique environmental contexts (Context A, Context B) as previously described. Responses on the active lever were reinforced under an FR1 schedule of cocaine reinforcement (cocaine hydrochloride; 0.15 mg/infusion, equivalent to ~0.5 mg/kg/infusion based on body weight; i.v; NIDA, Research Triangle Park, NC, USA). A 20s time-out period followed each 2s infusion. During the time-out period, lever responses were recorded, but had no programmed consequences, i.e. no response-contingent cues were presented. Responses on the inactive lever were recorded but had no programmed consequences. Training continued until the rats obtained $\geq$ 10 cocaine infusions/session on at least 10 training days (i.e., acquisition criterion).

Extinction Training

After meeting the acquisition criterion, rats received daily 2h extinction training sessions in the context (Context A or B) that distinctly differed from the cocaine self-administration training context. Lever presses were recorded, but had no programmed consequences. Extinction training consisted of a minimum of 7 sessions plus additional sessions, as needed, until the rats reached the extinction criterion (≤ 25 active lever presses/session during two consecutive sessions). Immediately prior to the behavioral session on extinction day 4, rats were acclimated to the intracranial infusion procedure. To this end, injection cannulae (33 Ga, Plastics One) were inserted into the rats' guide cannulae to a depth of 2 mm below the tip of the guide cannulae in the OFC-cannulated and MC-cannulated groups. Injection cannulae were left in place for 4 minutes but no drug was infused.

Reinstatement Testing

After the rats reached the extinction criterion, cocaine-seeking behavior was assessed in the cocaine-paired and extinction contexts during four test sessions (**Fig 3.1A**). Immediately prior to testing, rats received bilateral microinfusions of the dopamine D1-like receptor antagonist, SCH23390 (OFC: 0.02 or 0.2 ug/0.5 ul per hemisphere; MC: 0.2 ug/0.5 ul per hemisphere), or phosphate-buffered saline VEH (OFC or MC: 0.5 µl/hemisphere). The doses of SCH23390 were informed by previous findings that a 0.25ug dose of SCH23390 in the OFC prevented foot shock-induced cocaine-seeking behavior without altering instrumental responding for sucrose reinforcement (Capriles et al., 2003). The intracranial infusions were delivered over 2 min, and the injection

cannulae were left in place for 1 min before and 1 min after the infusion. Immediately after the infusions procedure, rats were placed into the cocaine-paired or extinction context to assess lever responding, although lever responding had no programmed consequences. Session length was 1 h to allow for repeated testing without significant extinction learning occurring. The order of testing in the two contexts (cocaine-paired context, extinction context) and the order of intracranial treatments (0.02 µg or 0.2µg SCH23390; VEH) were counterbalanced based on mean active lever responding during the last three cocaine self-administration training days. Subjects received additional extinction training sessions in the extinction context between test sessions until they re-obtained the extinction criterion (see above).

Locomotor activity and food-reinforced instrumental behavior

Intracranial manipulations can produce non-specific motor deficits that impair instrumental performance. Hence, the effects of intra-OFC SCH23390 and VEH treatment on general locomotor activity and food-reinforced instrumental behavior were examined.

Locomotor Activity

Locomotor activity was assessed during two 1-h test sessions held 5 days apart, starting 48 h after the last reinstatement test session. Locomotor activity was measured in novel Plexiglas chambers (42 x 20 x 20 cm) equipped with an array of eight photodetectors and corresponding light sources. Prior to testing, rats received intracranial microinfusions of the behaviorally effective dose of SCH23390 (0.2 ug/0.5 ul per hemisphere) or VEH into

the OFC using the infusion procedures and treatment order applied in the reinstatement experiment. A computerized activity system (San Diego Instruments, San Diego, CA) recorded the number of consecutive photobeams interrupted by rats moving in the activity chamber during each test session.

Food-reinforced Instrumental Responding

Food-reinforced instrumental responding was assessed in a separate group of experimentally naïve rats (n=9) using a full within-subjects design in order to examine the effects of intra-OFC SCH23390 treatment on instrumental responding (**Fig 3.1B**). After overnight food training, stereotaxic surgery, and post-operative recovery (described above), the rats received additional daily 2h food self-administration training sessions in Context A or B until responding stabilized (i.e., ≤ 20% variability in active lever responding across two consecutive sessions), using previously described methods (Xie *et al*., 2010). Thereafter, three 1-h test sessions were conducted. Immediately before the test sessions, rats received SCH23390 (0.02 or 0.2 ug/0.5 ul per hemisphere) or VEH bilaterally into the OFC using the infusion procedure described above. Intracranial treatment order was counterbalanced across the three test sessions based on mean active lever responding during the last two food self-administration training sessions. During the training and test sessions, active lever presses were reinforced under an FR1 schedule of food reinforcement (45 mg, Purina) with a 20-s timeout period. Inactive lever presses were recorded, but had no programmed consequences. Between the test sessions, rats received a minimum of two additional food self-administration training sessions to re-establish baseline responding.

Histology

After the last experimental session, rats were overdosed using ketamine hydrochloride and 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, stored in 10% formaldehyde solution, and then sectioned at a thickness of 75 µm using a vibratome. The sections were stained using cresyl violet (Kodak, Rochester, NY, USA). Cannula placement was determined using light microscopy and was mapped onto schematics from the rat brain atlas of Paxinos and Watson (1997).

Data analysis

Only data from rats with correct cannula placements were included in the data analysis. Potential pre-existing differences between the treatment groups in (a) lever responses and cocaine intake during the last three days of self-administration training, (b) lever responses during the first seven days of extinction training, and (c) the number of days needed to reach the extinction criterion were analyzed using mixed factors ANOVAs with surgery group (OFC VEH and 0.02µg SCH23390 dose, OFC VEH and 0.2µg SCH23390 dose, MC VEH and 0.2µg SCH23390 dose) and subsequent treatment order (SCH23390 first, VEH first) as between-subjects factors and time (day) as the within-subjects factor, where appropriate.

To determine whether the vehicle data can be collapsed in the OFC-cannulated groups, non-reinforced active and inactive lever presses on the vehicle test days were

analyzed separately using mixed factors ANOVAs with additional treatment (SCH23390 0.02 µg, SCH233900.2 µg) and test order (extinction first, cocaine-paired first) as between subjects factors and testing context (extinction, cocaine-paired) as the within-subjects factor. To assess the effects of intra-OFC SCH23390 on the test days, active and inactive lever responses were analyzed separately using mixed factors ANOVAs with drug treatment (SCH23390 0.02µg, SCH23390 0.2ug, VEH) as the between-subjects factor and testing context (extinction, cocaine-paired) as the within-subjects factor. To assess the effects of intra-MC SCH23390 on the test days, active and inactive lever responses were analyzed separately using mixed factors ANOVAs with drug treatment (SCH23390 0.2ug, VEH) as the between-subjects factor and testing context (cocaine-paired context, extinction context) as the within-subjects factor.

The number of photobeam breaks during the locomotor activity tests were assessed separately using a repeated-measures ANOVA with drug treatment (SCH23390 0.2 ug, VEH) and time (20-min intervals) as within-subjects factors. Food-reinforced instrumental responding was assessed using a repeated measures ANOVA with drug treatment (SCH23390 0.02 ug, SCH23390 0.2 ug, VEH) and lever (active, inactive) as within-subjects factors. Significant main and interaction effects were investigated using simple main effects tests or Tukey *post hoc* tests, when appropriate. Alpha was set at 0.05. Only statistically significant effects are reported below.

RESULTS

Histology

Photomicrographs of representative cannula placements as well as schematics of the distribution of cannula placements are provided in **Fig 3.2**. The target brain regions were defined as the lateral and ventrolateral subregions of the OFC and the dorsally adjacent primary and secondary motor cortex (MC). High power microscopy confirmed that there was no evidence of abnormal tissue damage (i.e., extensive cell loss or gliosis) at the infusion sites. Data from rats with misplaced cannulae were excluded from the data analysis. For the remaining rats, the most ventral point of the injection cannula tracts were correctly located bilaterally within the OFC (VEH and SCH23390 0.02µg, n = 8; VEH and SCH23390 0.2µg, n = 10, food, n = 8) or the MC (n = 9).

Self-Administration Responding

Both the OFC- and MC-cannulated groups exhibited stable active lever responding for cocaine reinforcement over the last three days of cocaine self-administration training, with a within-subjects variability of <10% in daily cocaine intake. There was no difference between the subsequent treatment groups (OFC VEH and SCH23390 0.02 µg, OFC VEH and SCH23390 0.2 µg, MC) in active or inactive lever responding during the last three days of cocaine self-administration training (all group and day main and interaction effects, $F_{2\text{-}4,\ 24\text{-}48} = 0.25\text{-}1.92$, $P = 0.10\text{-}0.78$). Collapsed across groups, the mean active and inactive lever responding ± SEM was 51.67 ± 3.80 and 4.01 ± 1.28, respectively, and the mean daily cocaine intake ± SEM was approximately 10.44 ± 0.52 mg/kg per session based on body weight (20.88 ± 1.03 infusions) (data not shown).

Separate ANOVAs for each subsequent treatment group (OFC VEH and SCH23390 0.02 µg, OFC VEH and SCH23390 0.2 µg, MC) did not reveal significant effects of subsequent treatment order (SCH23390 first, VEH first) on these measures (data not shown).

Separate ANOVAs for each subsequent treatment group (OFC VEH and SCH23390 0.02µg, OFC VEH and SCH23390 0.2µg, MC) further indicated no pre-existing differences in active or inactive lever responding during the last three self-administration training days as a function of subsequent treatment order (SCH23390 first, VEH first) (all treatment order and day main and interaction effects, $F_{1\text{-}2,\ 6\text{-}16} = 0.01\text{-}2.30$, $P = 0.13\text{-}0.80$). Similarly, separate ANOVAs for the OFC-cannulated groups (OFC 0.02g, OFC 0.2µg) revealed no pre-existing differences in cocaine intake as a function of subsequent treatment order (all treatment order and day main effects and interactions, $F_{1\text{-}2,\ 6\text{-}16} = 0.03\text{-}1.98$, $P = 0.18\text{-}0.87$). The MC-cannulated group that subsequently received VEH first during reinstatement testing had slightly higher cocaine intake than the group that subsequently received SCH23390 first (treatment order main effect, $F_{1,7} = 17.65$, $P = 0.004$; VEH first, 11.04 ± 0.38 mg/kg per session; SCH23390 first, 8.84 ± 0.36 mg/kg per session, Tukey test, $P < 0.05$). However, this did not correspond with treatment order effects on lever responding during subsequent extinction and reinstatement test sessions.

Extinction Responding

Upon removal of cocaine reinforcement during extinction training, separate ANOVAs for active and inactive lever responding by the subsequent treatment groups (OFC VEH and SCH23390 0.02µg, OFC VEH and SCH23390 0.2µg, MC) indicated that

responding gradually declined on the active (day main effect, $F_{6, 144} = 5.57$, $P = 0.001$; day 1 > day 2-7, Tukey test, $P < 0.01$) and inactive levers (day main effect, $F_{6, 144} = 6.08$, $P = 0.001$; day 1 > day 5-7, Tukey test, $P < 0.05$). There were no differences between the subsequent treatment groups in active lever responding during the first seven days of extinction training (group and day main and interaction effects, $F_{1\text{-}12, 2\text{-}144} = 0.83\text{-}0.93$, $P = 0.44\text{-}0.52$). However, the MC and OFC SCH23390 0.02 µg groups exhibited more inactive lever presses than the OFC 0.02 µg group on the first day of extinction training (group X day interaction, $F_{12,144} = 5.98$, $P = 0.012$; Tukey test, $P<0.05$) after which subsequent treatment effects were not observed. Finally, there was no difference in the mean number of days ± SEM required to reach the extinction criterion (7.15 ± 0.10) based on subsequent treatment group (OFC VEH and SCH23390 0.02 µg, OFC VEH and SCH23390 0.2 µg, MC; $F_{2,24} = 2.82$, $P = 0.08$). Separate ANOVAs for each subsequent treatment group did not reveal significant effects of subsequent treatment order (SCH23390 first, VEH first) on these measures (data not shown). Hence, it is unlikely that pre-existing differences during extinction training accounted for group differences in reinstatement responding during the subsequent test sessions.

Separate ANOVAs for each subsequent treatment group (OFC VEH and SCH23390 0.02 µg, OFC VEH and SCH23390 0.2 µg, MC) further explored whether there were pre-existing differences in lever responding based on subsequent treatment order during reinstatement testing. In the OFC VEH and SCH23390 0.02 µg and MC groups, there were no pre-existing differences in active lever responding during extinction as a function of subsequent treatment order (all treatment order and day main and interaction effects; $F_{1\text{-}6, 6\text{-}42} = 0.21\text{-}1.46$, $P = 0.27\text{-}0.66$). However, rats in the OFC VEH and SCH23390 0.2

µg group that received VEH first during subsequent reinstatement testing exhibited more active lever responding on extinction day 1 than rats that received SCH23390 first (treatment order X day interaction, $F_{6,48} = 14.64$, $P < 0.000$; Tukey test, $P < 0.01$; day main effect, $F_{6,48} = 6.94$, $P < 0.000$) after which subsequent treatment order effects were not observed. Separate ANOVAs for each group (OFC VEH and SCH23390 0.02µg, OFC VEH and SCH23390 0.2µg, MC) revealed no differences in inactive lever responding as a function of subsequent treatment order (all treatment order and day main and interaction effects, $F_{1\text{-}6,6\text{-}48} = 0.01\text{-}2.29$, $P = 0.05\text{-}0.91$).

Site-specific Effects of SCH23390 Treatment on Drug Context-induced Reinstatement of Cocaine-seeking Behavior

Exposure to the previously cocaine-paired context reinstated active lever responding in rats following intracranial VEH pretreatment administered into the OFC or MC (**Fig 3.3**) regardless of test order (extinction or cocaine-paired context first), treatment order (VEH or SCH23390 first), or treatment history (SCH23390 0.02 ug or 0.2 ug on the other test day) (data not shown). Therefore, data were collapsed across test order, treatment order, and treatment history to create a single VEH condition, and treatment was treated as a between-subjects factor in all the subsequent statistical analyses.

Effects of Intra-OFC SCH23390 Treatment on Drug Context-induced Reinstatement of Cocaine-seeking Behavior

Intracranial infusions of SCH23390 into the OFC altered drug context-induced reinstatement in a dose-dependent manner (**Fig 3.3A**). Following VEH pretreatment, re-exposure to the previously cocaine-paired context enhanced active lever responding

relative to the extinction context (treatment X context interaction, $F_{2,35} = 8.17$, $P = 0.000$; context main effect, $F_{1,3)} = 63.87$, $P = 0.001$; treatment main effect, $F_{2,35} = 8.44$, $P = 0.001$). The 0.02 dose of SCH23390 failed to alter active lever responding in the cocaine-paired context or extinction context relative to VEH. Conversely, the 0.2ug dose of SCH23390 significantly attenuated active lever responding in the cocaine-paired context (Tukey test, $P < 0.01$), without altering responding in the extinction context, relative to VEH. As a result, following pretreatment with the 0.2µg dose of SCH23390, there was no difference in active lever responding in the cocaine-paired and extinction contexts.

Following VEH pretreatment, re-exposure to the cocaine-paired context failed to alter inactive lever responding relative to the extinction context (**Fig 3.3C**; context main effect, $F_{1,27} = 0.328$, $P = 0.571$). Furthermore, neither the 0.02ug nor the 0.2ug dose of SC23390 altered inactive lever responding in either the cocaine-paired context or the extinction context (treatment X context interaction, $F_{2,35} = 2.771$; $p = 0.076$; treatment main effect, $F_{2,35} = 0.731$; $P = 0.489$) relative to VEH.

Effects of Intra-MC SCH23390 Treatment on Drug Context-induced Reinstatement of Cocaine-seeking Behavior

Re-exposure to the previously cocaine-paired context following intra-MC vehicle pretreatment reinstated active lever responding relative to the extinction context (**Fig 3.3B;** context main effect, $F_{1,8} = 42.18$, $P = 0.00$). Furthermore, intra-MC infusions of the behaviorally effective dose of SCH23390 (0.2 ug per hemisphere) did not alter active lever responding relative to VEH in either context (treatment X context interaction, $F_{1,8} = 0.26$, $P = 0.62$; treatment main effect, $F_{1,8} = 0.37$, $P = 0.56$). Re-exposure to the cocaine-

paired context following intra-MC vehicle pretreatment did not alter responding on the inactive lever relative to the extinction context (**Fig 3.3D**; context main effect, $F_{1,8} = 5.03$, $P = 0.06$). Furthermore, SCH23390 at the 0.2ug dose did not alter inactive lever responding relative to VEH in either context (treatment X context interaction, $F_{1,8} = 0.90$, $P = 0.37$; treatment main effect, $F_{1,8} = 1.26$, $P = 0.26$).

Effects of Intra-OFC SCH23390 Treatment on Locomotor Activity

Intra-OFC administration of the behaviorally effective dose of SCH23390 (0.2 ug per hemisphere) failed to alter locomotor activity relative to VEH (**Fig 3.4A**). The number of photobeam breaks decreased across the three 20-min intervals of the 1-h locomotor test session (time main effect, $F_{2,20} = 28.29$,$P = 0.0001$; interval 1 > intervals 2-3; Tukey test, $P < 0.01$). SCH23390 at the 0.2ug dose did not alter the number of photobeam breaks relative to VEH (all treatment and time main and interaction effects, Fs ≤ 1.09, $P \geq 0.32$).

Effects of Intra-OFC SCH23390 Treatment on Food-reinforced Instrumental Behavior

Intra-OFC administration of SCH23390 failed to alter food-reinforced instrumental responding relative to VEH pretreatment (**Fig 3.5**). Active lever responding was significantly greater than inactive lever responding during the food-reinforced test sessions (lever main effect, $F_{1,17} = 17.32$, $P = 0.004$). Furthermore, intra-OFC SCH23390 pretreatment at either the 0.02ug or the 0.2µg dose did not alter food-reinforced active or inactive lever responding relative to VEH pretreatment (treatment main and lever main and interaction effects, all Fs < 0.06, $P > 0.69$).

DISCUSSION

Experiments in Chapter 3 explored whether the stimulation of dopamine D1 receptor populations in the OFC makes a fundamental contribution to drug context-induced motivation for cocaine. To evaluate this question, rats received intra-OFC infusions of the highly-selective dopamine D1-like receptor antagonist, SCH23390, prior to testing for drug context-induced cocaine-seeking behavior (**Fig. 3.3A**). Exposure to the cocaine-paired context significantly enhanced active lever responding relative to the extinction context. Furthermore, bilateral microinfusions of the 0.2µg – but not the 0.02µg – dose of SCH23390 into the OFC significantly attenuated drug context-induced cocaine-seeking behavior relative to VEH. While D1-like dopamine receptor antagonists have been shown to produce motor impairments (Fowler and Liou, 1994), the dose-dependent effects of intra-OFC SCH23390 treatment on drug context-induced cocaine seeking were not likely to reflect non-specific deficits in instrumental motor performance. Consistent with this, intra-OFC SCH23390 treatment failed to alter active lever responding in the extinction context (**Fig 3.3A**), inactive lever responding in either context (**Fig 3.3A/C**), general motor activity in a novel context (**Fig 3.4**), or food-reinforced instrumental behavior (**Fig 3.5**), similar to the lack of effects of intra-OFC SCH23390 treatment on responding for sucrose reinforcement in an earlier study (Capriles et al., 2003). The ability of SCH23390 to attenuate drug context-induced cocaine-seeking behavior was also anatomically-specific to the lateral OFC. In this respect, we have previously demonstrated that the OFC is functionally heterogeneous in its contribution to drug context-induced cocaine seeking in that GABA agonist-induced functional inactivation of the lateral, but not the medial, OFC prevents a cocaine-paired context from eliciting

cocaine-seeking behavior (Lasseter et al., 2009). Furthermore, bilateral infusions of the behaviorally effective dose of SCH23390 into the MC – a region dorsally adjacent to the lateral OFC and thus in the most likely path of unintended SCH23390 diffusion along the cannula tract (Neisewander et al., 1998) – did not alter drug context-induced cocaine-seeking behavior (**Fig 3.3B**). This latter finding is consistent with previous studies indicating that dopamine D1 receptor stimulation in the MC is critical for the acquisition, but not the expression, of reward-related motor behaviors (Luft and Schwarz, 2009; Molina-Luna et al., 2009; Hosp et al., 2011). Taken together, these results provide the first evidence that dopamine D1-like receptor stimulation in the OFC is necessary for the motivational effects of a drug-paired environmental context on goal-directed behavior. These results are in concert with previous findings that the OFC is part of the mesocorticolimbic neurocircuitry that regulates drug-paired CS- and context-induced cocaine-seeking behaviors (Fuchs et al., 2004; Lasseter et al., 2009; Lasseter et al., 2011) and complement evidence that dopamine in the OFC is necessary for the reinstatement of stress-induced cocaine seeking (Capriles et al., 2003).

The OFC is thought to contribute to reward-related behaviors by maintaining an internal representation of a reward's motivational value and updating this information in the face of changing outcome-expectancies in order to guide behavior. Based on its pattern of anatomical connectivity, the OFC is well-positioned to integrate sensory information from the primary sensory cortices with reward-related information from mesocorticolimbic brain regions in order to guide goal-directed behaviors (Price, 1986; Carmichael and Price, 1995b; Carmichael and Price, 1995a; McDannald et al., 2004). Accordingly, individual neurons within the OFC exhibit cue-specific firing as a function

of the sensory properties or of the predicted reward value of a CS and alter their activity during changing stimulus-reward contingencies (Rolls et al., 1996; Schoenbaum et al., 1998; Schultz et al., 1998; Schultz et al., 2000; Tremblay and Schultz, 2000; Saddoris et al., 2003; Schoenbaum et al., 2009; Takahashi et al., 2009). In preclinical studies, damage to the OFC preferentially impairs responding when task performance depends on the ability to update information about the value of predicted outcomes. For example, OFC lesions impair performance following reinforcer devaluation, inhibits rapid reversal learning, and produce perseverative responding for a conditioned reinforcer (Gallagher et al., 1999; Pickens et al., 2003; Izquierdo et al., 2004; Izquierdo and Murray, 2005; Izquierdo et al., 2005). Hence, the OFC may promote drug context-induced cocaine seeking by representing the motivational significance of drug-paired environmental stimuli.

Evidence further suggests that dopamine input from the VTA to the OFC facilitates the ability of the OFC to maintain the value of reward-related conditioned stimuli. Preclinical studies have demonstrated that dopaminergic neurotransmission in the OFC is necessary for behavioral responding maintained by conditioned reinforcement. Consistent with this, dopamine utilization is increased in the OFC during presentation of reward-predictive conditioned stimuli in a delay discounting task (Winstanley et al., 2006). Dopamine D1-like receptor antagonism in the OFC also increases impulsive decision making in this task, but only when a CS is presented to bridge the gap between response selection and reward delivery (Zeeb et al., 2010). From a neurochemical perspective, the dynamics of dopamine neurotransmission in the OFC synapse may be particularly well suited for encoding the value of conditioned stimuli.

Unlike in the striatum and many other brain regions, synapses in prefrontal cortical areas contain relatively low density of dopamine transporters and are characterized by prolonged increase in dopamine concentration following cue-induced dopamine release (Sesack et al., 1998; Garris et al., 1993). This protracted elevation in extracellular dopamine levels may permit the OFC to hold and manipulate information that is necessary to direct behavioral responding (Seamans and Yang, 2004). Additionally, dopamine neurotransmission in the OFC may play a neuromodulatory role by either suppressing or enhancing the effects of other neurotransmitters, such as GABA, serotonin, or glutamate (Kiyatkin and Rebec, 1996b; Kiyatkin and Rebec, 1996a; Floresco et al., 1998; Seamans et al., 2001a; Wang and O'Donnell, 2001).

In particular, dopamine may create a robust motivational representation of drug-paired contextual stimuli by facilitating strong glutamatergic inputs to the OFC while simultaneously inhibiting weaker inputs (Cepeda et al., 1998; Schultz, 2002; Seamans et al., 2003; Lapish et al., 2006). Dopamine D1 receptors are located in close proximity to ionotropic glutamate receptors at asymmetric synapses on the dendritic spines of pyramidal neurons (Seamans and Yang, 2004; Paspalas and Goldman-Rakic, 2005) and dopamine and D1 agonists have been shown to potentiate NMDA- and AMPA-mediated excitatory post-synaptic potentials in the prefrontal cortices (Liu et al., 2011; Seamans et al., 2001b; Gonzalez-Islas and Hablitz, 2003). Hence, dopamine release in the OFC may increase the salience of the drug-paired context by enhancing the effects of glutamatergic input from other brain regions known to contribute to drug context-induced cocaine-seeking behaviors. Such critical glutamatergic input may come from the dorsomedial PFC or BLA given that the functional integrity of these brain regions is necessary for

drug context-induced cocaine-seeking behavior and that the OFC and BLA exhibit functional interactions in the control of cocaine seeking (Fuchs et al., 2005; Lasseter et al., 2011). Interestingly, the PFC – including the OFC – is the only cortical area that provides dense projections to the VTA. These fibers synapse onto the same dopamine neurons that project back to the OFC as well as onto GABAergic VTA neurons that project to the NA (Campbell et al., 1999; Carr and Sesack, 2000), a structure that is implicated in the execution of cocaine-seeking behavior (Bossert et al., 2007; Fuchs et al., 2008a). Therefore, future studies will be necessary to parse out the precise neural mechanisms by which dopamine D1 receptor stimulation in the OFC promotes drug context-induced cocaine seeking as well as to explore the larger neural circuitry within which the OFC and VTA may interact to direct the motivational effects of a drug-paired context on conditioned goal-directed behavior.

It is possible that SCH23390 treatment in the OFC impairs drug context-induced cocaine seeking by a mechanism other than dopamine D1-like receptor antagonism. Based on its receptor affinities, SCH23390 is considered to be the pharmacological gold standard for differentiating between the pharmacological effects mediated by dopamine D1-like (K_D ~0.4nM) versus D2-like (K_D ~631nM) receptors (Bourne, 2001). Thus, SCH23390 has been routinely used to assess the contribution of dopamine D1-like receptor signaling to the reinstatement of drug-seeking behaviors (Caggiula et al., 2001; Alleweireldt et al., 2002; Sun and Rebec, 2005; Berglind et al., 2006; Bossert et al., 2007; Bossert et al., 2009). However, SCH23390 also exhibits moderate affinity for the serotonin receptor 2c (5-HT2c) subtype (K_D ~20nM) *in vitro* and acts as a 5-HT2c receptor *agonist* (Rupniak et al., 1986; Kalkman et al., 1998). 5-HT2c receptors co-

localize with dopamine receptors in the same cortical layers of the rat OFC that contain the greatest dopamine D1 receptor density (Pazos et al., 1985; Lidow et al., 1989). Furthermore, systemic administration of 5-HT2c receptor agonists attenuates explicit cue-induced reinstatement and context-induced renewal of cocaine-seeking behaviors (Neisewander and Acosta, 2007; Fletcher et al., 2008). Hence, SCH23390 may exert its behavioral effects, at least in part, via 5-HT2c receptor stimulation. Unfortunately, performing agonist-antagonist experiments – for instance co-administering SCH23390 with a D1 receptor agonist or a 5-HT2c receptor antagonist – cannot adequately address this question given that dopamine and serotonin receptor-mediated signaling mechanisms in the OFC may co-regulate drug context-induced reinstatement. Furthermore, either a D1 agonist or a 5-HT2c antagonist may be sufficient to reinstate lever responding independent of re-exposure to the drug-paired context (Burmeister et al., 2004; Bachtell et al., 2005; Mashhoon et al., 2009). Thus, this and similar questions will have to be re-investigated in future studies, pending the availability of new test compounds with significantly greater affinity for D1-like receptors relative to 5-HT2c receptors.

In summary, SCH23390 pretreatment in the OFC produced profound attenuation in drug context-induced cocaine seeking in the current study. This highlights the role that the OFC plays in regulating the motivational effects of drug-paired stimuli on goal-directed instrumental responding and provides evidence that dopamine D1 receptor-mediated stimulation in the OFC, alone or in combination with 5-HT2c receptor-mediated stimulation, is necessary for this behavior. These findings are important for informing our understanding about the neurobiological mechanisms of drug relapse.

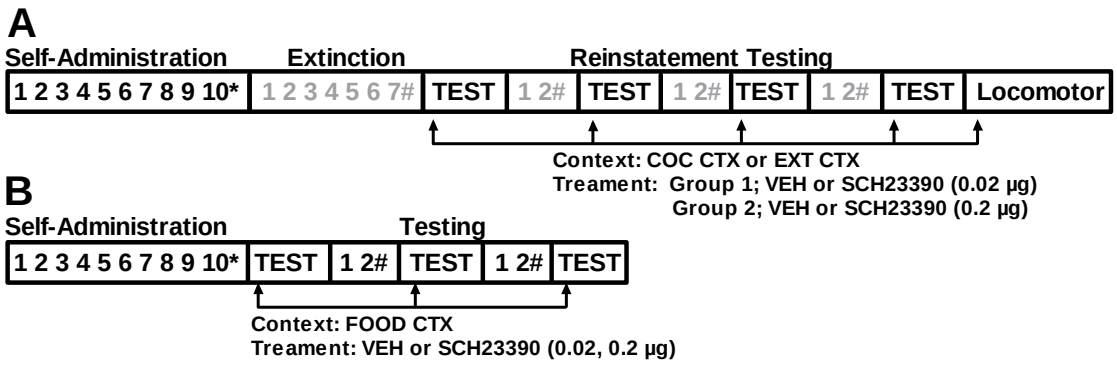


Fig 3.1 Schematic representation of the timeline for the drug context-induced reinstatement experiments (***A***) and the food-maintained instrumental control experiments (***B***). Arrows on the schematics identify sessions in which VEH or SCH23390 was administered immediately prior to testing. During reinstatement testing, the order of context exposure (COC CTX; EXT CTX) and the order of drug treatment (group 1: VEH, SCH23390 0.02µg, group 2: VEH, SCH23390 0.2µg) was counterbalanced. In the control experiments, the order of drug treatment was fully counterbalanced (VEH, SCH23390 0.02 µg, SCH23390 0.2µg). *Asterisks* indicate that the rats had to reach an acquisition criterion (≥ 10 infusions per session for minimum 10 sessions) to complete self-administration training and had to satisfy our extinction criterion (≤ 25 active lever presses per session for two consecutive sessions) before each test session. *Pound signs* indicate that rats had to reach a stability criterion (≤ 10% variability in active lever presses for two consecutive sessions) before each test session.

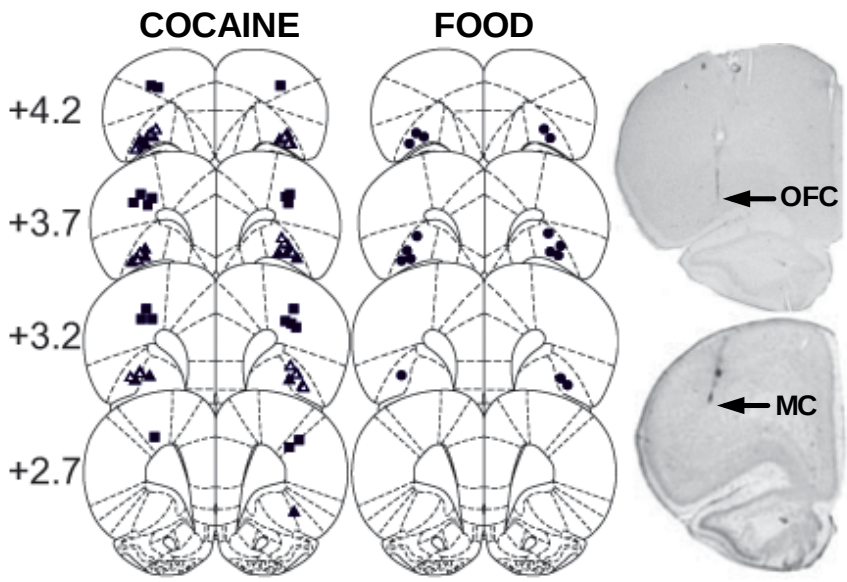


Fig 3.2 Schematic and photographic representation of injection cannula placements. The *arrows* on the photomicrographs identify the most ventral point of the infusion cannula tracts on representative cresyl violet-stained brain sections. The symbols on the schematics (Paxinos and Watson, 1997) represent the most ventral point of the infusion cannula tracts for rats that received bilateral microinfusions into the OFC (cocaine self-administration: *open triangles* – VEH /SCH23390 0.02µg, *closed triangles* – VEH/SCH23390 0.2 µg; food-maintained responding: *closed circles* – VEH/SCH23390 0.2 µg,) or into the MC (VEH/SCH23390 0.2 µg, *closed squares*). Numbers indicate the distance from bregma in millimeters.

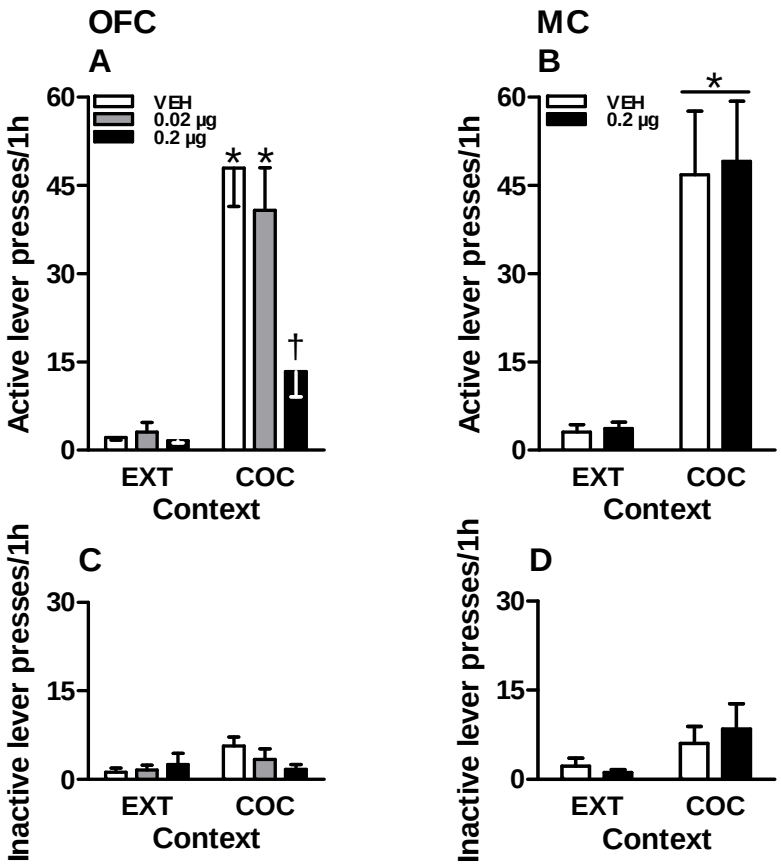


Fig 3.3 SCH23390 administration dose-dependently attenuates drug context-induced reinstatement of cocaine-seeking behavior when administered bilaterally into the OFC but not the overlying MC anatomical control brain region. The panels depict non-reinforced active and inactive lever responses (mean/1h ± SEM) during testing in the extinction context (EXT context) and the previously cocaine-paired context (COC context). Immediately before testing, VEH or SCH23390 (0.02 µg or 0.2 µg) was infused bilaterally into the OFC (***A,C***) or into the MC (***B,D***). *Asterisks* represent significant difference relative to responding in the extinction context (***A****:* ANOVA context simple main effect, $p < 0.05$; ***B:*** ANOVA context main effect, $p < 0.05$). *Dagger* represents significant difference relative to VEH pretreatment (***A:*** ANOVA treatment simple main effect, $p < 0.05$).

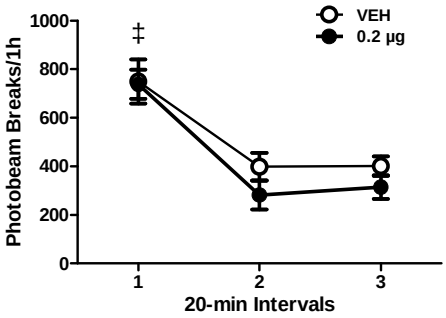


Fig 3.4 SCH23390 administration into the OFC does not alter general motor activity. Photobeam breaks (mean 1h ± SEM) were triggered by the movement of subjects in a novel context during a 1-h locomotor activity test. Immediately before testing, groups received VEH or SCH23390 infusions bilaterally into the OFC. *Double dagger* represents a significant difference relative to all other time points (ANOVA time simple main effect, interval 1> intervals 2-3, $p < 0.05$).

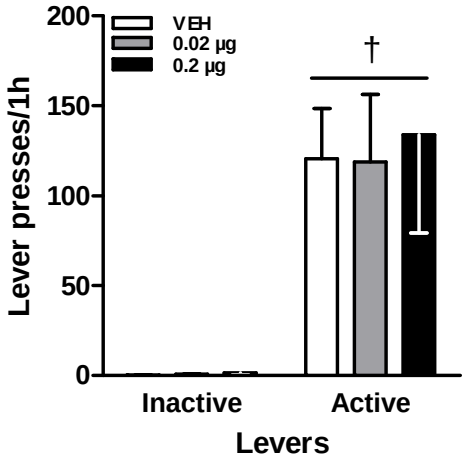


Fig 3.5 SCH23390 administration into the OFC does not alter food-reinforced instrumental responding. The panels depict active and inactive lever responses (mean/1h ± SEM) during testing in the food self-administration context. Immediately before testing, groups received VEH or SCH23390 infusions bilaterally into the OFC. SCH23390 treatment did not alter active or inactive lever responding relative to VEH treatment. *Dagger* represents a significant difference in responding relative to responding on the inactive lever (ANOVA lever main effect, $p < 0.01$).

CHAPTER 4

A MESOCORTICOLIMBIC CIRCUITRY CONSISTING OF THE VENTRAL TEGMENTAL AREA, ORBITOFRONTAL CORTEX, AND BASOLATERAL AMYGALA REGULATES THE MOTIVATIONAL EFFECTS OF A DRUG-PAIRED CONTEXT ON COCAINE-SEEKING BEHAVIOR

INTRODUCTION

The lateral OFC and BLA represent regions of the mesocorticolimbic neural circuitry that regulate the motivational effects of a drug-paired context on cocaine-seeking behavior. Exposure to cocaine-paired stimuli elicits enhanced neural activity in the OFC and BLA in abstinent cocaine users concomitant with increases in cocaine craving (Grant et al., 1996; Childress et al., 1999; London et al., 1999; Duncan et al., 2007), whereas neural inactivation of either the OFC or the BLA prevents the reinstatement of cocaine-seeking behavior in laboratory animals (See et al., 2001a; Kantak et al., 2002; McLaughlin and See, 2003; See et al., 2003; Fuchs et al., 2004; Fuchs et al., 2005; Lasseter et al., 2009). Findings from experiments in Chapter 2 further indicate that the OFC and BLA display obligatory functional interactions in the control of drug context-induced motivation for cocaine such that disrupting either inter- and intrahemispheric interactions between these brain regions attenuates drug context-induced cocaine seeking (Lasseter et al., 2011). However, the larger neural circuitry within which the OFC and BLA interact to promote drug context-induced cocaine seeking remains to be ascertained.

Importantly, dopaminergic neurotransmission in the frontal cortices critically regulates higher cognitive functions, including reward-related processing by the OFC,

such that dysregulation of the dopamine system may contribute to compulsive and impulsive cocaine-seeking behaviors (Ragozzino et al., 1999; Cetin et al., 2004; Dalley et al., 2004; Kheramin et al., 2004; Ward et al., 2009; Winter et al., 2009). Abstinent cocaine users consistently exhibit significant decreases in dopamine D2 receptor availability and dopamine release in the ventral striatum, and these abnormalities are correlated with hypoactivity in the OFC (London et al., 2000; Volkow and Fowler, 2000; Volkow et al., 2002; Volkow et al., 2009). In contrast, when methylphenidate is experimentally administered to increase extracellular dopamine levels, thereby simulating the effects of cocaine-paired cues or cocaine itself, abstinent drug users exhibit hyperactivity in the OFC relative to healthy controls, and this enhanced neural activity is positively correlated with increases in cocaine craving (Volkow et al., 2005). Experiments in Chapter 3 indicate that dopamine D1-like receptor-mediated signaling in the OFC is critical for the expression of drug context-induced cocaine seeking. Therefore, dopaminergic input to the OFC – via D1 receptor stimulation – may regulate interactions between the OFC and BLA that promote the motivational effects of drug-paired environmental stimuli on instrumental behavior.

Utilizing the fact that the VTA is the sole source of dopamine to the OFC (Berger et al., 1991), the current study employed a triple functional disconnection procedure to investigate whether the VTA regulates intrahemispheric or interhemispheric interactions between the OFC and BLA that promote cocaine-seeking behavior. To bilaterally disrupt intrahemispheric neural communication within the putative VTA-OFC-BLA circuit, rats received unilateral infusions of SCH23390 into the OFC paired with infusions of BM into the contralateral BLA immediately before assessing drug context-induced cocaine

seeking. To bilaterally disrupt interhemispheric communication within the VTA-OFC-BLA circuit, additional groups received unilateral infusions of SCH23390 into the OFC paired with infusions of BM into the ipsilateral BLA. Based on our previous findings (Lasseter et al., 2011), we predicted that blocking either intrahemispheric or interhemispheric communication within the putative VTA-OFC-BLA circuit would similarly attenuate drug context-induced cocaine seeking without altering inactive lever responding in the drug-paired context or responding in the extinction context. Such results would indicate that dopamine D1-like receptor stimulation in the OFC mediates both interhemispheric and intrahemispheric interactions between the OFC and BLA that promote drug context-induced motivation for cocaine.

METHODS

Animals

Male Sprague-Dawley rats (n = 20; 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 Rats* (Institute of Laboratory Animal Resources on Life Sciences, 1996) and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Food training

To expedite cocaine self-administration training, all rats (n = 20) were trained to lever press on a continuous reinforcement schedule of food reinforcement (45 mg pellets; Noyes, Lancaster, NH, USA) in sound-attenuated operant conditioning chambers (26 x 27 x 27 cm high; Coulbourn Instruments, Allentown, PA, USA) during a 16-h overnight session. Active lever responses resulted in the delivery of one food pellet only; inactive lever responses had no programmed consequences. During food training, contextual stimuli subsequently used for cocaine conditioning were not present.

Surgery

Forty-eight h after food training, all rats were fully anesthetized using ketamine hydrochloride and xylazine (66.6 and 1.33 mg/kg, i.p., respectively). Chronic indwelling jugular catheters were constructed in house and were surgically implanted into the right jugular vein, as described previously (Fuchs et al., 2007). Rats were stereotaxically implanted with stainless-steel guide cannulae (26 gauge; Plastics One) aimed dorsal to the left or right BLA (-2.7 mm AP, +/-5.2 mm ML, -6.7 mm DV, relative to bregma) and the left or right OFC (+3.5 mm AP, +/-3.0 mm ML, -3.4 DV) using standard procedures. Stainless steel screws and cranioplastic cement secured the guide cannulae to the skull. Stylets (Plastics One) and Tygon caps sealed the guide cannulae and catheter, respectively. To extend catheter patency, the catheters were flushed daily with an antibiotic solution of cefazolin (10.0 mg/ml; Schein Pharmaceuticals, Albuquerque, NM, USA) and heparinized saline (70 U/ml; Baxter Health Care Corp, Deerfield, IL, USA), as described previously (Fuchs et al., 2007). Rats were given a 5-day post-operative

recovery period before the start of the experiment. Catheter patency was evaluated periodically using propofol (1mg/0.1ml, i.v. Eli Abbott Lab, North Chicago, IL, USA), which produces a rapid loss of muscle tone only when administered intravenously.

Cocaine Self-administration Training

Cocaine self-administration and extinction training sessions (**Fig 4.1**) were conducted in operant conditioning chambers configured to one of two unique environmental contexts (Context 1, Context 2) that differed along visual, auditory, tactile, and olfactory modalities, as described previously (Fuchs et al., 2005; Fuchs et al., 2007; Fuchs et al., 2008a). Rats had no exposure to these contextual stimuli prior to cocaine self-administration training; these stimuli were presented throughout each session independent of responding.

The rats were randomly assigned to receive daily 2h cocaine self-administration training sessions in Context 1 or 2 during their dark cycle. Responses on one (active) lever were reinforced under an FR1 schedule of cocaine reinforcement (cocaine hydrochloride; 0.15 mg/infusion, equivalent to ~0.50 mg/kg/infusion; i.v; NIDA, Research Triangle Park, NC, USA). A 20s time-out period followed each 2s infusion during which lever responses were recorded, but had no programmed consequences. Responses on the other (inactive) lever were recorded but had no programmed consequences. Training continued until the rats obtained ≥10 cocaine infusions/session on at least 10 training days (i.e., acquisition criterion).

Extinction Training

After meeting the acquisition criterion, rats received daily 2h extinction training sessions in the context (Context 1 or 2) that distinctly differed from the cocaine self-administration training context. Lever presses were recorded, but had no programmed consequences. Immediately prior to the behavioral session on extinction day 4, rats were acclimated to the intracranial infusion procedure. To this end, injection cannulae (33 Ga, Plastics One) were inserted into the rats' guide cannulae to a depth 2 mm below the tip of the guide cannulae and were left in place for 4 minutes. No drug was infused. Extinction training consisted of a minimum of 7 sessions plus additional sessions, as needed, until the rats reached the extinction criterion (≤ 25 active lever presses/session for two consecutive sessions).

Reinstatement Testing

After the rats reached the extinction criterion, cocaine seeking was assessed in the cocaine-paired and extinction contexts during four test sessions (**Fig 4.1**). Immediately prior to testing, rats received microinfusions of BM (1.0/0.1 mM; 0.5 µl/hemisphere, respectively; pH ~7.0) or phosphate buffered saline VEH (0.5 µl/hemisphere) unilaterally into the BLA plus infusions of the dopamine D1-like receptor antagonist SCH23390 (0.2 µg; 0.5 µl/hemisphere) or VEH into the contralateral OFC. This BM dose was selected based on previous findings that bilateral administration of this dose into the OFC or BLA impairs drug context-induced cocaine-seeking behavior in a brain-region specific manner (Fuchs et al., 2007; Lasseter et al., 2009). The dose of SCH23390 was selected based on our current findings (**Chapter 2**) that bilateral administration of this dose into the OFC

impairs drug context-induced cocaine seeking without altering locomotor behavior in a novel context or food-reinforced instrumental responding. Infusions were delivered over 2 min, and the injection cannulae were left in place for 1 min before and 1 min after the infusion. During the test sessions, lever responding had no programmed consequences. Session length was 1 h to allow for repeated testing without significant extinction learning. Both the order of testing in the two contexts and the order of intracranial treatments (intra-OFC SCH23390+intra-BLA BM, VEH+VEH) were counterbalanced based on mean active lever responding during the last three self-administration training days. Subjects received additional extinction sessions in the extinction context between test sessions until they re-obtained the extinction criterion (see above). Based on our previous findings that both intra- and interhemispheric interactions between the OFC/BLA control drug context-induced cocaine seeking (Lasseter et al., 2011), we predicted that blocking either intrahemispheric or interhemispheric communication within the putative VTA-OFC-BLA circuit would similarly attenuate drug context-induced cocaine seeking (**Fig 4.2**).

Histology

After the last experimental session, rats were overdosed using ketamine hydrochloride and 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, stored in 10% formaldehyde solution, and then sectioned at a thickness of 75 µm using a vibratome. The sections were stained using cresyl violet (Kodak, Rochester, NY, USA). Cannula

placements were verified using light microscopy and were mapped onto schematics from the rat brain atlas (Paxinos and Watson, 1997).

Data analysis

Only data from rats with correct cannula placements were included in the data analysis. Potential pre-existing differences between the treatment groups in (a) lever responses and cocaine intake during the last three days of self-administration training, (b) lever responses during the first seven days of extinction training, and (c) the number of days needed to reach the extinction criterion were analyzed using mixed factors ANOVAs with surgery condition (contralateral, ipsilateral) and subsequent treatment order (intra-OFC SCH23390+intra-BLA BM first, VEH+VEH first) as between-subjects factors and time (day) as the within-subjects factor, where appropriate. The effects of intra-OFC SCH23390+intra-BLA BM and VEH+VEH infusions on lever responses during the contextual reinstatement test sessions were assessed using mixed factors ANOVAs with surgery condition (ipsilateral, contralateral) as the between-subjects factor and treatment (intra-OFC SCH23390+intra-BLA BM, VEH+VEH) and testing context (cocaine-paired context, extinction context) as the within-subjects factor. Because the variables BLA hemisphere (left, right) and OFC hemisphere (left, right) are not orthogonal, the hemispheric laterality of significant effects was analyzed separately for the contralateral and ipsilateral surgery groups using planned t-tests. Significant main and interaction effects were investigated using simple main effects tests or Tukey *post hoc* tests. Alpha was set at 0.05.

RESULTS

Histology

Photomicrographs of representative cannula placements as well as schematics of the distribution of cannula placements are provided in **Fig 4.3**. The target brain regions were defined as the basolateral and lateral nuclei of the BLA and the lateral and ventrolateral subregions of the OFC. High power microscopy confirmed that there was no evidence of abnormal tissue damage (i.e., extensive cell loss or gliosis) at the infusion sites. Data from rats with misplaced cannulae were excluded from data analysis. For the remaining cocaine-trained rats (n=22), the most ventral point of the cannula tract was correctly located within the target brain regions of the contralateral group (left OFC/right BLA, n=6; right OFC/left BLA, n=6) and the ipsilateral group (left OFC/BLA, n=4; right OFC/BLA, n=6).

Self-Administration Responding

The groups with cannulae aimed at the contralateral or ipsilateral OFC/BLA exhibited stable active lever responding for cocaine reinforcement over the last three days of self-administration training, with a within-subjects variability of ≤ 10% in daily cocaine intake. There was no difference between these groups in active lever responding (all day and surgery type main effects and interactions, all Fs ≤ 0.77, $p \geq 0.14$ or inactive lever responding (all day and surgery type main effects and interactions, all Fs ≤ 0.21, $p \geq 0.14$) during the last three days of self-administration training. Collapsed across groups, the mean active and inactive lever responding ± SEM was 58.83 ± 9.52 and 10.55 ± 3.60, respectively, while the mean cocaine intake ± SEM was approximately 11.23 ± 0.73

mg/kg per session (22.45 ± 1.46 infusions) (data not shown). Separate ANOVAs further indicated that there were no pre-existing differences in either lever responding or cocaine intake during the last three self-administration training days as a function of subsequent treatment order (intra-OFC SCH23390+intra-BLA BM first, VEH+VEH first) (all treatment order main effects and interactions, all Fs ≤ 2.74, $p \geq 0.13$). Similarly, there were no pre-existing effects of hemispheric laterality on mean lever responding or cocaine intake over the three days of self-administration (all ts ≤1.85, $p \geq 0.09$).

Extinction Responding

Upon removal of cocaine reinforcement during extinction training, active and inactive lever responding gradually declined (active lever: day main effect, $F_{(6, 120)} = 19.01$, $p = 0.0001$; day 1 > day 2-7, Tukey test, $p < 0.01$; inactive lever: day main effect, $F_{(6, 120)} = 6.56$ $p = 0.0001$; day 1 > day 2-7, Tukey test, $p < 0.05$). There was no difference between the contralateral and ipsilateral OFC/BLA-cannulated groups in active or inactive lever responding during the first seven days of extinction training (all surgery type main effects and surgery type X day interactions, all Fs ≤ 27, $p \geq 0.61$). Separate ANOVAs indicated that there were no differences in active or inactive lever responding as a function of subsequent treatment order for either the contralateral or ipsilateral OFC/BLA-cannulated groups (all treatment order main effects and treatment order X day interactions, all Fs ≤ 1.60, $p \geq 0.16$). There was also no difference in the mean number of days ± SEM required to reach the extinction criterion (7.18 ± 0.14) as a function of surgery condition (contralateral, ipsilateral; $F_{(1,20)} = 1.40$, $p = 0.25$) or subsequent treatment order (all ts≤1.00, $p \geq 0.54$). Similarly, there was no difference between the groups in the mean

number of days ± SEM needed to re-obtain the extinction criterion between reinstatement test sessions (2.05 ± 0.05). Hence, it is unlikely that pre-existing differences accounted for group differences in reinstatement responding during the test sessions.

Effects of VTA-OFC-BLA functional disconnections on drug context-induced reinstatement of cocaine-seeking behavior

Following VEH pretreatment, the contralateral and ipsilateral OFC/BLA-cannulated groups exhibited enhanced non-reinforced active lever responding upon exposure to the previously cocaine-paired context relative to responding in the extinction context (**Fig 4.4**; context main effect, $F_{(1,20)} = 74.10$, $p = 0.0001$). However, intra-OFC SCH23390+intra-BLA BM pretreatment impaired active lever responding relative to VEH+VEH pretreatment in a context-specific manner following administration into either the contralateral or ipsilateral OFC and BLA (treatment X context interaction, $F_{(1,20)} = 44.60$, $p = 0.0001$; treatment main effect, $F_{(1,20)} = 43.23$, $p = 0.0001$; no surgery condition interaction or main effects). Specifically, independent of surgery condition, OFC SCH23390+intra-BLA BM pretreatment attenuated active lever responding relative to VEH+VEH pretreatment in the cocaine-paired context (Tukey test, $p < 0.001$) without altering responding in the extinction context. As a result, following intra-OFC SCH23390+intra-BLA BM pretreatment into the contralateral or ipsilateral OFC and BLA, there was no difference between responding in the cocaine-paired context and the extinction context. The effect of intra-OFC SCH23390+intra-BLA BM pretreatment on active lever responding in the cocaine-paired context was independent of the particular

hemisphere in which SCH23390 was administered into the OFC ($t_{(20)} = 0.79$, $p = 0.44$) or in which BM was administered into the BLA ($t_{(20)} = 0.16$, $p = 0.88$).

Intra-OFC SCH23390+intra-BLA BM pretreatment administered in either the contralateral or ipsilateral OFC and BLA failed to alter inactive lever responding in the previously cocaine-paired context or in the extinction context (all treatment main effects and interactions, Fs ≤ 2.62, $p \geq 0.12$). Irrespective of drug pretreatment, both the contralateral and ipsilateral groups exhibited a slight increase in inactive lever responding following exposure to the previously cocaine-paired context relative to responding in the extinction context (**Fig 4.4;** context main effect, $F_{(1,20)} = 9.57$, $p = 0.006$). The contralateral OFC/BLA-cannulated group exhibited more inactive lever responses than the ipsilateral OFC/BLA-cannulated group (surgery condition main effect, $F_{(1,20)} = 9.03$, $p = 0.007$), which was driven by a trend for increased responding by the contralateral OFC/BLA-cannulated group in the cocaine-paired context relative to the ipsilateral group following VEH+VEH pretreatment

DISCUSSION

The experiment in Chapter 4 explored whether a putative mesocorticolimbic circuit consisting of the VTA-OFC-BLA mediates drug context-induced cocaine-seeking behavior such that dopaminergic input from the VTA to the OFC, via dopamine D1 stimulation, regulates both intrahemispheric and interhemispheric interactions between the OFC and BLA that promote this behavior. To this end, the dopamine D1-like receptor antagonist, SCH23390, or VEH was administered unilaterally into the OFC paired with the administration of BM or VEH into the contralateral or ipsilateral BLA immediately before drug context-induced cocaine-seeking behavior. Exposure to the

cocaine-paired context significantly enhanced active lever responding relative to responding in the extinction context. However, consistent with our previous findings (**Chapter 2**), unilateral SCH23390 treatment into the OFC paired with BM treatment into either the contralateral or ipsilateral BLA significantly impaired active lever responding in the drug-paired context relative to responding following VEH treatment (**Fig. 4.4A-B).** Some drug context-induced cocaine-seeking behavior was also recorded on the inactive lever (**Fig. 4.4C-D**). This phenomenon is often observed and likely reflects the expression of an alternate form of cocaine-seeking behavior (Fuchs et al., 2007; Fuchs et al., 2009; Lasseter et al., 2010). Importantly, the decrease in drug context-induced cocaine seeking was unlikely to reflect non-specific deficits in motor performance given that intra-OFC SCH23390+intra-BLA BM treatment did not alter inactive lever responding in the drug-paired context or responding in the extinction context. In concert with this, experiments in Chapters 2 and 3, as well as in previous studies, have established that both BM treatment into the OFC plus BLA and SCH23390 treatment into the OFC fails to alter sucrose- or food-maintained instrumental responding in an alternate context or general motor activity in a novel context (Capriles et al., 2003; Lasseter et al., 2011). Overall, these findings indicate that dopamine D1 receptor stimulation in the OFC critically gates interactions between the OFC and BLA that regulate drug context-induced cocaine seeking. Such results are consistent with extensive research indicating the OFC and BLA are critical components of the mesocorticolimbic neural circuitry known to direct CS- and context-induced cocaine-seeking behavior (Grimm and See, 2000; Neisewander et al., 2000; See et al., 2001a; Kantak et al., 2002; McLaughlin and See, 2003; Fuchs et al., 2004; Fuchs et al., 2005; Atkins et al., 2008; Crombag et al., 2008;

Fuchs et al., 2008a; Hamlin et al., 2008; Zavala et al., 2008; Lasseter et al., 2009; Lasseter et al., 2011). These findings also expand upon evidence that dopamine neurotransmission in the OFC is necessary for stress-induced (Capriles et al., 2003) and context-induced cocaine seeking (**Chapter 2**). Because the VTA provides the sole source of dopamine to the OFC, the present data further indicate that input from the VTA regulates interactions between the OFC and BLA that control cocaine-seeking behavior and that this newly characterized VTA-OFC-BLA neural circuit promotes drug context-induced motivation for cocaine.

When interpreting findings that disrupting interactions within a VTA-OFC-BLA circuit attenuates drug context-induced cocaine seeking, it is important to note that rats exhibit robust drug context-induced cocaine-seeking behavior following unilateral neural inactivation of the BLA (Fuchs et al., 2007). Furthermore, unilateral BLA manipulations are insufficient to prevent reversal learning (Saddoris et al., 2005) or the impede the expression of drug context-induced cocaine seeking or sucrose-conditioned place preference (Everitt et al., 1991; Fuchs et al., 2007). Hence, it is unlikely that the robust attenuation in drug context-induced cocaine seeking following SCH23390 into the OFC paired with BM into the BLA reflects the *additive* effects of D1-like receptor antagonism in one OFC plus functional inactivation of one BLA. However, an experiment examining the effects of unilateral intra-OFC SCH23390 treatment on drug context-induced cocaine seeking is currently underway to rule out the possibility that additive effects of separate unilateral OFC and unilateral BLA manipulations account for the current findings.

Because drug context-induced cocaine seeking was equally impaired following ipsilateral or contralateral intra-OFC SCH23390+intra-BLA BM treatment, dopaminergic

input from the VTA to the OFC must critically regulate both intrahemispheric and interhemispheric interactions between the OFC and BLA, which were bilaterally disrupted by the contralateral and ipsilateral SCH23390/BM manipulations, respectively. This explanation is supported by anatomical evidence that the VTA sends dense, topographically-organized projections to the same cortical layers V and VI of the OFC that contain the densest population of dopamine D1-like receptors and that project back to the VTA (Berger et al., 1991; Dunnett and Robbins, 1992; Frankle et al., 2006; Reynolds et al., 2006; Sesack and Grace, 2010). In addition, the OFC and BLA are known to share direct, reciprocal intra- and interhemispheric projections (Krettek and Price, 1977a; McDonald, 1991; Carmichael and Price, 1995a; Ghashghaei and Barbas, 2002) as well as indirect connections that are relayed through the MDT (Demeter et al., 1990; Cavada et al., 2000; Ghashghaei and Barbas, 2002; Macey et al., 2003; Miyashita et al., 2007). Therefore, a substantial anatomical substrate provides for extensive functional interactions between elements of the putative VTA-OFC-BLA neural circuit.

Cocaine-seeking behavior is a complex behavioral phenomenon that stems from an aggregate of cognitive, sensory, and motor processes. Within this context, the OFC and BLA may make a critical contribution to reward-related behaviors by interacting to represent and process the incentive motivational properties of reward-predictive stimuli that are used to guide goal-directed behavior. From an anatomical perspective, the OFC and BLA are well-positioned to integrate sensory information from the primary and associative sensory cortices with reward-related information from mesocorticolimbic brain regions and then to interact to process this information in order to regulate behavioral responding (Price, 1986; Carmichael and Price, 1995b; Carmichael and Price,

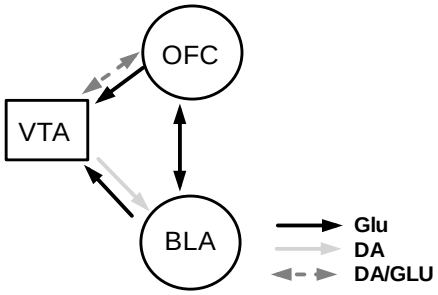
1995a; Sah et al., 2003; McDannald et al., 2004). Projections from the OFC and BLA may be direct (monosynaptic) or they may converge on similar populations of neurons in the MDT. Electrophysiological studies have further demonstrated that neurons in the OFC and BLA exhibit cue-specific firing as a function of the sensory properties or predicted reward value of a CS. The OFC and BLA exhibit high rates of activity in anticipation of an expected reward and alter their activity during changes in stimulus-reward contingencies (Rolls et al., 1996; Schoenbaum et al., 1998; Schultz et al., 1998; Schultz et al., 2000; Tremblay and Schultz, 2000; Saddoris et al., 2003; Schoenbaum et al., 2009; Takahashi et al., 2009). In this respect, the OFC facilitates cognitive flexibility by promoting the updating of associative encoding in downstream brain regions, such as the BLA, that is necessary for goal-directed instrumental responding. Following lesions to the OFC, BLA neurons fail to exhibit rapid associative encoding during cue sampling both before and after reversal learning, and there are smaller populations of outcome-expectant BLA neurons that respond during the delay before reward delivery (Saddoris et al., 2005). Similarly, bilateral lesions of the OFC impair performance under reward reversal conditions (Schoenbaum et al., 2002; Schoenbaum et al., 2003b), likely due to altered neural activity in the BLA given that these deficits are actually abolished by subsequently administering BLA lesions (Stalnaker et al., 2007a). Damage to the BLA decreases the population of OFC neurons that exhibit outcome-expectant encoding during the presentation of a reward-related stimulus (Schoenbaum et al., 2003a). Moreover, the BLA may be critical for rapidly encoding reward-related information given that neurons in the BLA develop cue-selective neural encoding more rapidly and in greater populations than OFC neurons during acquisition training and following reward reversals

(Schoenbaum et al., 1999; Schoenbaum et al., 2003b). Hence, while the OFC is generally thought to be critical for promoting the updating of associative encoding in downstream brain areas, the BLA may be similarly important for facilitating encoding by the OFC. In preclinical studies, functional disconnection of the OFC and BLA impairs responding on tasks that require one to update information about the value of predicted outcomes. For example, functional disconnection of the OFC plus BLA produces inflexible behavioral responding during odor reversal tasks in rats (Churchwell et al., 2009) and prevents monkeys from altering behavioral responses based on the changing motivational value of reward-predictive cues (Baxter et al., 2000; Izquierdo et al., 2004). Therefore, the OFC and BLA may promote drug context-induced cocaine seeking by interacting to represent the motivational significance of drug-paired environmental stimuli.

Given that dopamine receptor antagonism in the OFC attenuates drug context-induced cocaine-seeking behavior (**Chapter 2**), dopaminergic neurotransmission in the OFC may play an important role in maintaining the motivational value of cocaine-paired stimuli. However, it is important to note that dopaminergic input from the VTA to the BLA, via dopamine D1 receptor stimulation, may be equally important for maintaining the representation of the motivational salience of cocaine-paired contextual stimuli. Consistent with this, dopamine D1-like receptor antagonism in the BLA prevents explicit CSs from eliciting cocaine-seeking behavior (See et al., 2001a; Alleweireldt et al., 2005; Berglind et al., 2006; Mashhoon et al., 2009). Thus, interactions within the putative VTA-OFC-BLA circuit may be quite complex. Unfortunately, the disconnection procedure does not permit one to identify the exact sequence of information processing within a neural circuit. For instance, while input from the VTA to the OFC may alter

interactions between the OFC and BLA that subserve drug context-induced cocaine seeking, back-projections from the OFC to the VTA may both alter dopaminergic input to itself as well as regulate interactions between the VTA and BLA that promote behavioral responding (**Illustration 1**).

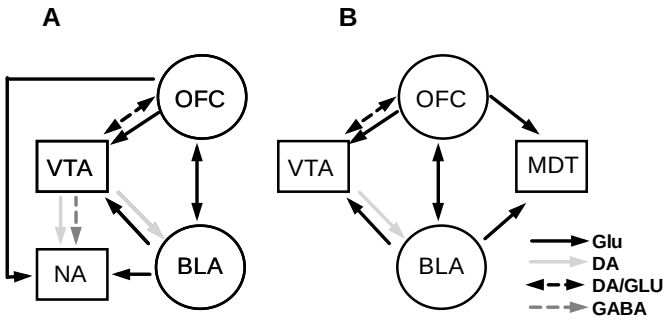
Figure 1. Potential interactions within the VTA-OFC-BLA neural circuit



Moreover, interactions between the VTA-OFC-BLA neural circuit and the NA may be critical for the expression of cocaine-seeking behavior given that the NA is implicated in the execution of reward-related behaviors and represents a critical component of the context-based reward circuitry (Brog et al., 1993; Sah et al., 2003; Fuchs et al., 2008a; Xie et al., 2010). Glutamatergic output from the OFC and BLA converge on dendritic spines in the NA (**Illustration 2A**) that also receive dopaminergic input from the VTA (Kelley et al, 1982; Bouyer et al., 1984; Haber et al., 1995). Dopamine enhances synaptic efficacy of strong glutamatergic inputs while simultaneously reducing efficacy of weak glutamatergic inputs (Hernandez et al., 1988; O'Donnell et al., 1999). Therefore, concurrent glutamatergic input to the NA from the BLA or OFC may gate the throughput of information to the limbic loop of the basal ganglia that promotes conditioned responding (Cepeda et al., 1993; Kiyatkin and Rebec, 1996b; Cepeda and Levine, 1998). In concert with this, unilateral inactivation of the BLA paired with dopamine D1-like receptor antagonism in the NA reduces behavioral

responding to sucrose-predictive cues (Ambroggi et al., 2008) and optogenetic inhibition of glutamatergic projections from the BLA to the NA prevents cue-maintained sucrose consumption (Stuber et al., 2011). This latter finding may stem, in part, from reduced dopamine neurotransmission in the NA given that the BLA is capable of enhancing conditioned dopamine release and that optogenetic stimulation of glutamatergic projections from the BLA to the NA only reinforces behavioral responding when NA

Illustration 2: Potential Interactions by the VTA-OFC-BLA Neural Circuit with the NA (A) and MDT (B)



dopamine D1 receptors are concurrently stimulated (Stuber et al., 2011).

Furthermore, direct and indirect communication between the OFC and NA may regulate the expression of reward-related behavior given that the OFC projects directly to the NA as well as to GABAergic neurons in the VTA that innervate the NA (Campbell et al., 1999; Carr and Sesack, 2000). However, while functional disconnection of the BLA from the NA disrupts explicit cue-induced cocaine-seeking behavior (Di Ciano and Everitt, 2004), the potential contribution of an OFC-NA circuit has yet to be explored with respect to drug context-induced cocaine seeking. Nevertheless, disrupting communication within this newly characterized VTA-OFC-BLA circuit may prevent drug context-induced cocaine seeking by reducing glutamatergic input from the BLA and/or the OFC to the NA.

Finally, functional disconnection of the VTA-OFC-BLA circuit may attenuate drug context-induced cocaine seeking by disrupting communication between the OFC-BLA circuit and the MDT (**Illustration 2B**). Consistent with this, functional disconnection of the OFC-BLA circuit from the MDT – but not the NA – impaired reward-related behaviors on a reinforcer devaluation task (Izquierdo and Murray, 2010). However, these effects may simply reflect that the MDT regulates behavior based on the motivational value of reward-related conditioned stimuli rather than playing a more general role in directing goal-directed behavior, or that interactions within a OFC-BLA-NA subcircuit are not necessary for responding during reinforcer devaluation (Cardinal et al., 2004; Izquierdo and Murray, 2010). Either way, the neural circuitry recruited by the reinforcer devaluation task may be distinctly different from the circuit that regulates drug context-induced cocaine seeking. Therefore, future studies will be necessary to explore whether the VTA-OFC-BLA neural circuit interacts with either the MDT or NA in the control of drug context-induced motivation for cocaine.

In conclusion, results from the current study provide the first evidence that a mesocorticolimbic subcircuit exists in which dopaminergic input from the VTA to the OFC, via dopamine D1 receptor stimulation, regulates both intra- and interhemispheric interactions between the OFC and BLA that promote drug context-induced cocaine seeking. Future studies will be necessary to characterize the precise neural mechanisms by which the VTA, OFC, and BLA interact to control this behavior as well as to identify other elements of the context-based relapse circuitry with which this newly characterized VTA-OFC-BLA circuit interacts to direct the expression of a drug context-induced motivation for cocaine.

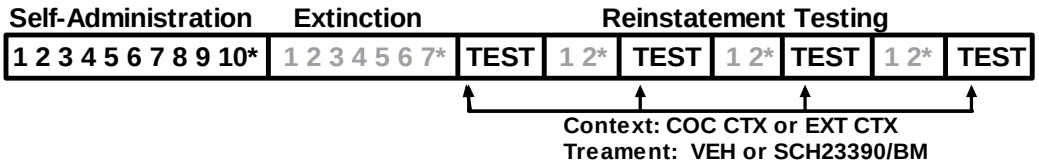


Fig 4.1 Schematic representation of the timeline for the drug context-induced reinstatement experiment. Arrows identify sessions in which drug (intra-OFC SCH23390+intra-BLA BM) or vehicle (VEH+VEH) or was administered into the OFC and BLA immediately prior to testing. The order of context exposure (COC CTX; EXT CTX) and the order of drug treatment (intra-OFC SCH23390+intra-BLA BM; VEH+VEH) were counterbalanced during reinstatement testing. *Asterisks* indicate that the rats had to reach an acquisition criterion (≥ 10 infusions per session for minimum 10 sessions) to complete self-administration training and had to satisfy the extinction criterion (≤ 25 active lever presses per session for two consecutive sessions) before each test session.

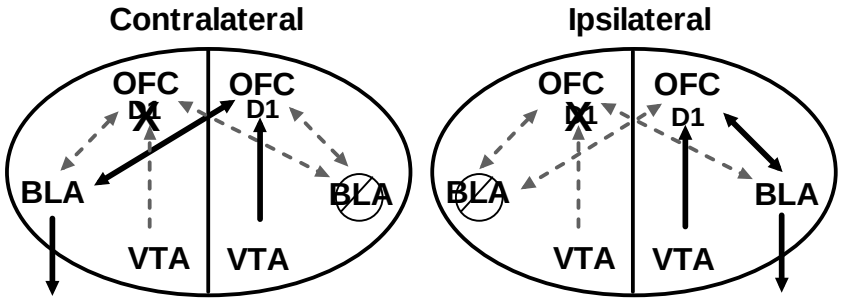


Fig 4.2 Schematic representation of the modified functional disconnection procedure. Following the contralateral manipulation, putative intrahemispheric processing within the VTA-OFC-BLA circuit is disrupted bilaterally, while interhemispheric processing is spared in one hemisphere. Following the ipsilateral manipulation, putative interhemispheric information processing within the VTA-OFC-BLA circuit is disrupted bilaterally, while intrahemispheric processing is spared in one hemisphere. Solid lines represent communication that is preserved between two intact brain regions. Dotted lines represent communication that is transiently disrupted between the target brain regions following the functional disconnection procedure.

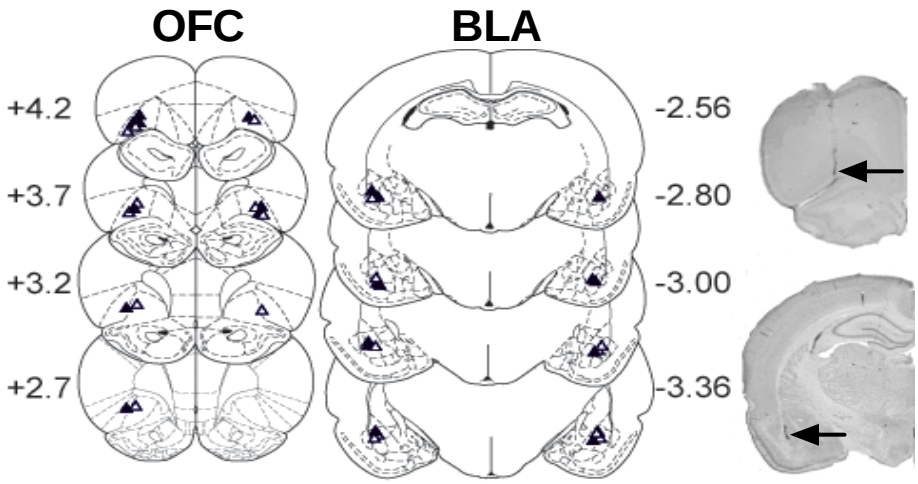


Fig 4.3 Schematic and photographic representation of injection cannula placements. The *arrows* on the photomicrographs identify the most ventral point of the infusion cannula tracts on representative cresyl violet-stained brain sections. The symbols on the schematics (Paxinos and Watson, 1997) represent the most ventral point of the infusion cannula tracts for rats that received unilateral microinfusions into the OFC plus the contralateral BLA (*closed triangles*) or the ipsilateral BLA (*open triangles*). Numbers indicate the distance from bregma in millimeters.

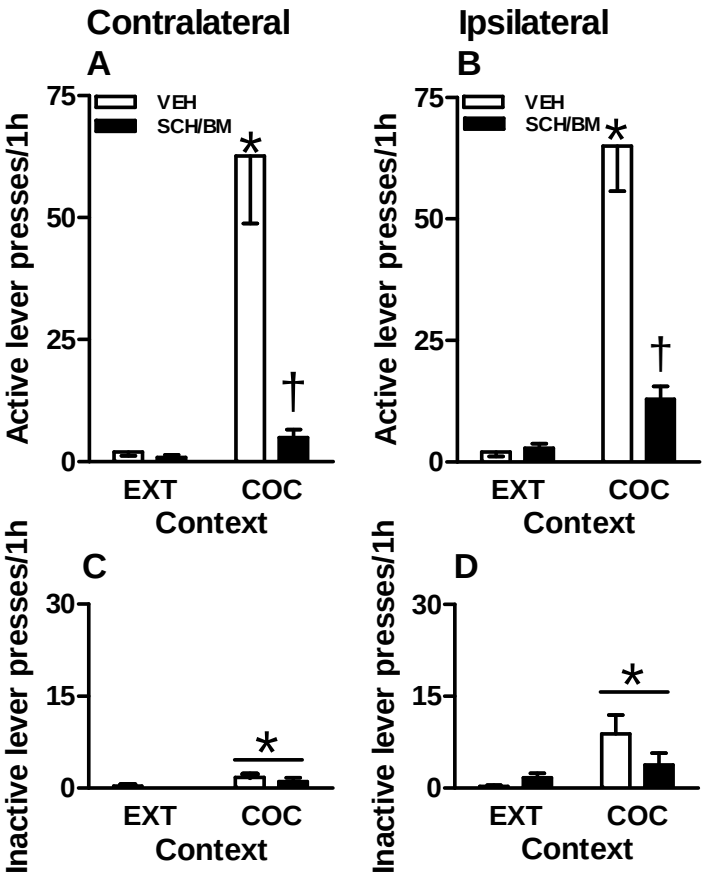


Fig 4.4 Bilateral disruption of intrahemispheric or interhemispheric communication within a VTA-OFC-BLA circuit similarly impairs drug context-induced reinstatement of cocaine-seeking behavior. The panels depict non-reinforced active and inactive lever responses (mean/1h ± SEM) during testing in the extinction context (EXT context) and the previously cocaine-paired context (COC context). Immediately before testing, groups received infusions of (a) SCH23390 unilaterally into the OFC paired with BM into the contralateral or ipsilateral BLA or (b) VEH unilaterally into the OFC and into the contralateral or ipsilateral BLA. *Asterisks* represent significant difference relative to responding in the extinction context (***A,B****:* ANOVA context simple main effect, $p < 0.01$; ***C,D:*** ANOVA context main effect, $p < 0.05$). *Daggers* represent significant difference relative to VEH pretreatment (***A,B:*** ANOVA treatment simple main effect, $p < 0.01$).

CHAPTER 5

GENERAL DISCUSSION

Summary of Experimental Findings

Extensive work has identified various elements of a mesocorticolimbic neural circuitry that contributes to the reinstatement of drug context-induced cocaine seeking. These elements include the OFC, BLA and dopaminergic brain regions (Crombag et al., 2002; Fuchs et al., 2005; Lasseter et al., 2009). To expand upon these findings, the first series of experiments discussed in Chapter 2 utilized a functional disconnection procedure to explore whether the OFC and BLA exhibit obligatory functional interactions in the control of drug context-induced cocaine seeking. Anatomical evidence indicates that the OFC and BLA share dense, reciprocal intra- and interhemispheric anatomical projections (Krettek and Price, 1977b), while behavioral studies demonstrate that interactions between the OFC and BLA are necessary for a variety of reward-related behaviors (Baxter et al., 2000; Churchwell et al., 2009). Thus, we hypothesized that both inter- and intrahemispheric processing by the OFC and BLA contributes to drug context-induced cocaine-seeking behavior in a previously drug-paired environmental context. To evaluate this hypothesis, rats received infusions of BM or VEH unilaterally into the OFC plus into either the contralateral or ipsilateral OFC immediately before assessing cocaine-seeking behavior. Relative to VEH treatment, BM-induced functional inactivation of the OFC plus either the contralateral or ipsilateral BLA impaired drug

context-induced reinstatement of cocaine seeking. The attenuation in cocaine seeking following contralateral or ipsilateral inactivation of the OFC and BLA was superadditive relative to the effects of unilateral inactivation of the OFC plus unilateral inactivation of the BLA. Furthermore, contralateral or ipsilateral inactivation of the OFC and BLA did not alter general motor activity or food-maintained instrumental responding, relative to the VEH treatment. Together, these findings indicate that sequential information processing by the OFC and BLA regulates the motivational effects of a drug-paired context on instrumental behavior and provides the first evidence that both intrahemispheric and interhemispheric interactions between these brain regions critically contribute to cocaine-seeking behavior.

Dopamine neurotransmission in the OFC may regulate to some forms of drug-seeking behavior given that dopamine D1-like receptor antagonism in the OFC attenuates stress-induced cocaine seeking (Capriles et al., 2003) and that systemic blockade of dopamine D1-like receptors impairs drug context-induced cocaine seeking (Crombag et al., 2002). However, whether dopamine in the OFC regulates activity within the OFC-BLA neural circuit remained to be ascertained. As a first step towards evaluating this question, the second series of experiments reported in Chapter 3 were designed to explore the contribution of dopamine D1-like receptor stimulation in the OFC to drug context-induced cocaine seeking. Rats received bilateral infusions of the highly selective D1-like receptor antagonist, SCH23390, or VEH into the OFC or into the MC – an anatomical control brain region – immediately before the reinstatement test sessions. Results from these experiments indicated that SCH23390 treatment into the OFC produced a dose-dependent attenuation in drug context-induced cocaine seeking relative to VEH

treatment. While dopamine D1-like receptor antagonists have been shown to impair motor activity (Fowler and Liou, 1994), the effects of intra-OFC SCH23390 treatment on cocaine-seeking behavior were unlikely to reflect non-specific motor deficits given that the behaviorally effective dose of SCH23390 in the OFC did not alter general motor activity in a novel context or food-maintained instrumental responding. Furthermore, the behavioral effects of SCH23390 were specific to the OFC to the extent that infusions of SCH23390 into the MC – the brain region most likely affected by unintended diffusion of SCH23390 along the cannula tract – did not alter the reinstatement of drug context-induced cocaine seeking. Taken together, these findings support previous studies showing that the OFC regulates the motivational effects of drug-paired contextual stimuli on instrumental responding (Fuchs et al., 2004; Lasseter et al., 2009) and provide the first evidence that stimulation of dopamine D1-like receptors in the OFC is necessary for this behavior.

Based on the above findings, the third series of experiments reported in Chapter 4 were designed to assess whether a putative neural circuit consisting of the VTA, OFC, and BLA critically contributes to drug context-induced cocaine seeking. Because the VTA provides the sole source of dopamine to the OFC (Berger et al., 1991), it was hypothesized that dopaminergic input from the VTA to the OFC, via dopamine D1-like receptor stimulation, may regulate interactions between the OFC and BLA that promote this behavior. To test this hypothesis, a triple disconnection procedure was employed in which rats received unilateral microinfusions of SCH23390 or VEH into the OFC paired with infusions of BM or VEH into the contralateral or ipsilateral BLA immediately before assessing cocaine-seeking behavior. Consistent with our previous findings

(Lasseter et al., 2011), unilateral SCH23390 treatment in the OFC paired with BM treatment in either the contralateral or ipsilateral BLA significantly impaired the reinstatement of drug context-induced cocaine seeking relative to VEH treatment. These findings indicate that dopaminergic input from the VTA to the OFC, via dopamine D1-like receptor stimulation, regulates both interhemispheric and intrahemispheric interactions between the OFC and BLA and that this newly characterized VTA-BLA-OFC neural circuit promotes drug context-induced motivation for cocaine.

Effects of Dopamine Receptor Stimulation in the OFC

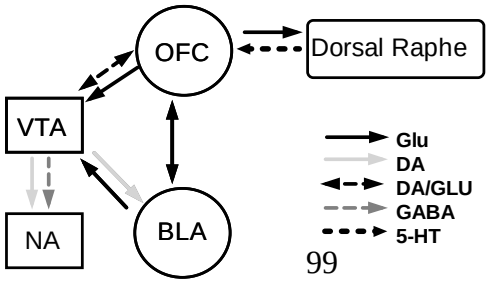
While it is known that the OFC receives dense dopaminergic efferents from the VTA (Berger et al. 1991; Dunnett and Robbins 1992; Frankle et al. 2006; Geisler et al. 2007; Sesack and Grace 2010), little work has been done to explore the synaptic mechanisms of dopamine modulation of neural activity in OFC neurons. Throughout the prefrontal cortices, including the OFC, dopamine D1-like receptors are significantly more abundant than dopamine D2-like receptors (Boyson et al. 1986; Dawson et al. 1986; Lidow et al. 1989), highlighting the importance of dopamine D1-like receptors in regulating OFC neural activity. Dopamine release can exert either excitatory or inhibitory influences on the neural activity of pyramidal neurons in the prefrontal cortices depending on the relative distribution of D1-like and D2-like receptors on these and neighboring non-pyramidal neurons (Seamans and Yang 2004; Sun and Rebec 2005; Vijayraghavan et al. 2007). D1-like receptors are primarily expressed on the dendritic spines and shafts of pyramidal neurons, while both D1-like and D2-like receptors are localized on inhibitory GABAergic interneurons and presynaptic glutamatergic terminals

(Muly et al. 1998; Sesack et al. 1995; Vincent et al. 1995). In general, D1-like receptor stimulation on pyramidal neurons enhances, whereas D1-like receptor stimulation on GABAergic interneurons inhibits, pyramidal neuronal activity (Seamans and Yang, 2004). Furthermore, dopamine D2-like receptor stimulation generally has an inhibitory influence on pyramidal neurons, but it can also attenuate inhibition maintained by GABAergic interneurons (Seamans and Yang, 2004). Hence, the overall effect of mesocortical dopamine release on behavioral performance (Floresco and Magyar 2006; Granon et al. 2000; Williams and Goldman-Rakic 1995; Zahrt et al. 1997) can be difficult to predict, and it can depend on the specific cortical layer(s) in which dopamine is released, on the dynamics of its release, and on the dopamine receptor populations that are being stimulated (for review, see Seamans and Yang, 2004). Consistent with this, D1-like and D2-like receptor antagonists have had differential effects on drug-seeking behavior following administration into the PFC (Capriles et al. 2003; See 2009; Sun and Rebec 2005). Contributing to this line of research, the current findings from Chapter 3 and 4 suggest that dopamine D1-like receptor-mediated signaling in the OFC is necessary for drug context-induced cocaine seeking, perhaps by mediating interactions between the OFC and BLA that control this behavior. However, subsequent research is needed to explore the precise neural mechanisms by which dopamine D1-like receptor antagonism alters cocaine-seeking behaviors. Specifically, it will be of interest to employ electrophysiological recordings and optogenetic techniques to examine how SCH23390 microinjections in the OFC affect neural activity in different cortical layers and cell types, respectively.

Contribution of 5-HT2c Receptor-mediated Signaling in the OFC to Drug Context-induced Cocaine Seeking

While results from the experiments in Chapter 3 and 4 indicate that dopamine receptor stimulation in the OFC contributes to the ability of a drug-paired context to elicit motivation for cocaine, dopamine plays a neuromodulatory role in that its effect on behavior depends on the presence of other neurotransmitters, such as GABA, serotonin, or glutamate (Kiyatkin and Rebec, 1996b; Kiyatkin and Rebec, 1996a; Floresco et al., 1998; Seamans et al., 2001a; Wang and O'Donnell, 2001). The potential contribution of serotonin receptor-mediated signaling is of particular interest given that the highly selective dopamine D1-like receptor antagonist, SCH23390, used in the experiments in Chapters 3 and 4, also exhibits a moderate affinity for the 5-HT2c receptor subtype (K_D ~20nM) *in vitro* and acts as a 5-HT2c receptor *agonist* (Rupniak et al., 1986; Kalkman et al., 1998). Notably, the OFC receives dense serotonergic innervation from the dorsal raphe nucleus and sends direct, glutamatergic input to GABAergic interneurons within the dorsal raphe nucleus (Arnsten and Goldman-Rakic, 1984; Hajos et al., 1998; Hajos et al., 1999) (**Illustration 3**). As a result, the OFC can regulate serotonergic input onto itself as well as to the rest of the forebrain such that activation of the prefrontal cortices

Illustration 3. Potential Contribution of 5-HT2c Receptor-mediated Signaling to Drug Context-induced Cocaine-Seeking Behavior



inhibits serotonin release via this negative feedback loop (Hajos et al., 1999; Celada et al., 2001; Varga et al., 2001).

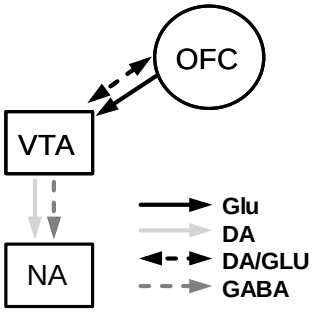
Because SCH23390 exhibits moderate affinity for 5-HT2c receptors, intra-OFC infusions of SCH233390 in the current experiments may have impaired drug context-induced cocaine seeking by either blocking dopamine D1-like receptors or by stimulating 5-HT2c receptors. Interestingly, 5-HT2c receptors are located in the cortical layers of the OFC that contain the greatest density of dopamine D1-like receptors (Pazos et al., 1985; Lidow et al., 1989), indicating that input from both the serotonergic and dopaminergic systems may interact to control reward-related behaviors. Serotonin receptor signaling in the OFC has been shown to make an important contribution to reward-related behaviors such that serotonin depletion in the OFC impairs reversal learning, whereas 5-HT2c receptor antagonism in the OFC improves performance on reward reversal tasks (Clarke et al., 2004; Clarke et al., 2005; Clarke et al., 2007; Boulougouris and Robbins, 2010). Furthermore, both systemic and intra-PFC administration of 5-HT2c receptor agonists attenuate explicit CS-induced reinstatement and context-induced renewal of cocaine-seeking behaviors (Neisewander and Acosta, 2007; Fletcher et al., 2008; Pentkowski et al., 2010). In conclusion, given that both dopamine D1-like antagonists and serotonin 5-HT2c receptor agonists are capable of disrupting cocaine-seeking behaviors (Capriles et al., 2003; Neisewander and Acosta, 2007; Fletcher et al., 2008), SCH23390 may have exerted its behavioral effects in the current experiments by acting at either receptor population. Therefore, it will be of interest to further explore the neural mechanisms by which SCH23390 in the OFC

impairs drug context-induced cocaine-seeking behaviors as well as to assess the selective contribution of 5-HT2c receptors to this behavior.

Contribution of a Corticomesal Projection to Drug Context-induced Cocaine-Seeking Behaviors

The current experiments indicate that dopaminergic input from the VTA to the OFC regulates interactions between the OFC and BLA that promote drug context-induced cocaine seeking. However, input from the OFC to the VTA may also make a contribution to reward-related behaviors by regulating neural activity in the VTA (**Illustration 4**). The prefrontal cortex – including the OFC – provides dense projections

Illustration 4. Potential Contribution of a Corticomesal Projection to Drug Context-induced Cocaine-seeking Behaviors



to the VTA (Campbell et al., 1999; Carr and Sesack, 2000). Glutamatergic projections from the OFC synapse onto the same VTA dopamine neurons that project back to the OFC and onto GABAergic neurons within the VTA that project to the NA, a structure implicated in the motor execution of drug-seeking behavior (Bossert et al., 2007; Fuchs et al., 2008a), as well as onto local axonal collaterals within the VTA (Campbell et al., 1999; Carr and Sesack, 2000). Electrical stimulation of the prefrontal cortices can enhance glutamate and dopamine release in the VTA and NA, respectively, both of which

can be reversed by ionotropic glutamate receptor antagonism in the VTA (Taber et al., 1995; Rossetti et al., 1998; You et al., 1998). Importantly, recent evidence indicates that infusions of ionotropic glutamate antagonists into the VTA abolish the ability of a cocaine-paired context to maintain responding in the absence of cocaine reinforcement (You et al., 2007). Moreover, glutamate release in the VTA may regulate the motivational effects of a drug-paired context on goal-directed behavior given that systemic and intra-VTA infusions of the group II metabotropic glutamate autoreceptor agonist, LY379268, attenuates drug context-induced heroin-seeking behavior (Bossert et al., 2004). However, in addition to glutamatergic afferents from the prefrontal cortices, dopamine neurons in the VTA receive multiple sources of glutamatergic input from subcortical structures, including the subthalamic nucleus, pedunculopontine nucleus, bed nucleus of the stria terminalis, hypothalamus (Georges and Aston-Jones, 2002; Sesack et al., 2003; Reynolds et al., 2006), and BLA (Reynolds et al., 2006), as well as from VTA glutamatergic neurons (Yamamoto et al., 2001). Therefore, the critical source of VTA glutamate that contributes to drug context-induced reinstatement remains to be determined.

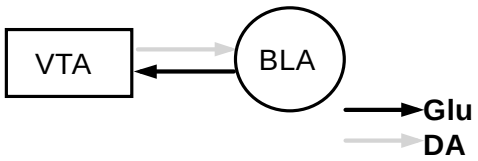
Surprisingly, electrophysiological data indicates that burst-like stimulation of OFC neurons *inhibits* 50% of VTA dopamine neurons followed by phasic activation of 40% of VTA dopamine neurons, indicating that OFC activation of VTA dopaminergic neurons is secondary to activation of GABAergic neurons (Lodge, 2011). However, in the current series of experiments, dopamine receptor stimulation in the OFC appears critical for the expression of cocaine-seeking behavior (Chapter 3) as well as for the control of interactions between the OFC and BLA that subserve drug context-induced

motivation for cocaine (Chapter 4). Therefore, exploring the precise neural mechanisms by which the OFC regulates VTA output – both with respect to itself as well as to other brain regions, including the NA – will be critical for characterizing the neural circuitry that regulates cocaine-seeking behaviors.

Contribution of BLA Dopamine Afferents to Drug Context-induced Cocaine-Seeking Behavior

While the current findings suggest that dopaminergic projections from the VTA to the OFC contribute to cocaine seeking-behavior by supporting the ability of interactions between the OFC and BLA to maintain a representation of the motivational salience of the drug-paired environmental context (Winstanley et al., 2006; Sesack and Grace, 2010; Zeeb et al., 2010), dopaminergic projections from the VTA to the BLA are also likely critical for the expression of drug context-induced cocaine seeking (**Illustration 5**).

Illustration 5. Potential Contribution of a Mesolimbic Projection to Drug Context-induced Cocaine-seeking Behavior



Dopamine from the VTA stimulates dopamine receptors in the BLA (Mansour et al., 1991; Meador-Woodruff et al., 1991; Ford et al., 2006) that are expressed postsynaptically on glutamatergic projection neurons as well as presynaptically on glutamatergic terminals (Rosenkranz and Grace, 2002; Bissiere et al., 2003; Loretan et al., 2004). Systemic dopamine D1 receptor antagonism prevents a discriminative stimulus plus explicit CS from eliciting cocaine-seeking behavior concomitant with

decreased expression of the activity-dependent early-immediate gene, *c-fos*, in the BLA (Ciccocioppo et al., 2001). Moreover, dopamine D1-like receptor antagonism in the BLA decreases cocaine intake when drug self-administration is maintained under a second-order schedule of reinforcement (Mashhoon et al., 2009) and impairs the expression of explicit CS-induced reinstatement of cocaine-seeking behavior (See et al., 2001a; Alleweireldt et al., 2005; Berglind et al., 2006; Mashhoon et al., 2009). While *systemic* infusions of the glutamate AMPA/kainite receptor antagonist, NBQX, attenuate drug cue-induced cocaine-seeking behavior concomitant with decreased c-Fos expression in the BLA, site-directed infusions of the NMDA receptor antagonist, AP-5, the AMPA receptor antagonist CNQX, or AP-5+CNQX into the BLA fail to prevent a cocaine-paired explicit CS from eliciting cocaine-seeking behavior (See et al., 2001b; Zavala et al., 2008), which highlights the critical role of *dopamine* neurotransmission in some forms of relapse behaviors.

Interestingly, dopamine input from the VTA to the BLA may facilitate OFC-BLA interactions that are necessary to represent the motivational value of reward-related conditioned stimuli. This is a possibility given that stimulation of BLA dopamine receptors potentiates strong sensory inputs to the BLA and simultaneously decreases inhibitory inputs from the PFC, thereby facilitating sensory-driven associative learning processes, such as the formation of context-response-cocaine associations (Rosenkranz and Grace, 1999; Rosenkranz and Grace, 2001). Overall, these studies indicate that dopaminergic neurotransmission in the BLA likely plays a role in conditioned goal-directed behaviors; however, future studies will be necessary to parse out the precise

contribution of dopamine receptor-mediated signaling in the BLA to drug context-induced cocaine seeking.

The Emerging Mesocorticolimbic Neural Circuitry of Drug Context-induced Cocaine-seeking Behavior

Recent evidence has identified elements of a mesocorticolimbic neural circuitry that regulates the ability of drug-paired environmental stimuli to produce motivation for cocaine reinforcement (**Illustration 6**). The reinstatement of drug-context induced cocaine seeking depends on the functional integrity of the dorsal lateral caudate putamen (dlCPu), BLA, DH, VH, PFC, OFC, NA core and shell, and lateral septum (LS) (Fuchs et al., 2005; Di Pietro et al., 2006; Fuchs et al., 2006; Crombag et al., 2008; Fuchs et al., 2008a; Lasseter et al., 2010; Luo et al., 2010; Mashhoon et al., 2010), as well as serial information processing within the BLA-DH, BLA-PFC, BLA-OFC, and DH-LS-VTA subcircuits (Fuchs et al., 2007; Luo et al., 2010; Lasseter et al., 2011). In addition, correlational evidence indicates that the lateral hypothalamus (LH) contributes to drug context-induced cocaine seeking (Hamlin et al., 2006; Hamlin et al., 2008). However, the ability of a cocaine-paired context to elicit neural activity in the LH is independent of its connections with the VTA, NA, or PFC, suggesting that the LH may contribute to drug context-induced cocaine seeking via a distinct neural circuitry, perhaps consistent with its more general involvement in reward-seeking behaviors (Hamlin et al., 2006; Hamlin et al., 2008).

The VTA exerts a powerful influence on the activity of this neural circuitry. Re-exposure to drug-paired contextual stimuli elicits cocaine-seeking behavior by enhancing

the activity of the VTA, which in turn increases dopamine release in cortical and limbic terminal regions (Crombag et al., 2008; Di Chiara and Imperato, 1988; Kiyatkin et al., 1993; Weiss et al., 2000; Di Ciano et al., 2001; Phillips et al., 2003; Schiffer et al., 2009). In turn, the DH, BLA, and other elements of the mesocorticolimbic relapse circuitry communicate primarily via glutamatergic and GABAergic projections in order to process the motivational significance of drug-paired contextual stimuli and initiate the most appropriate behavior in response to these stimuli (Sesack and Pickel, 1990; Cepeda et al., 1993; Meredith, 1999). The results of this processing are thought to be integrated at the level of the prelimbic and infralimbic subregions of the PFC, which may direct the expression of cocaine-seeking behavior via their respective interactions with the NA core and shell (Kalivas and McFarland, 2003; Peters et al., 2008). The NA, in turn, gates the throughput of information to the limbic loop of the basal ganglia that promotes conditioned responding (Cepeda et al., 1993; Kiyatkin and Rebec, 1996b; Cepeda and Levine, 1998).

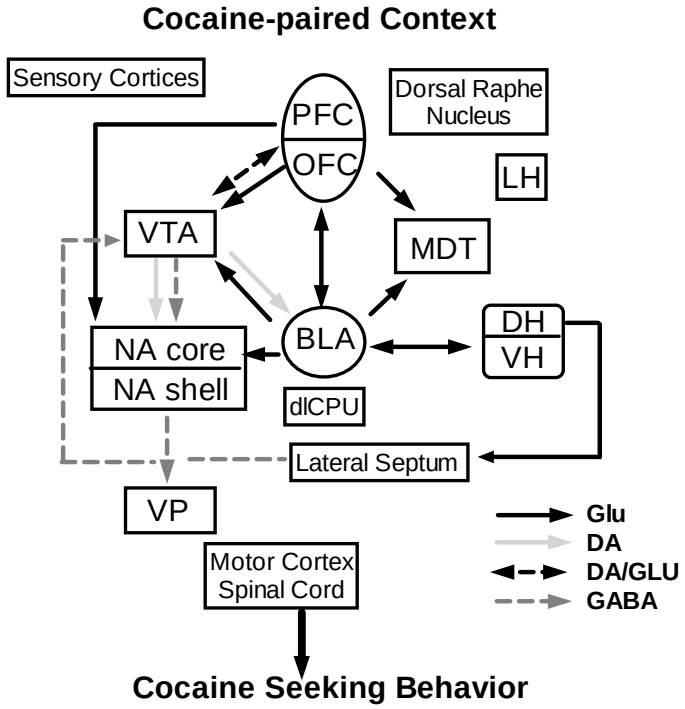
It is important to note that different neural substrates, such as the dlCPu, may come to control relapse behaviors, whereas other brain regions, including the PFC and BLA, may no longer mediate drug-seeking behavior following abstinence periods as opposed to explicit extinction training (Fuchs et al., 2006). All experiments in the current project utilized the context-based extinction-reinstatement model of addiction in order to explore the neural circuitry that contributes to drug context-induced cocaine seeking in rats. This model offers excellent translational value for examining the neural underpinnings of human drug addiction as it possesses both strong face and predictive validity. For instance, re-exposure to cocaine-paired contexts consistently reinstates

drug-seeking behavior in this model, mirroring clinical findings that, even after successful inpatient detoxification plus cue-exposure therapy, abstinent drug users exhibit high rates of relapse after returning to previously drug-associated contexts (Hunt et al., 1971; Carter and Tiffany, 1999; Drummond, 2000). Extinction training in this model is necessary in order to isolate the influence of the cocaine-paired context on the reinstatement of instrumental responding, even though human cocaine addicts seldom receive explicit extinction training during abstinence from drug taking (Katz, 2001). Importantly, extinction training is an active learning process that produces neurobiological adaptations (Self and Nestler, 1998; Katz, 2001), and it may reshape the neural system recruited for drug context-induced cocaine seeking behavior.

Different relapse triggers, such as contextual stimuli, explicit CSs, small amounts of cocaine, and stress, appear to mediate cocaine seeking through distinct, yet partially over-lapping mesocorticolimbic subcircuits (Shaham et al., 2000a; Kalivas and McFarland, 2003; Crombag et al., 2008). While the DH plays a selective role in drug context-induced reinstatement, both the BLA and OFC are critical for drug context-induced and CS-induced reinstatement, and the VTA, NA, VH, and PFC make a general contribution to drug context-induced, CS-induced, and drug-primed reinstatement. (Grimm and See, 2000; Shaham et al., 2000b; McFarland and Kalivas, 2001; See et al., 2001b; Capriles et al., 2003; Fuchs et al., 2004; McFarland et al., 2004b; Fuchs et al., 2005; Rogers and See, 2007; Lasseter et al., 2009). Furthermore, stress-induced reinstatement recruits the VTA, NA, PFC, and OFC as well as some unique neural substrates, including the lateral tegmental nucleus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis (Erb et al., 2000; Shaham et al., 2000b;

Capriles et al., 2003; McFarland et al., 2004a). Importantly, the mesocorticolimbic neural circuitry also contributes to a variety of adaptive, goal-directed motor behaviors, and hence does not specifically promote pathological drug-seeking behavior in and of itself. Rather, chronic cocaine exposure is thought to induce neural changes in elements of this neural circuitry that subsequently facilitate the emergence of perseverative cocaine-seeking behavior in rats as well as the transition from casual drug use to drug addiction in human cocaine users, as well be described below (Koob and Volkow, 2010).

Illustration 6. Emerging Neural Circuitry Underlying Drug Context-induced Cocaine-seeking Behavior



Neuroadaptations in the Drug Context-induced Relapse Circuitry

Central to the theory of drug addiction is that chronic drug exposure produces pathological changes in the brain that facilitate the transition from recreational drug use

to drug addiction and produce a long-lasting vulnerability to drug relapse (Koob and Volkow, 2010). In particular, cocaine addicts typically present with abnormalities in the prefrontal cortical areas, including decreased gray matter density in the OFC and anterior cingulate, diminished baseline blood glucose metabolism in the frontal cortex, and enhanced cue-evoked activation of the OFC, some of which may be proportional to drug use (Volkow et al., 1991; London et al., 2000; Volkow and Fowler, 2000; Franklin et al., 2002; Bolla et al., 2003a; Matochik et al., 2003b). Furthermore, extended access to cocaine produces long-lasting reductions in the density of neurons and oligodendrocytes in the PFC as well as in oligodendrocytes in the OFC, both of which are associated with persistent deficits in working memory (George et al., 2008). Interestingly, OFC damage in drug-naïve individuals produces behavioral impairments similar to those seen in cocaine addicts, including maladaptive decision-making, impulsive behavior, and perseveration of non-rewarding responses (O'Doherty et al., 2001; Bechara et al., 1994). Therefore, whether compulsive and impulsive drug seeking and taking behaviors exhibited by drug addicts results from an underlying neural sensitivity predisposing one to drug addiction or develops as result of chronic drug exposure remains to be determined (Volkow *et al.*, 1992; Franklin *et al.*, 2002; Volkow *et al.*, 2002).

Importantly, chronic cocaine use may produce behavioral impairments at least in part by altering gene transcription in the brain regions that contribute to drug context-induced cocaine seeking. Following chronic cocaine self-administration, the NA and PFC exhibit an accumulation of the transcription factor ΔFosB, a truncated product of the *fosB* gene that is highly resistant to degradation and remains in the brain for weeks after the last drug exposure (Nye et al., 1995; Nestler, 2001; Nestler, 2004; Winstanley, 2007).

While ΔFosB appears to protect against cognitive impairments produced by acute cocaine challenge in cocaine-experienced vs. cocaine-inexperienced rats (Winstanley, 2007), virally-mediated over-expression of ΔFosB increases impulsive responding during periods of experimenter-enforced abstinence as assessed by the 5-choice serial reaction time task (Winstanley et al., 2009). Thus, increases in ΔFosB may represent an adaptive mechanism that enables animals to better function in the presence of cocaine; however, this neuroplasticity simultaneously contributes to the loss of control over cocaine seeking during withdrawal (Volkow and Fowler, 2000; Winstanley et al., 2009).

In concert with the above neuroadaptations, chronic drug use produces dysregulation of the glutamatergic system in the NA, which may facilitate the ability of drug-paired cues and contexts to elicit cocaine-seeking behavior (Kalivas and O'Brien, 2008). For instance, the activity of the cystine–glutamate antiporter in the NA decreases following withdrawal from repeated cocaine exposure (McBean, 2002). Normally, the antiportal permits the uptake of cystine in exchange for glutamate, thereby producing sufficient glutamatergic tone in the synapse to stimulate mGluR autoreceptors and to decrease synaptic glutamate release (Moran et al., 2005). Chronic cocaine-induced decreases in cystine-glutamate antiporter expression may facilitate relapse to cocaine-seeking behaviors by increasing the probability of glutamate release. In concert with this, normalizing antiporter activity and thus extracellular glutamate levels with *N*-acetyl cysteine prevents cocaine-primed (Baker et al., 2003b; Baker et al., 2003a) and drug context-induced reinstatement of drug-seeking behavior (Xie and Fuchs, unpublished findings). Alterations in glutamate release during cocaine exposure also produce enduring post-synaptic structural changes in NA neurons, including enhanced dendritic

spine density and dendritic branching on medium spiny neurons, and the development of these is correlated with both the degree of cocaine intake and the development of behavioral sensitization to cocaine (Robinson and Kolb, 1999; Robinson et al., 2001; Li et al., 2004; Robinson and Kolb, 2004).

Importantly, laboratory animals exhibit *incubation*, a time-dependent increase in the magnitude of cocaine-seeking behavior during the first two months of experimenter-enforced abstinence (Grimm et al., 2001; Grimm et al., 2003; Lu et al., 2004). Incubation may occur due to neuroadaptations that develop during withdrawal from cocaine. For instance, studies have shown that there is enhanced extracellular dopamine overflow in the BLA following a 1-month withdrawal period concomitant with increases in drug-seeking behavior (Tran-Nguyen et al., 1998). Similarly, withdrawal from chronic cocaine exposure produces long-lasting increases in AMPA and D3 receptor binding in the NA (Neisewander et al., 2004; Conrad et al., 2008; Ferrario et al., 2010; Wolf and Ferrario, 2010) as well as a progressive increase in the expression of the plasticity-associated gene, brain-derived neurotrophic factor, in the VTA, NA, and BLA after cocaine, but not sucrose, withdrawal (Grimm et al., 2003). Taken together, these findings indicate that both chronic cocaine exposure as well as abstinence from cocaine can produce long-term changes in the VTA-OFC-BLA circuit as well as in the larger mesocorticolimbic neural circuitry. This neuroplasticity may contribute to an enhanced ability of drug-associated environmental stimuli to produce compulsive drug-seeking behavior and thereby facilitate the transition from casual drug use to drug dependence.

Conclusions

This dissertation presents novel evidence that dopaminergic input from the VTA to the OFC, via dopamine D1-like receptor stimulation, regulates interactions between the OFC and the BLA that promote drug context-induced cocaine-seeking behavior. These observations extend a growing body of literature that indicate both the OFC and BLA are necessary for the motivational salience of drug-paired contexts to guide behavioral responding and provides novel evidence that the BLA and OFC exhibit interhemispheric and intrahemispheric interactions with respect to reward-related behaviors. Additionally, these experimental findings provide the first evidence that dopaminergic stimulation in the OFC is both necessary for a drug-paired context to elicit cocaine-seeking behaviors and that dopamine regulates interactions between the OFC and BLA that control drug context-induced cocaine seeking. Because the VTA provides the sole source of dopamine to the OFC, these experiments characterize – for the first time – a putative neural circuit consisting of the VTA, OFC, and BLA that critical regulates drug context-induced cocaine seeking. Overall, the experimental findings in the current project lay the groundwork for future endeavors that can characterize the precise neural mechanisms by which the VTA-OFC-BLA circuit promotes cocaine-seeking behaviors, identify critical neuroadaptations within this circuitry that promote drug dependence, and inform the development of novel pharmacotherapeutic interventions for cocaine addiction.

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