INVOLVEMENT OF THE LATERAL ORBITOFRONTAL CORTEX IN CONTEXT-INDUCED AND COCAINE-PRIMED REINSTATEMENT OF COCAINE SEEKING BEHAVIOR IN RATS

Heather C. Lasseter

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Psychology (Behavioral Neuroscience)

Chapel Hill
2009

Approved by

Advisor: Rita A. Fuchs-Lokensgard, Ph.D.

Reader: Regina M. Carelli, Ph.D.

Reader: Todd E. Thiele, Ph.D.

ABSTRACT

HEATHER C. LASSETER: Involvement of the lateral orbitofrontal cortex in context-induced and cocaine-primed reinstatement of cocaine-seeking behavior in rats
(Under the direction of Rita A. Fuchs-Lokensgard)

Orbitofrontal cortex (OFC) damage produces impaired decision-making, impulsivity, and perseveration of maladaptive behaviors and it potentially contributes to compulsive drug seeking in cocaine users. To investigate whether lOFC damage contributes to enhanced context-induced cocaine seeking in an animal model of drug relapse, the effects of lOFC temporary functional inactivation, pre-training lesions, and post-training lesions were assessed on the reinstatement of previously extinguished cocaine-seeking behavior (i.e., non-reinforced responses on a previously cocaine-paired lever). All rats were trained to lever press for intravenous cocaine infusions (0.2 mg/infusion) in a distinct environmental context followed by extinction training in a different context where cocaine was not available. In experiment 1 we assessed whether acute loss of lOFC output alters context-induced cocaine-seeking behavior by infusing either the GABA receptor agonists, baclofen and muscimol (1.0 mM, 0.5 µl/side), or vehicle into the lOFC or mOFC anatomical control region immediately before re-exposure to the cocaine-paired context. To evaluate the effects of long-term loss of lOFC output on this behavior, in experiment 2 we assessed the effects of pre-training bilateral NMDA (0.1 M, 0.6 µl/side) or sham lesions of the lOFC on cocaine-seeking behavior elicited by either re-exposure to the cocaine-paired context or a cocaine priming injection (0 or 10 mg/kg, i.p.) administered immediately before exposure to the extinction

context. GABA agonist-induced functional inactivation of the lOFC, but not mOFC, significantly attenuated context-induced cocaine seeking (Fuchs et al., 2004). In contrast, pre-training lOFC lesions enhanced cocaine context-induced cocaine seeking, but failed to alter cocaine-primed cocaine seeking. To identify whether the timing of the lOFC manipulation underlies this discrepancy, in experiment 3 we assessed the effects of post-training lOFC lesions on context-induced cocaine-seeking behaviors. In contrast to the effects of pre-training lesions and functional inactivation, post-training lOFC lesions failed to alter context-induced cocaine-seeking behavior. Overall, the results of the functional inactivation experiment suggest that the lOFC promotes context-induced cocaine-seeking behavior. However, prolonged loss of lOFC output may enhance the motivational salience of the cocaine-paired environmental stimuli by eliciting compensatory neuroadaptations, which may develop over time such that the effects of post-training lOFC lesions reflect an intermediate state of compensatory neuroplasticity.

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my advisor, Dr. Rita A. Fuchs-Lokensgard, for the invaluable guidance, wisdom, and encouragement she provided over the course of this project. In addition, I would like to thank Dr. Regina M. Carelli and Dr. Todd E. Thiele for their contributions to this manuscript and their support as members of my master's thesis committee. This research also integrally depended on the contributions of my colleagues, Dr. Donna R. Ramirez and Dr. Xiaohu Xie, who provided exceptional intellectual contributions and technical assistance.

TABLE OF CONTENTS

Page

LIST OF FIGURES

CHAPTER I
INTRODUCTION

Significance of the Problem

Cocaine addiction remains a prominent health and social issue in the United States. According to the 2007 National Survey on Drug Use and Health (NIDA), approximately 22.3 million people were classified as having substance abuse or dependence problems. 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 some form of medical or social support for their problem from such specialty treatment facilities.

The successful treatment of cocaine addiction is severely impeded by a high propensity for relapse seen in former drug users, even after they have completed detoxification and rehabilitation programs. Hence, drug addiction typically manifests as a chronic relapsing disorder characterized by compulsive drug seeking and drug craving that can be precipitated by exposure to drug-associated explicit cues or environmental contexts even after prolonged abstinence periods (Ehrman et al., 1992; Foltin and Haney, 2000; Rohsenow et al., 2007). 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). Thus, from an addiction treatment perspective it is critical to understand the neural mechanisms underlying the loss of control in drug seeking.

Modeling Drug Relapse

Over the course of chronic drug use, environmental stimuli are repeatedly paired with the effects of the drug. Through associative learning processes, these previously neutral stimuli can acquire conditioned rewarding properties such that the stimuli themselves become reward, conditioned reinforcing properties that maintain behavior, and/or incentive motivational properties in that stimuli elicit motivation for drug reinforcement. Given that re-exposure to drug-associated contexts is a major factor precipitating relapse in humans, several *in vivo* models have been developed to assess environmentally induced incentive motivation for cocaine reinforcement.

One of the most commonly used animal models for studying drug seeking and relapse behaviors is the extinction-reinstatement model. In this model, subjects are trained to respond for drug reinforcement in a distinct environmental context or drug reinforcement is explicitly paired with the presentation of a response-contingent conditioned stimulus (CS).

After animals reach an arbitrary response acquisition criterion, responding is extinguished in a distinctly different environmental context or in the absence of the response-contingent CS, respectively. To assess cue-induced relapse to drug seeking, animals are given a reinstatement test during which they are re-exposed to either the cocaine-paired context or the response-contingent CS in the absence of cocaine reinforcement. Responding during the reinstatement test is thought to reflect context- or CS-induced cocaine-seeking behavior, respectively. The contextual reinstatement model offers several advantages over the CS-induced reinstatement model. Namely, subjects receive uniform cocaine-cue exposure and, due to the lack of response-contingent CS presentations, responding likely provides an index of cocaine context-induced incentive motivation rather than conditioned reinforcement. Nevertheless, the contextual model has some limitations. The drug-associated context must be multi-modal in order to elicit robust reinstatement and to permit repeated testing using a within-subjects design. As a result, experimental results may not be readily generalizable to other cue types.

Similar to drug-associated cues, exposure to small amounts of drug can increase drug craving in human drug users and elicit drug seeking in laboratory animals (Jaffe et al., 1989; de Witt and Stewart, 1981). Drug primed reinstatement has been traditionally modeled by giving intraperitoneal injections of drug immediately before exposing subjects to the previous drug-paired operant chamber in the absence of the drug-paired CS. However, when drug priming is administered in the previously drug-paired operant chamber, the motivational effects of the drug-paired context may interact with the motivational effects of the drug prime to produce drug-seeking behavior. Hence, studying the effects of drug priming in a non-drug

paired context may provide a better model for isolating the motivational effects of drug priming on relapse behaviors.

While reinstatement models are highly similar to the human condition in that drug is self-administered, extinction training unfortunately reduces the face validity of the models given that humans seldom undergo explicit extinction training prior to relapse (Katz and Higgins, 2003). However, some extinction experience may be accrued in humans whenever cocaine use is not possible despite the presence of drug-related stimuli. Therefore, extinction-reinstatement models provide 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. Hence, in the present study we utilized the contextual reinstatement and drug-primed models to further investigate the involvement of the orbitofrontal cortex (OFC) in drug context-induced and drug-primed relapse.

Anatomy of the orbitofrontal cortex

The likely involvement of the OFC in drug-seeking behaviors stems from its functional connectivity with cortical and limbic brain regions. Rose and Woolsey (1948) suggested that homologous brain regions could be identified between different species based on the similarity of anatomical connections. Specifically, they proposed that the prefrontal cortex could be defined by mediodorsal thalamus (MD) afferents. Within the PFC, the orbital and agranular insular areas of the rat prefrontal cortex are thought to be homologous to the primate orbitofrontal cortex based on the pattern of input received from the medial and central areas of the MD, as well as their connectivity with the amygdala and ventral striatum (Rose and Woolsey, 1948; for review, see Price 2007). The primate OFC receives robust

sensory input from the olfactory cortex, gustatory cortex, somatosensory areas in the insula and parietal cortex, and visual association areas in the inferior temporal cortex, while the rodent OFC is strongly connected to the olfactory system and likely receives gustatory, somatosensory, and visual inputs, making this structure crucial for the integration of sensory information (Carmichael and Price, 1995; Price, 1985). Moreover, both the primate and rat OFC share robust connections with several elements of the known brain relapse circuitry. Most notably, the OFC has extensive reciprocal connections with the basolateral amygdala, a structure implicated in the attachment of motivational significance to environmental cues (Everitt et al., 1999). In addition, it shares both direct and indirect (via the thalamus) connections to the cingulate gyrus, hippocampus, prelimbic cortex, basal ganglia, nucleus accumbens, and lateral hypothalamus (Krettek and Price, 1977; Groenewegen et al., 1990; Ray and Price, 1992, 1993; Carmichael and Price, 1995; Haber et al., 1995; Oades and Halliday, 1987). Because many of these brain regions have been implicated in relapse to drug seeking (Fuchs et al., 2005; 2007; 2008; Lasseter et al., in prep; McFarland and Kalivas, 2001; McLaughlin and See, 2003; See et al., 2001; Sun and Rebec, 2003), the OFC is anatomically well positioned to integrate information from sensory and limbic regions and then use this information to generate outcome expectancies that guide subsequent behavioral responses, including drug seeking behavior (Holland and Gallagher, 2004).

Role of the orbitofrontal cortex in drug-seeking behavior

Numerous lines of evidence suggest that structural, physiological, and functional abnormalities in the frontal cortex may facilitate addictive behavior. Cocaine users exhibit abnormalities in frontal cortical regions, including decreased gray matter density in the

orbitofrontal cortex and anterior cingulate, diminished baseline blood glucose metabolism in the frontal cortex, and enhanced cue-evoked activation of the orbitofrontal cortex, some of which are proportional to drug use (Volkow and Fowler, 2000; Volkow et al., 1991; Franklin et al., 2002; Bolla et al., 2003; Matochick et al., 2003; London et al., 2000). Additionally, 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). Humans with OFC damage perform poorly on tasks that assess impulsivity, such as the Iowa Gambling Task, and are unable to use performance feedback following response selection to either modulate their emotional response or alter their response strategy (Bechara et al., 1994; Camille et al., 2004).

Similar to humans with OFC damage, animal with lateral OFC (lOFC) lesions exhibit deficits on reinforcer devaluation tasks, rapid reversal learning, and extinction learning (Gallagher et al., 1999; Pickens et al., 2003; Pickens et al., 2005; Izquierdo et al., 2004; Izquierdo and Murray, 2005). However, OFC damage does not appear to induce fundamental deficits in learning or primary motivation given that primates and rats with OFC lesions display normal responding for food reward and acquire novel visual and odor discriminations (Izquierdo et al., 2005; McDannald et al., 2005; Schoenbaum et al., 2002). Hence, observed deficits appear to reflect either an impairment in error detection, an inability to update outcome expectancies in the face of changing reward contingencies, or an inability to express updated expectancies in behavioral responses.

Involvement of the lOFC in drug relapse

Recent studies have suggested that cocaine-seeking behavior is mediated by a corticolimbic "brain relapse circuitry" comprised of the hippocampus, amygdala, prefrontal cortex, nucleus accumbens, as well as the OFC. In a previous study we investigated the effects of lOFC lesions and functional inactivation on CS-induced and drug-primed reinstatement of cocaine seeking (Fuchs et al., 2004). In this study, pre-training lOFC lesions greatly potentiated cocaine-primed reinstatement to cocaine seeking in a preservative manner during a reinstatement test session held in the previously cocaine-paired operant chamber (Fuchs et al., 2004). Conversely, temporary functional inactivation of the lOFC produced by infusions of either $GABA_A$ and $GABA_B$ agonists or tetrodotoxin (TTX) failed to alter cocaine-primed reinstatement (Fuchs et al., 2004; Capriles et al., 2006). This pattern of findings suggests that chronic loss of the lOFC output may elicit neuroadaptations in other elements of the brain relapse circuitry that are responsible for the observed lesion-induced functional impairment in cocaine-primed cocaine-seeking behavior.

Several lines of evidence suggest that the ability of cocaine-paired cues to evoke cocaine-seeking depends on the functional integrity of the lOFC. Cocaine-experienced rats exhibit enhanced expression of the activity-dependent immediate-early genes (IEG) *c-fos*, *zif-268, BDNF,* and *arc* in the OFC following exposure to a cocaine-paired context relative to IEG expression observed in saline-yoked controls exposed to a saline-paired context or cocaine-experienced rats exposed to an alternate context (Hearing et al., 2008). Consistent with this, temporary inactivation of the lOFC prevents CS-induced cocaine seeking, suggesting that the functional integrity of the lOFC is necessary for cocaine-paired cues to elicit motivation for cocaine reinforcement (Fuchs et al., 2004). Moreover, repeated cocaine

intake or self-administration experience may produce enduring neuroadaptations in the OFC given that *Arc* expression is enhanced in cocaine-experienced rats regardless of whether rats were exposed to cocaine-paired cues (Zavala et al., 2008).

Cellular and molecular changes in orbitofrontal cortical neurons induced by cocaine self-administration may reflect aberrant neuroadaptations in neuronal processing related to learning and memory. Recent neurophysiological evidence suggests that cocaine exposure induces inflexible encoding in OFC neurons during an odor discrimination task and that such abnormalities are associated with impaired reversal learning (Stalnaker et al., 2006). Importantly, behavioral deficits resulting from OFC lesions do not stem directly from abnormal OFC output, but rather reflect inflexible encoding in basolateral amygdala (BLA) neurons. Consistent with this, unilateral OFC lesions impair cue-selective firing in the BLA during reversal learning (Saddoris et al., 2005). Moreover, OFC lesion-induced deficits in reversal learning are actually rescued by BLA lesions (Stalnaker et al., 2007). These findings are consistent with the idea that OFC damage in humans or lab animals produces inflexible behavior. However, it has yet to be determined whether OFC damage produces compulsive responding to cocaine or cocaine-paired conditioned stimuli via similar mechanisms.

Interestingly, lOFC lesions fail to enhance reinstatement elicited by an explicit, response-contingent CS, which contrasts with evidence that lOFC lesions potentiate cocaine-primed reinstatement (Fuchs et al., 2004). While this suggests the lOFC may play a different role in explicit CS-induced and cocaine-primed cocaine-seeking behavior, the apparent inability of the CS to induce perseverative cocaine seeking may stem from ceiling effects related to steady cocaine-seeking behavior maintained by conditioned reinforcement in the sham control group. Unlike in the CS-induced reinstatement model, responding is unlikely

to be maintained by conditioned reinforcement in the context-induced reinstatement model because cues are passively presented. Hence, the latter model may be more suitable for evaluating cue-induced incentive motivation for cocaine given that putative perseveration in lOFC-lesioned rats is not obscured by CS-maintained responding in sham animals. Furthermore, it is worthwhile to re-investigate the effects of lOFC lesions on cocaine-primed reinstatement of cocaine seeking when cocaine-priming injections are administered in a non-cocaine-paired context to exclude potential context-cocaine interactions and thereby isolate the effects of lOFC lesions on cocaine-induced incentive motivation.

Hypothesis and Predictions

To evaluate whether lOFC damage contributes to enhanced context-induced cocaine-seeking behavior in the absence of conditioned reinforcement, the present study investigated whether bilateral lOFC functional inactivation (experiment 1) or pre-training lesions (experiment 2) alter the reinstatement of cocaine-seeking behavior following re-exposure to a distinct cocaine-predictive environmental context after extinction training in a different context (i.e. extinction context). Based on previous research (Fuchs et al., 2004), we hypothesized that lOFC functional inactivation would attenuate context-induced cocaine seeking in experiment 1 given that the functional integrity of the lOFC appears to be necessary for the expression of explicit CS-induced cocaine-seeking behavior. In addition, we hypothesized that lOFC lesions would fail to impair the acquisition of cocaine self-administration or extinction learning in experiment 2, but would potentiate both context-induced and cocaine-primed reinstatement of cocaine-seeking behaviors in a perseverative manner (Fuchs et al., 2004). Unlike in our previous study, the cocaine-primed reinstatement

test was conducted in the extinction context to eliminate potential interactions between the motivational effects of cocaine and those of the previously cocaine-paired contextual stimuli (Fuchs et al., 2004).

In experiments 1 and 2, lOFC functional inactivation and lesions had distinctly different effects on context-induced reinstatement. Importantly, functionally inactivating the lOFC is a fundamentally different manipulation from administering permanent excitotoxic lesions in two respects: first, the manipulations, by necessity, occur at different time points relative to associative learning processes and second, the manipulations have different neurochemical effects. For instance, while both manipulations attenuate glutamate output from the lOFC, functional inactivation with $GABA_A$ and $GABA_B$ agonists increases – while excitotoxic lesions disrupt – GABA neurotransmission (Beal et al., 1991; Matsumoto et al., 2003). To investigate the source of discrepancy between experiments 1 and 2, experiment 3 was designed to minimize differences in the timing (i.e. pre- or post-training) of the functional inactivation and lesion manipulation. To this end, lOFC lesions were induced following self-administration and extinction training, and the effects of these post-training lOFC lesions were assessed on context-induced reinstatement of cocaine-seeking behavior. Consistent with the hypothesis that pre-training lOFC lesions potentiate context-induced cocaine seeking due to neuroadaptations that occur following lesion induction, we predicted that post-training lOFC lesions would either A) produce similar effects as lOFC functional inactivation if the loss of lOFC output during self-administration training critically underlies these effects or B) have similar effects as pre-training lOFC lesions if lesion-induced neuroadaptations are sufficient to enhance context-induced motivation for cocaine.

CHAPTER II
METHODS AND MATERIALS

Subjects

Male Sprague-Dawley rats (n = 79), weighing 250-300 g at the start of the experiment, were individually housed in a temperature- and humidity-controlled vivarium on a reversed light-dark cycle. Rats were maintained on 20-25 gm of rat chow per day with water available *ad libitum*. The housing and treatment of the rats followed guidelines outlined in the *Guide for the Care and Use of Laboratory Rats* (Institute of Laboratory Animal Resources on Life Sciences, 1996) and the study protocol was approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Procedures

Food Training. Rats were acclimated to handling 2 days before being trained to lever press on a fixed ratio 1 (FR1) 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 Institute, Allentown, PA, USA) during a 16-h overnight food training session. The chambers were equipped with two retractable levers, a stimulus light above each lever, a food pellet dispenser between the levers, a house light on the wall opposite to the levers, and a speaker connected to a tone generator (Coulbourn Institute, Allentown, PA, USA). During the food training session, stimuli subsequently used for contextual cocaine conditioning were not present. Each active (right) lever response resulted in delivery of one food pellet;

inactive (left) lever responses had no programmed consequences. Food pellet dispensers were removed from the chambers after food training.

Surgery. At least 48-h after food training, rats was pre-anesthetized using ketamine hydrochloride and xylazine (66 and 1.33 mg/kg, i.p., respectively). Full anesthesia was maintained during surgery with pentobarbital sodium (50mg/kg, i.p.) so that ketamine would not inhibit the development of NMDA-induced excitotoxic lesions induced immediately after surgery in Experiment 1. The rats received the same anesthesia regimen in all experimental groups. Chronic indwelling catheters were constructed in-house using bent-steel cannulae with a screw-type connector (Plastics One, Roanoke, VA, USA), SILASTIC tubing (10 cm, inner diameter, 0.64 mm; outer diameter, 1.19 mm; Dow Corning, Midland, MI, USA), Prolite monofilament mesh (Atrium Medical Corp., Hudson, NH, USA) and cranioplastic cement, as described before (Fuchs et al., 2007). The end of the catheter was inserted 3.25 mm into the right jugular vein and secured with suture to surrounding tissue. The catheter ran subcutaneously and exited the back between the scapulae. Immediately following catheterization, rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL, USA) and bilateral stainless-steel guide cannulae (26 gauge; Plastics One) were aimed dorsal to the target brain structure in the rats' brain using standard stereotaxic procedures. The guide cannulae were secured to the skull using three screws and cranioplastic cement. All rats were cannulated regardless of experimental manipulation so that differences in surgical history could not account for potential differences across the experiments.

To extend catheter patency during the recovery period, catheters were flushed daily with 0.1 ml of an antibiotic solution of cefazolin (10.0 mg/ml; Schein Pharmaceuticals,

Albuquerque, NM, USA) dissolved in heparinized saline (70 U/ml; Baxter Health Care Corp, Deerfield, IL, USA). Thereafter, catheters were flushed with 0.1 ml of heparinized saline (10 U/ml) before each self-administration session and with 0.1 ml of the cefazolin solution and 0.1 ml of heparinized saline (70 U/ml) after each session. Stylets (Plastics One) were placed in catheters and cannulae to prevent both occlusion and infection. Catheter patency was periodically verified by infusing 0.1 ml of propofol (10 mg/ml, i.v. Eli Abbot Lab, North Chicago, IL, USA), a fast-acting barbiturate that produces a rapid loss of muscle tone only when administered intravenously.

Excitotoxic lesions and intracranial drug infusions. For all intracranial infusions, stainless-steel injection cannulae (33 gauge; Plastics One) were inserted to a depth of 1 mm (mOFC) or 2 mm (lOFC) below the tip of the guide cannulae. The injection cannulae were connected to 10 µl Hamilton syringes (Hamilton, Reno, NV) mounted on an infusion pump (KD Scientific, Holliston, MA). Either the $GABA_B$/$GABA_A$ agonist cocktail baclofen/muscimol (BM; 1.0 and 0.1 mM, respectively), N-methyl-D-aspartic acid (NMDA; 0.1 M; pH ~7.0), or phosphate buffered saline vehicle (VEH) was then infused bilaterally into the lOFC or mOFC (control region) over 2 min at a volume of 0.6 or 0.3 µl per hemisphere, respectively. The injection cannulae were left in place for 1 min before and 1 min (BM) or 4 min (NMDA) after the infusion to minimize diffusion dorsally along the cannulae shaft. The doses of BM and NMDA were selected based on previous research showing these intra-lOFC doses alter explicit CS-induced and drug-primed cocaine-seeking behavior, respectively (Fuchs et al., 2004). The timing of the above intracranial manipulations was varied from experiment to experiment as described below in the specific methods section for experiments 1, 2, and 3.

Operant Conditioning Contexts. Self-administration and extinction sessions were conducted in operant conditioning chambers configured to one of two unique environmental contexts that differed along four sensory modalities. Context 1 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 2 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 self-administration training. As in our previous studies, these stimuli were presented throughout each session independent of responding (Fuchs et al. 2007; Fuchs et al. 2008).

Self-Administration Training. Subjects were assigned randomly to receive self-administration training in Context 1 or 2. Self-administration training was conducted during the rats' dark cycle in one of the two distinct environmental contexts during 2-h sessions. The rats' indwelling catheters were connected to liquid swivels (Instech, Plymouth Meeting, PA, USA) via polyethylene 20 tubing that was incased in steel spring leashes (Plastics One). The swivels were suspended above the operant conditioning chambers and were connected to infusion pumps (Coulbourn Institute, Allentown, PA, USA). Rats were trained to press on the right active lever on an FR1 schedule of cocaine reinforcement (0.2 mg/0.1 ml of cocaine hydrochloride, duration 4 s, i.v.; NIDA, Research Triangle Park, NC, USA). Responses on

the left, inactive lever were recorded but had no programmed consequences. A 20-sec time-out period followed each infusion during which lever responses were recorded, but had no programmed consequences. Training continued until the rats successfully obtained ≥10 cocaine infusions per session on at least 10 training days (i.e., acquisition criterion).

Extinction Training. After meeting the acquisition criterion for self-administration, rats underwent daily 2-h extinction training sessions in the environmental context that distinctly differed from the self-administration context. Active and inactive lever presses were recorded, but had no programmed consequences. Extinction training continued for a minimum of 7 sessions plus additional extinction training sessions, as needed, until the rats reached the extinction criterion (≤25 active lever presses per session on 2 consecutive sessions).

Locomotor Activity Testing. Motor side effects of intracranial manipulations can affect instrumental behavior. To assess the general motor effects of the experimental manipulations, locomotor activity was measured in a novel Plexiglas chamber (42 x 20 x 20 cm) equipped with an array of eight photodetectors and corresponding light sources. 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 a 2-h test session. Locomotion was assessed within 72-h of the reinstatement test as described below in the specific methods sections for experiments 1-3.

Experiment 1

Effects of functional inactivation of the OFC on drug context-induced cocaine-seeking behavior. Experiment 1 was designed to evaluate whether BM-induced functional inactivation of the lOFC would disrupt drug context-induced reinstatement of cocaine-seeking behavior in accordance with previous findings that lOFC functional inactivation attenuates CS-induced cocaine seeking (Fuchs et al., 2007). Because BM spread cannot be visualized, anatomical control groups received BM or VEH infusions into the mOFC to assess whether the effects were sub-region specific within the OFC.

Following surgery, rats underwent a 5-day post-operative recovery period before undergoing self-administration training in one context and extinction training in a different context. On extinction day 4, rats were acclimated to the intra-OFC infusion procedure. During the adaptation procedure, rats were held gently by the experimenters and injection cannulae were bilaterally inserted into the rats' guide cannulae and left in place for 4 minutes, but no drug was infused. Immediately following the adaptation procedure, rats were placed into the operant chamber for an extinction session.

After the rats reached the extinction criterion, reinstatement of cocaine-seeking behavior was assessed in the cocaine-paired context or extinction context over the course of 4 test sessions using a fully counterbalanced within-subjects testing design. The order of the tests in the extinction context and the cocaine context, as well as the order of intracranial treatments (BM, VEH), were counterbalanced based on previous cocaine intake during self-administration training. On the test day, intracranial infusions were administered while rats were gently held by the experimenter. Immediately thereafter, rats were placed into the operant conditioning chamber for a 1-h test session during which active and inactive lever

responses were recorded, but had no programmed consequences. Session length was 1-h to allow for repeated testing without significant extinction learning. Subjects received additional extinction sessions between test sessions until they reached the extinction criterion (≤25 lever presses per session for 2 consecutive days). Rats were given two, 1-h locomotor activity test sessions 24-h after the last test session. Immediately before each locomotor test, rats received either a BM or VEH infusion consistent with the order of treatment received during the reinstatement test sessions.

Experiment 2

Effects of pre-training lesions of the lOFC on context-induced and cocaine-primed cocaine-seeking behavior. Experiment 2 was designed to evaluate the effects of prolonged loss of lOFC output on the reinstatement of cocaine-seeking behavior. Immediately after stereotaxic surgery, rats received infusions of either NMDA or VEH into the lOFC as described above, with lesion groups assignment randomized. Rats were then given a 7-day post-operative recovery period to allow the lesions to develop followed by self-administration in one context and extinction training in a different context. 72-h prior to the first reinstatement test session, locomotor activity was assessed in all rats in order to examine the effects of lesion and sham manipulations on general activity at the approximate time of reinstatement testing.

Context-induced and cocaine-primed reinstatement of cocaine-seeking behavior was assessed in the same subjects over 2 test sessions. Between test days rats received a minimum of 2 extinction sessions until they reached the extinction criterion (≤25 lever presses per session for 2 consecutive days). During the context-induced reinstatement test, rats were re-exposed to the cocaine context in the absence of cocaine reinforcement for a 2-h test session

during which lever responses had no programmed consequences. Responding in the extinction context 24-h before the cocaine-context reinstatement test served as the measure of lesion effects on baseline operant responding. For the cocaine-primed reinstatement test, rats received an i.p. injection of cocaine (10 mg/kg, i.p.) or saline (1 ml/kg, i.p.) using a between-subjects design. Assignment to cocaine or saline priming injections was based on previous cocaine intake during self-administration training. After the i.p. injection, rats were placed into the extinction context for a 2-h test session during which lever responses had no programmed consequences. The cocaine priming dose was selected based on previous studies demonstrating that it produces robust reinstatement with minimal variability in responding (Lynch and Carroll, 2000; Fuchs et al. 2004; Placenza et al., 2005).

Experiment 3

Effects of post-training lOFC lesions on drug context-induced cocaine-seeking behavior. Functional inactivation of lOFC attenuated context-induced reinstatement of cocaine seeking in experiment 1, whereas pre-training lOFC lesions potentiated cocaine context-induced cocaine seeking in experiment 2. To determine whether differential effects of lOFC lesions and functional inactivation stemmed from the timing of the manipulation relative to associative learning, experiment 3 was designed to evaluate the effects of *post-training* lOFC lesions on context-induced reinstatement. After reaching self-administration and extinction criteria, rats received infusions of either NMDA or VEH into the lOFC, with assignment to lesion group counterbalanced based on previous cocaine intake during self-administration training. Rats were given a 7-d post-operative recovery period to allow the lesions to develop. Thereafter, rats received a minimum of 2 extinction sessions to re-establish

extinction baselines to pre-lesion levels and eliminate spontaneous recovery before a context-induced reinstatement test was conducted. During the context-induced reinstatement test, rats were re-exposed to the cocaine-paired context in the absence of cocaine reinforcement for 2-h during which lever responses had no programmed consequences. Responding in the extinction context 24-h before the context-induced reinstatement test served as the measure of lesion effects on baseline operant responding. Locomotor activity was assessed 72-h prior to the reinstatement test in order to examine the effects of lesions on general activity at the approximate time of reinstatement testing, as in Experiment 1.

Histology

Immediately following the last test session, rats were fully anesthetized with 0.2 ml ketamine (66 mg/ml, i.v.) after which they were decapitated and their brains were dissected out. Brains of rats in Experiments 2 and 3 were flash-frozen in methylbutane (J.T. Baker, Phillipsburg, NJ) and stored at -70°C until coronal sections (14 μm) were taken on a cryostat. The extent of the lesions and/or cannula placements were verified under a light microscope. The pattern of cell loss or the most ventral point of each cannula track was mapped onto schematics of the appropriate plates from the rat brain atlas of Paxinos and Watson (1997).

Statistical Analysis

Only data from rats with correctly placed lesions and cannula placements were included in data analysis. In experiment 1, repeated measures ANOVAs were used to analyze lever responses on the test days with treatment (BM, VEH), context (extinction context), and time (three, 20-min intervals) as factors, where appropriate. Locomotor

activity was assessed using a repeated measures ANOVA with treatment (BM, VEH) and time (three, 20-min intervals) as factors. Significant main and interaction effects were investigated using simple main-effects test (Tukey test) and Tukey post-hoc tests with the alpha set at 0.05. In experiments 2 and 3, mixed-factorial ANOVAs were used to analyze lever responses and cocaine intake during self-administration training and extinction training with lesion (sham, lesion) and group (sham, lesion) as the between-subjects factors and day as the within-subjects factors. Mixed-factorial ANOVAs were used to analyze lever responses on the context-induced reinstatement test days with lesion (sham, lesion) as the between subjects factors and context (extinction context, reinstatement context) and time (six, 20-min intervals) as the within-subjects factors. In experiment 2, two-factorial ANOVAs were used to analyze lever responses on the cocaine-primed reinstatement test day with lesion (sham, lesion) and priming (saline, cocaine) as the between-subjects factors. Locomotor activity was assessed using mixed-factorial ANOVAs with lesion (sham, lesion) as the between-subjects factors and time (six, 20-min intervals) as within-subjects factors. Interaction effects were investigated, where appropriate, using Tukey *post hoc* tests with alpha set at 0.05.

CHAPTER III
RESULTS

Histological Analysis

The photomicrographs in **Fig 1A** are of representative brains from rats that received BM- or VEH-infusions into the lOFC or mOFC, as well as photomicrographs of representative lOFC lesions. Furthermore, the schematic diagrams in **Fig 1B** depict the distribution of injection cannula placements in the brains of rats from experiments 1-3 as well as the extent and the location of the smallest and largest lesions in experiment 2-3. The lOFC target region was defined as an aggregate of the lateral and ventrolateral subregions of the OFC based on the atlas of Paxinos and Watson (1997). After lOFC lesions, cell loss was observed in the ventrolateral and lateral regions of the lOFC as well as in the adjacent agranular insular (AIC) and frontal cortices in a subset of rats. The mOFC target region was defined as the combination of medial and ventromedial subregions of the OFC based on the atlas of Paxinos and Watson (1997). The most ventral points of the cannulae tracts were bilaterally located within the lOFC or mOFC for all rats whose data were included in the analyses. Data obtained from rats with misplaced cannulae or with lesions in unintended brain regions were excluded. For experiment 1, the resulting groups (sample sizes) were as follows: lOFC functional inactivation, $n = 10$; mOFC functional inactivation, $n = 8$. For experiment 2, the resulting groups were: pre-training lOFC lesion (cocaine priming, $n = 11$; saline priming, $n = 11$); pre-training lOFC sham (cocaine priming, $n = 9$; saline priming, $n =$

10). For experiment 3, the resulting groups were: post-training lOFC lesion, *n* = 9; post-training lOFC sham, *n* = 11.

Experiment 1

Self-Administration and Extinction Responding. Groups received intra-lOFC or intra-mOFC BM or VEH treatment immediately prior to two reinstatement test sessions, with treatment order counterbalanced based on cocaine intake during self-administration training. Consequently, for the lOFC-cannulated subjects, active lever responding, inactive lever responding, and cocaine intake during the last 7 days of self-administration did not vary as a function of treatment order. The mean active and inactive lever responding was 33.97 ± 5.15 and 2.97 ± 1.56, respectively, while the mean cocaine intake was 17.40 ± 1.245 infusions (11.60 ± 0.83 mg/kg per session). Similarly, for the mOFC-cannulated subjects, active lever responding, inactive lever responding, and cocaine intake during the last 7 days of self-administration did not vary as a function of treatment order. The mean active and inactive lever responding was 34.00 ± 3.374 and 1.88 ± 1.38, respectively, while the mean cocaine intake was 23.38 ± 2.421 infusions (15.58 ± 1.493 mg/kg per session).

There were no pre-existing differences in active or inactive lever responding during extinction training as a function of treatment order. In lOFC-cannulated subjects, as well as in mOFC-cannulated subjects, the mean number of days (mean ± SEM) to reach the extinction criterion was 7.00 ± 0.00 (data not shown).

Effects of lOFC Functional Inactivation on Context-induced Reinstatement of Cocaine-seeking Behavior. Re-exposure to the previously cocaine-paired context enhanced active lever responding relative to responding in the extinction context, and lOFC functional

inactivation impaired responding in a lever-selective manner. The ANOVA of active lever responses following intra-lOFC BM or VEH pretreatment before exposure to either the cocaine-paired or the extinction context revealed significant treatment X context interaction ($F_{(1, 9)} = 52.494$, $p < 0.001$), treatment main ($F_{(1,9)} = 40.218$, $p < 0.001$), and context main effects ($F_{(1,9)} = 39.439$, $p < 0.001$) (**Fig 2A**). Thus, re-exposure to the cocaine-paired context increased active lever responding following VEH pretreatment, but not BM pretreatment, relative to responding in the extinction context (Tukey, $p < 0.001$). Moreover, BM pretreatment administered into the lOFC significantly attenuated active lever responding in the cocaine-paired context relative to VEH treatment (Tukey, $p < 0.01$) without altering active lever responding in the extinction context. The ANOVA of active lever responses across the three 20-min intervals of the 1-h reinstatement test confirmed that there were significant treatment main ($F_{(2,18)} = 64.310$, $p < 0.001$) and time main effects ($F_{(2,18)} = 5.926$, $p = 0.011$), but no treatment X time interaction effect ($F_{(2.18)} = 0.399$, $p = 0.677$). Hence, BM treatment administered into the lOFC attenuated active lever responding throughout the test session relative to VEH treatment (**Fig 2B).**

The ANOVA of inactive lever responses revealed no significant treatment X context interaction ($F_{(1, 9)} = 0.638$, $p = 0.139$), treatment main ($F_{(1,9)} = 0.098$, $p = 0.761$), or context main effect ($F_{(1,9)} = 0.455$, $p = 0.517$) (**Fig 2C**). Hence, exposure to the cocaine-paired context did not alter responding on the inactive lever relative to responding in the extinction context. Furthermore, intra-lOFC BM treatment failed to alter inactive lever responding relative to VEH treatment in either context.

Effects of mOFC Functional Inactivation on Context-Induced Reinstatement of Cocaine-seeking Behavior. Following re-exposure to the cocaine-paired context or extinction context, mOFC functional inactivation failed to alter lever responding (**Fig 3A**). The ANOVA of active lever responses following intra-mOFC BM or VEH pretreatment before re-exposure to either the cocaine-paired or extinction context revealed a significant context main effect ($F_{(1,7)} = 17.184$; $p = 0.004$), but no significant treatment X context interaction ($F_{(1,7)} = 0.370$, $p = 0.562$) or treatment main effect ($F_{(1,7)} = 0.057$, $p = 0.819$). In addition, the 2X3 ANOVA of active lever responses across three, 20-min intervals of the 1-h reinstatement test revealed a significant time main effect ($F_{(2,14)} = 9.088$, $p = 0.03$), but no treatment X time interaction ($F_{(2,14)} = 0.139$, $p = 0.872$) or treatment main effect ($F_{(1,7)} = 0.218$; $p = 0.650$) (**Fig 3B**). Hence, re-exposure to the cocaine-paired context enhanced lever responding to a similar extent following BM or VEH treatment administered into the mOFC relative to responding in the extinction condition. Additionally, BM pretreatment failed to alter active lever responding in the cocaine-paired context or extinction context relative to VEH pretreatment.

Finally, the ANOVA of inactive lever responses indicated no treatment X context interaction ($F_{(1,7)} = 0.517$, $p = 0.495$), treatment main ($F_{(1,7)} = 0.040$, $p = 0.0847$), or context main effect ($F_{(1,7)} = 1.197$, $p = 0.310$) (**Fig 3C**). Hence, re-exposure to the cocaine-paired context following BM or VEH treatment did not alter inactive lever responding relative to responding in the extinction context. Furthermore, intra-mOFC BM pretreatment failed to alter inactive lever responding relative to VEH pretreatment in either context.

Effects of lOFC and mOFC Functional Inactivation on Locomotor Activity: lOFC functional inactivation attenuated motor activity during the locomotor activity test relative to VEH treatment (**Fig 4A**). The 2X3 ANOVA of photobeam breaks during the three, 20-min intervals of the locomotor test indicated significant time main ($F_{(2,18)} = 61.162$, $p < 0.001$) and treatment main effects ($F_{(1,9)} = 5.895$, $p = 0.038$), but no treatment X time interaction effect ($F_{(2,18)} = 1.367$, $p = 0.280$). Thus, both groups exhibited a decrease in motor activity following the first 20-min interval of the locomotor test session (interval 1 > interval 2-3; Tukey $p < 0.01$). Furthermore, intra-lOFC BM treatment decreased locomotor activity relative to VEH treatment.

Unlike lOFC functional inactivation, mOFC functional inactivation failed to alter motor activity relative to VEH treatment. The 2X3 ANOVA of photobeam breaks indicated a significant time main effect ($F_{(2,14)} = 54.306$, $p < 0.001$), but no treatment X time interaction ($F_{(2,14)} = 0.415$, $p = 0.668$) or treatment main effect ($F_{(1,7)} = 0.037$, p = 0.853) (**Fig 4B**). Thus, following pretreatment with either VEH or BM, motor activity declined at a similar rate following the first 20-min interval of the locomotor test session (interval 1 > interval 2-3; Tukey $p < 0.01$).

Experiment 2

Self-Administration Responding: Pre-training lOFC lesions did not impair cocaine self-administration given that the lOFC lesion and sham groups exhibited similar levels of lever responding and cocaine intake. The mean ± SEM daily cocaine intake for the lOFC lesion and sham group was 24.56 ± 1.60 and 24.41 ± 1.58 infusions/session (16.37 ± 1.07 and 16.27± 1.05 mg/kg per session), respectively (**Fig 5**). The mixed factors ANOVA for active

lever responses over the last 7 days of cocaine self-administration training indicated no lesion X day interaction ($F_{(6,234)} = 1.029$, $p = 0.407$), day main ($F_{(6,234)} = 0.730$, $p = 0.626$), or lesion main effect ($F_{(1,39)} = 1.217$, $p = 0.266$). While the ANOVA of inactive lever presses indicated a significant day main effect ($F_{(6,234)} = 2.486$, $p = 0.024$), there was no significant lesion X day interaction effect ($F_{(6,234)} = 0.213$, $p = 0.974$) or lesion main effect ($F_{(1,39)} = 0.016$, $p = 0.937$). Hence, inactive lever presses decreased over time for both lesion and sham groups. Finally, the ANOVA of cocaine intake revealed no significant lesion X day interaction ($F_{(6,234)} = 1.572$, $p = 0.156$), day main ($F_{(6,234)} = 0.653$, $p = 0.688$), or lesion main effect ($F_{(1,39)} = 0.523$, $p = 0.474$). Overall, these results indicate there were no differences between the lesion and sham groups in lever responding or cocaine intake during cocaine self-administration training.

Extinction Responding: Pre-training lOFC lesions did not impair extinction learning upon removal of cocaine reinforcement (**Fig. 5B**). The lOFC lesion and sham controls groups did not differ in the mean number of days they needed to reach the extinction criterion ($t_{(39)} = 1.294$, $p = 0.214$; Sham mean = 7.26 $\pm$ 0.214, Lesion mean = 7.00 $\pm$ 0.00; data not shown). Furthermore, the ANOVA of active lever responses on the first 7 days of extinction training revealed a significant day main effect ($F_{(6,234)} = 26.747$, $p < 0.001$), but no lesion X day interaction effect ($F_{(6,234)} = 1.922$, $p = 0.072$) or lesion main effect ($F_{(1,39)} = 1.355$, $p = 0.251$). Hence, active lever responding declined following removal of cocaine reinforcement irrespective of lesion condition (day 1 > day 2-7, Tukey, $p < 0.001$). Similarly, the ANOVA of inactive lever responses revealed a significant day main effect ($F_{(6,234)} = 3.615$, $p = 0.002$), but no lesion X day interaction ($F_{(6,234)} = 2.413$ $p = 0.128$) or lesion main effect ($F_{(1,39)} =$

1.041, $p = 0.400$). The day main effect stemmed from higher levels of inactive lever responding on the first day of extinction training independent of lesion condition relative to responding on subsequent extinction days (day 1 > days 2-7, Tukey, $p < 0.01$). Overall, both groups exhibited high levels of active and inactive lever responding on the first day of extinction training after which responding declined at similar rates for both groups, suggesting there were no differences between the lOFC lesion and sham groups in lever responses during extinction training.

Context-induced Reinstatement of Cocaine-seeking Behavior: Re-exposure to the cocaine-paired context on the reinstatement test day increased lever responding in all groups relative to responding in the extinction context, while the pre-training lOFC lesions selectively altered active lever responding relative to the sham manipulation (**Fig 6**). The ANOVA of active lever responses on the reinstatement test day and preceding extinction day revealed a significant context X lesion interaction effect ($F_{(1,39)} = 5.461$, $p = 0.025$) as well as context main ($F_{(1,39)} = 130.748$, $p < 0.001$) and lesion main effects ($F_{(1,39)} = 6.663$, p = 0.014 5.461, $p = 0.025$) (**Fig 6A**). Thus, re-exposure to the previously cocaine-paired context elicited enhanced responding in all groups relative to responding the extinction context (Tuket's test, $p < 0.01$). However, the lOFC lesion group exhibited greater active lever responding in the cocaine-paired context relative to the sham controls (Tukey's test, $p < 0.01$). The 2X6 ANOVA of active lever responses across the six, 20-min intervals of the reinstatement test session revealed a significant time X lesion interaction effect ($F_{(5,195)} = 2.771$, $p = 0.019$) as well as time main ($F_{(5,195)} = 17.243$, $p < 0.001$) and lesion main effects ($F_{(1,39)} = 6.244$, $p =$

0.017) (**Fig 6B**). Thus, lOFC lesions increased active lever responding relative to the sham lesion during the first 20-min test interval of the reinstatement test (Tukey $p < 0.05$).

The ANOVA of inactive lever responses on the reinstatement test day and preceding extinction day revealed a significant context main effect ($F_{(1,39)} = 5.666$, $p = 0.022$), but no context X lesion interaction effect ($F_{(1,39)} = 0.742$, $p = 0.342$) or lesion main effect ($F_{(1,39)} = 1.195$, $p = 0.661$) (**Fig 6C)**. Thus, re-exposure to the cocaine-paired context increased inactive lever responding relative to responding in the extinction context independent of lesion condition. Furthermore, there were no differences between the lOFC lesion and sham groups in inactive lever responding in the cocaine paired context relative to responding in the extinction context.

Cocaine-primed Reinstatement of Cocaine-seeking Behavior: Exposure to an intraperitoneal cocaine priming injection produced a robust increase in active lever responding in both the lOFC lesion and sham lesion groups relative to saline priming injections (**Fig 7**). The 2X2 ANOVA of active lever responses exhibited by the lOFC lesion and sham group following pretreatment with either cocaine-priming or saline injections prior to exposure to the extinction context revealed a priming injection main effect ($F_{(1,41)} = 43.693$, $p < 0.001$), but no priming injection X lesion interaction ($F_{(1,37)} = 0.378$, $p = 0.542$) or lesion main effect ($F_{(1,37)} = 1.566$, $p = 0.219$) (**Fig 7A**). Hence, cocaine-priming injections enhanced active lever responding in both groups in the extinction context relative to saline injections. Furthermore, there were no differences in active lever responding between the lOFC lesion and sham control groups following cocaine-priming or saline injections relative to the sham control group.

The 2X2 ANOVA of inactive lever responses exhibited by the lOFC lesion and sham group following pretreatment with either cocaine-priming or saline injections on the cocaine-primed reinstatement day indicated no priming injection X lesion ($F_{(1,37)} = 0.378$, $p = 0.542$), priming injection main ($F_{(1,37)} = 2.042$, $p = 0.161$), or lesion main effect ($F_{(1,37)} = 1.566$, $p = 0.219$) (**Fig 7B)**. Hence, cocaine-priming injections did not alter inactive lever responding relative to saline injections in either group. Furthermore, there were no differences in inactive lever responding between the lOFC lesion and sham groups.

Locomotor Activity: The ANOVA of photobeam breaks across the six, 20-min intervals of the locomotor test session revealed a time main effect ($F_{(5,195)} = 78.827$, $p < 0.001$), but no lesion X time interaction effect ($F_{(5,195)} = 1.458$, $p = 0.807$) or lesion main effect ($F_{(1,39)} = 1.038$, $p = 0.315$) (**Fig 8**). Both lOFC lesion and sham groups exhibited a decrease in motor activity after the first 20-min interval of the locomotor test session (interval 1 > interval 2-6; Tukey $p < 0.001$). Furthermore, there was no difference between the lesion and sham groups in motor activity.

Experiment 3

Self-Administration Responding: There were no pre-existing differences in lever responding or cocaine intake between groups that subsequently received the lOFC lesion or sham manipulation (**Fig 9**). The mean ± SEM daily cocaine intake for the post-training lOFC lesion and sham group was 22.00 ± 2.10 and 24.60 ± 3.29 infusions, respectively (14.66 ± 1.40 and 16.40 ± 2.19 mg/kg per session). Consistent with this, the ANOVA of active lever responses for the last 7 days of self-administration training indicated no pre-existing group X

day interaction ($F_{(6,108)} = 0.813$, $p = 0.562$), day main ($F_{(6,108)} = 1.564$, $p = 0.165$) or group main effects ($F_{(1,18)} = 0.462$, $p = 0.506$) (**Fig 9A**). Similarly, the ANOVA of inactive lever responses indicated no group X day interaction ($F_{(6,108)} = 0.753$, $p = 0.608$), day main ($F_{(6,108)} = 0.989$, $p = 0.436$), or group main effect ($F_{(1,18)} = 2.998$, $p = 0.100$) (**Fig 9B**). Finally, the ANOVA for daily cocaine intake revealed a significant day main effect ($F_{(6,108)} = 4.550$, $p < 0.001$), but no group X day interaction ($F_{(6,108)} = 1.243$, $p = 0.290$) or group main effect ($F_{(1,18)} = 0.466$, $p = 0.504$). Thus, both the lOFC lesion and sham control groups exhibited a similar escalation in cocaine intake over the last 7 days of cocaine self-administration training (Tukey, $p < 0.05$; day 7 > day 1-3). However, there were no pre-existing differences in cocaine intake between groups that subsequently received the lOFC lesion or sham manipulation.

Extinction Responding: There were no pre-existing differences in extinction learning between groups that subsequently received the lOFC lesion or sham manipulation (**Fig. 9B**). Both groups needed a similar mean number of days to reach the extinction criterion ($t_{(18)} = 0.900$, p = 0.380; Sham mean = 7.09 $\pm$ 0.30, Lesion mean = 7.00 $\pm$ 0.00; data not shown). Similarly, the ANOVA of active lever responses on the first 7 days of extinction training revealed a significant day main effect ($F_{(6,108)} = 17.234$, $p = 0.001$), but no group X day interaction ($F_{(6,108)} = 0.508$, $p = 0.485$) or group main effect ($F_{(1,18)} = 0.193$, $p = 0.666$). Hence, active lever responses declined across extinction sessions at similar rates in both groups (day 1 > day 2-7; Tukey, $p < 0.01$). In contrast, the ANOVA of inactive lever responding indicated a significant group X day interaction effect ($F_{(6,108)} = 2.503$, $p = 0.026$) as well as a day main effect ($F_{(6,108)} = 8.251$, $p < 0.000$), but no group main effect ($F_{(1,18)} =$

2.890, p = 0.106). Hence, the group that subsequently received sham lesions made significantly more inactive lever responses on extinction day 1 than the group that subsequently received NMDA-induced lOFC lesions (day 1 > day 2-7; Tukey, $p < 0.05$). However, there were no significant differences between groups on extinction days 2-7.

Following the induction of post-training NMDA or sham lesions, extinction responding exhibited by the lesion and sham groups was similar to the previously established extinction baseline. In order to re-obtain the extinction criterion (≤ 25 active lever presses/session for 2 consecutive sessions), both groups required a similar mean number of days ($t_{(20)} = 0.102$, p = 0.920; Sham mean = 2.82 $\pm$ 0.519, Lesion mean = 2.75 $\pm$ 0.313; data not shown).

Context-induced Reinstatement of Cocaine-seeking Behavior: Re-exposure to the cocaine-paired context elicited robust lever responding in both the lOFC lesion and sham groups (**Fig 10**). The ANOVA of active lever responses on the reinstatement test day and preceding extinction day revealed a significant context main effect ($F_{(1,18)} = 54.450$, p < 0.00), but no context X lesion interaction ($F_{(1,18)} = 1.277$, $p = 0.273$) or lesion main effect ($F_{(1,18)} = 1.259$, $p = 0.277$). Hence, both groups exhibited more active lever responding upon exposure to the cocaine-paired context relative to the extinction context, and lOFC lesions failed to alter active lever responding relative to the sham lesions. A 2X6 ANOVA of active lever responses across the six 20-min intervals of the reinstatement session further confirmed that there was a significant time main effect ($F_{(5,90)} = 16.469$, $p < 0.001$), but no time X lesion interaction ($F_{(5,90)} = 0.591$, $p = 0.707$) or lesion main effect ($F_{(1,18)} = 1.282$, $p = 0.272$) (**Fig 10B**). Hence, active lever responses declined at a similar rate in both groups during the

reinstatement session (interval 1 > intervals 3-6; Tukey, $p < 0.01$). Furthermore, there was no difference between the lOFC lesion and sham groups in active lever responding.

The ANOVA of inactive lever responding on the reinstatement test day and preceding extinction day indicated no significant context X lesion interaction ($F_{(1,18)} = 0.056$, $p = 0.815$), context main ($F_{(1,18)} = 3.341$, $p = 0.085$), or lesion main effect ($F_{(1,18)} = 1.774$, $p = 0.200$) (**Fig 10C**). Thus, the groups did not exhibit a change in inactive lever responding in the cocaine-paired context relative to the extinction context. Furthermore, there was no difference between the lOFC lesion and sham groups in inactive lever responding.

Locomotor Activity Testing: A 2X6 ANOVA of photobeam breaks across the six 20-min intervals of the locomotor test session revealed a significant time main effect ($F_{(5,90)} = 63.456$, $p < 0.001$), but no lesion X time interaction ($F_{(5,90)} = 0.204$, $p = 0.960$) or lesion main effect ($F_{(1,18)} = 0.052$, $p = 0.822$) (**Fig 11**). Both the lOFC lesion and sham groups exhibited a similar decrease in motor activity following the first 20-min interval of the locomotor test session (interval 1 > interval 2-6; Tukey $p < 0.001$). Furthermore, there was no difference between lesion and sham groups in motor activity during the locomotor activity test.

CHAPTER IV
DISCUSSION

The findings in the present study highlight the complex role that the lOFC – a structure functionally homologous to the human medial PFC – plays in guiding drug-seeking behavior, providing the first evidence that the lOFC is critical for regulating context-induced reinstatement of cocaine seeking (Gallagher et al., 1999; Goldstein et al., 1997; Elliot et al., 2000). Functional inactivation of the lOFC – but not the mOFC – disrupted the ability of a cocaine-paired context to reinstate extinguished cocaine-seeking behavior (**Fig 2, 3**). In contrast, pre-training lOFC lesions augmented reinstatement of cocaine seeking in the cocaine-paired context, but failed to alter cocaine-primed reinstatement in the extinction context (**Fig 5, 6**). Finally, lOFC lesions induced after self-administration and extinction training failed to alter context-induced cocaine-seeking behavior (**Fig 10**). While these complex patterns of effects may seem contradictory, they likely reflect the intricate constellation of cognitive impairments produced by OFC damage in humans. Moreover, they suggest that manipulations of the lOFC have profoundly different effects on motivation for cocaine based on either the type or timing of the lOFC manipulation, as will be discussed in the subsequent paragraphs.

Functional inactivation of the lOFC, but not mOFC, impairs context-induced reinstatement of cocaine seeking

In the present study, temporary functional inactivation of the lOFC severely impaired the expression of cocaine context-induced cocaine-seeking behavior. Conversely, functional inactivation of the mOFC failed to alter context-induced cocaine seeking. Such results are consistent with our previous findings that lOFC functional inactivation – but not mOFC functional inactivation – prevents explicit cocaine-paired CSs from eliciting cocaine seeking (Fuchs et al., 2004). Taken together, these findings suggest that the rat OFC is a functionally heterogeneous brain region with respect to guiding cocaine seeking and imply the selective involvement of the lOFC in this behavior. Furthermore, cocaine cue-induced motivation for cocaine reinforcement critically relies on the functional integrity of the lOFC when the lOFC is intact during the formation of cocaine-cue associations. It is unlikely that BM-induced functional inactivation of the lOFC decreased cocaine-seeking behavior due to non-specific reductions in motor behavior even though this manipulation slightly depressed motor activity in a novel context. Namely, decreased motor activity was not observed during the first 20-min interval of the locomotor test (**Fig 3A**) when functional inactivation of the lOFC produced the most robust impairment in active lever responding (**Fig 2B**). In addition to the different time course of effects on motor activity and active lever responding, lOFC functional inactivation failed to alter inactive lever responding. Thus, overall, the present findings suggest that neural activity in the lOFC is necessary for recalling the motivational significance of cocaine-conditioned stimuli or utilizing this information to guide cocaine-seeking behaviors.

Pre-training lOFC lesions fail to alter either cocaine self-administration or extinction training

While the functional inactivation experiment provides critical information about the acute role of the lOFC in guiding cocaine seeking, cocaine users typically present with protracted structural, physiological, and functional abnormalities in prefrontal cortical regions (Volkow and Fowler, 2000; Volkow et al., 1991; Franklin et al 2002; Bolla et al., 2003; Matochick et al., 2003; London et al., 2000). These abnormalities may chronically alter OFC output and underlie pathological drug-seeking and drug-taking behaviors observed in former cocaine addicts. Hence, we also examined the effects of lOFC lesions on context-induced cocaine seeking in order to provide a better model for the human condition. In accordance with our earlier study, the present findings suggest that pre-training lOFC lesions failed to alter either the acquisition or maintenance of cocaine-reinforced instrumental behavior (Fuchs et al., 2004). Thus, long-term loss of lOFC output does not alter the primary reinforcing effects of cocaine nor does it impede the acquisition of response-drug, context-response, and context-drug associations that are theorized to maintain cocaine self-administration behavior (Stewart, 1983). Such results are consistent with previous studies examining the effects of lOFC lesions on the acquisition of cocaine self-administration as a function of cocaine dose (Hutcheson and Everitt, 2003; for review, Schoenbaum and Shaham, 2008) and on the acquisition of responding for natural reinforcers (Gallagher et al., 1999; McDannald et al., 2005; Ostlund and Balleine, 2007; Schoenbaum et al., 2002).

Similar to the lack of effects of lOFC lesions on cocaine-reinforced lever responding, pre-training lOFC lesions failed to alter either the extinction of lever responding in a novel context or the mean number of days required to reach the extinction criterion. While this

finding is consistent with results from our previous study (Fuchs et al., 2004), it appears to contrast with some reports that OFC damage causes perseveration of non-rewarding responses in humans and impairs performance on reinforcer devaluation and reversal learning tasks in animals (Bechara et al., 1994; Hatfield et al., 1996; Gallagher et al., 1999; Pickens et al., 2003, 2005; Izquierdo et al., 2004) as well as producing a resistance to extinction (Izquierdo and Murray, 2005). However, perseverative errors induced by lOFC lesions in devaluation and reversal tasks primarily reflect an inability to shift behavioral responding to a previously unrewarded stimulus, which requires the modification of existing CS-no reward association rather than a deficit in inhibiting non-rewarded responses (Tait and Brown, 2007). Hence, lOFC-lesioned rats might have relied on an intact ability to either form *new* context-response, no-reward associations or utilize state-dependent learning, i.e. the presence or absence of cocaine-related interoceptive cues in the present study, to adaptively inhibit lever responding.

Pre-training lOFC lesions enhance context-induced reinstatement of cocaine-seeking behavior

In contrast to the effects of lOFC functional inactivation on context-induced cocaine seeking, pre-training lOFC lesions augmented context-induced reinstatement of cocaine-seeking behaviors relative to sham lesions. This effect appeared to stem from enhanced context-induced motivation for cocaine rather than perseverative responding. Consistent with this, lOFC lesions significantly potentiated responding during the first 20 minutes of cocaine-context re-exposure rather than decreasing the rate of decline, or extinction, in cocaine-seeking behaviors during the course of the test session. Because findings from the

lOFC functional inactivation experiment indicated the lOFC regulates the motivational effects of cocaine-conditioned contextual cues, the mechanism by which pre-training lOFC lesions enhanced cue-induced reinstatement bears explication. Unlike transient, functional inactivation of the lOFC, NMDA-induced lesions permanently eliminate lOFC neural output to other elements of the relapse circuitry. Prolonged cell loss in the lOFC may elicit compensatory neural adaptations that, in turn, contribute to heightened context-induced incentive motivation for cocaine. Previous studies have suggested that other behavioral deficits commonly associated with lOFC damage, such as behavioral inflexibility, may stem from neuroplasticity in brain regions connected with the lOFC. For instance, neurophysiological evidence indicates that neural activity in the lOFC indirectly promotes behavioral flexibility by facilitating associative encoding in the amygdala (Patton et al., 2006). As a result, unilateral lesions of the lOFC impair cue-selective firing in the basolateral amygdala during reversal learning, and lOFC lesion-induced impairments in reversal learning are rescued by BLA lesions (Schoenbaum et al., 1999; Stalnaker et al., 2007). Hence, compensatory neuroadaptations may develop in regions of the mesocorticolimbic reward circuitry following lOFC lesions and this may account for potentiated context-induced cocaine seeking observed in the present study, as well as enhanced cue-induced motivation for cocaine in former cocaine users (McLaughlin and See, 2003; Fuchs et al., 2005; Bonson et al., 2002).

Interestingly, the behavioral effects of pre-training lesions reported here appear to contrast with our previous study in which lOFC lesions did not alter explicit CS-induced cocaine-seeking behaviors (Fuchs et al., 2004). However, the differential effects of lOFC lesions on context- vs CS-induced cocaine seeking may stem from critical differences

between these studies in the type of cue being utilized. While response-contingent explicit CSs can maintain drug seeking by providing conditioned reinforcement or by signaling imminent drug effects, contexts act as occasion setters or discriminative stimuli that signal drug availability contingent upon responding (Bouton and Bolles, 1979, Crombag and Shaham, 2002; Fuchs et al., 2005). Explicit CSs and contexts engage partially distinct neural systems to guide the expression of cocaine-seeking behavior (Fuchs et al., 2005; Bossert et al., 2007). Thus, lOFC lesions may produce compensatory neuroadaptations that differentially affect these distinct neural systems. Accordingly, lOFC lesions appear to impair behavior maintained by conditioned reinforcement given that lOFC lesions disrupt responding for cocaine on a second-order reinforcement schedule, produce an insensitivity to CS omission on a second-order task when primary reinforcement is available, and prevent Pavlovian cues from facilitating instrumental performance (Hutcheson and Everitt, 2003; Pears et al., 2003; Ostlund and Balleine, 2007). In contrast, lOFC lesions do not prevent the processing of discriminative stimuli given that lOFC-lesioned rats exhibit normal acquisition of instrumental discrimination learning, perform odor discriminations in a go, no-go task, and displayed normal acquisition of lever pressing for unsignalled cocaine in the present study (Chudasama and Robbins, 2003; Schoenbaum et al., 2002). However, lOFC lesion-induced neuroadaptions may enhance context-induced motivation for cocaine reinforcement, which manifests differently depending on the presence or absence of an explicit cocaine-paired CS. Hence, lOFC lesion-induced enhancement in context-induced motivation for cocaine may have been obscured in the previous study by lOFC lesion-induced attenuation in responding maintained by conditioned reinforcement. However, this effect is observed in the absence of

a CS in the current study, resulting in an overall augmentation of context-induced cocaine seeking in the lOFC lesion group.

Pre-training lOFC lesions fail to alter cocaine-primed reinstatement of cocaine-seeking behavior

Much like lOFC lesions did not alter the primary reinforcing properties of cocaine during self-administration training, lOFC lesions failed to potentiate reinstatement initiated by a single cocaine priming injection. These findings suggest that lOFC lesions specifically enhance context-induced motivation for cocaine, but fail to alter the reinforcing significance and motivational properties of cocaine itself. Interestingly, this finding contrasts with previous evidence that lOFC lesions induce perseveration in drug seeking behavior following cocaine priming (Fuchs et al., 2004). However, procedural differences may underlie this apparent discrepancy. Importantly, in the previous study cocaine-priming injections were administered immediately before exposure to a cocaine-paired context (i.e. the extinguished drug-associated context), whereas in the current study cocaine-primed injections were administered before exposure to a no-cocaine-paired context (i.e. the extinction context). Hence, perseverative responding in the prior study may have stemmed, at least in part, from an interaction between (A) the primary motivational properties of cocaine and (B) the effects of lOFC lesions on conditioned motivational properties of the cocaine-paired context. Because both GABA agonist-induced and tetrototoxin-induced functional inactivation of the lOFC fails to alter cocaine-primed cocaine seeking, lOFC output does not appear critical for cocaine-primed reinstatement (Fuchs et al., 2004; Capriles et al., 2003). Thus, in summary, pre-training lOFC lesions may elicit neuroadaptations that specifically enhance context-

induced reinstatement without altering cocaine-primed reinstatement of cocaine-seeking behaviors.

Post-training lOFC lesions fail to alter context-induced reinstatement of cocaine-seeking behaviors

Pre-training lOFC lesions and post-training lOFC functional inactivation may have differentially altered context-induced cocaine seeking because 1) these manipulations occur at different points relative to the formation of context-response-cocaine associations, 2) compensatory neuroadaptations require time to develop after the lesioning procedure, or 3) these manipulations produce fundamentally different neurochemical effects. When assessing these possible explanations, the results of the post-training lOFC lesion experiment failed to support the first possibility given that post-training lOFC lesions, unlike lOFC functional inactivation, failed to attenuate context-induced cocaine-seeking behaviors relative to sham lesions. In fact, both the post-training lOFC lesion and sham group exhibited robust cocaine-seeking behaviors upon re-exposure to the cocaine-paired context. These effects may have stemmed from incubation, a reliable time-dependent increase in cue-induced cocaine-seeking behavior following experimenter-imposed abstinence from cocaine during the post-lesion recovery period (Tran-Nguyen et al., 1998; Grimm et al., 2001). Furthermore, it is unlikely that post-training lOFC lesions and functional inactivation differentially altered context-induced reinstatement due to differences in their neurochemical effects because this would not account for differences between the post-training and pre-training lOFC lesion groups. Specifically, post-training lOFC lesions, unlike pre-training lOFC lesions, failed to potentiate context-induced cocaine-seeking behaviors. Hence, we can

conclude that the lOFC lesions triggered neuroadaptations that enhanced context-induced motivation for cocaine, but that these neuradaptations require more time to develop than the period available between lesion induction and reinstatement testing in experiment 3. Therefore, animals with post-training lOFC lesions might display an intermediate state of neuroplasticity that was sufficient to increase motivation for cocaine and compensate for decreased cocaine-seeking behavior stemming from acute loss of lOFC function. Such findings suggest that long-term loss of OFC output in humans may underlie enhanced cue-induced neural reactivity observed in former cocaine users.

The role of the OFC in drug relapse behaviors

Overall, the preceding findings indicate that the lOFC exerts a complex regulatory influence over the incentive motivational effects of cocaine-paired cues (Jentsch and Taylor 1999). The finding that the lOFC appears to play a different role in explicit CS-induced, context-induced, and cocaine-primed cocaine-seeking behavior is consistent with the idea that different reinstatement triggers induce drug-seeking behavior via partially distinct neural mechanisms. Because context-induced cocaine-seeking behavior is attenuated by acute lOFC functional inactivation, but is enhanced by chronic loss of lOFC output, neuroadaptations elicited in other elements of the relapse circuitry during associate learning processes may account for enhanced motivation for cocaine reinforcement. Importantly, the lOFC may regulate cocaine seeking via its robust connections with the basolateral amygdala (BLA), hippocampus, prefrontal cortex, thalamus, basal ganglia, and nucleus accumbens core (Krettek and Price, 1977; Groenewegen et al., 1990; Ray and Price, 1992, 1993; Carmichael and Price, 1995; Haber et al., 1995). Of these brain regions, the dorsal hippocampus plays a

selective role in context-induced reinstatement (Fuchs et al., 2005; 2007), the amygdala and ventral hippocampus are critical for context-induced and CS-induced reinstatement (Sun and Rebec, 2003, See et al., 2001, Fuchs et al., 2005; Lasseter et al., in prep), and the prefrontal cortex and nucleus accumbens are necessary for both drug-primed, CS-induced, and context-induced reinstatement (McFarland and Kalivas, 2001; McLaughlin and See, 2003; Fuchs et al., 2005; 2007; 2008). The differential effects of pre-training lOFC lesions on these forms of reinstatement suggest that different reinstatement triggers may engage distinct subcircuits within the lOFC, and these may, in turn, develop a different set of neuroadaptations following lOFC damage. We hypothesize that the existence of such subcircuits may explain the concomitant presence of chronic hypofrontality and enhanced cocaine-cue neural activation in the OFC in humans and rats (Volkow and Fowler, 2000; Franklin et al 2002, Bolla et al., 2003, Matochick et al., 2003, London et al., 2000, Zavala et al., 2007; Hearing et al., 2008). Exploring how lOFC damage contributes to cognitive and behavioral impairments in the lOFC-lesioned rat may help elucidate potential treatment strategies for humans dealing with addiction to cocaine. Future studies will be necessary to determine which of the above brain regions exhibits an obligatory functional interaction with the lOFC in regulating cue-induced cocaine-seeking behaviors. Of particular interest will be to systematically investigate the nature of lOFC lesion-induced neuroadaptive changes in the relapse circuitry and to assess the distinct contribution of these putative neuroadaptations to addictive behavior.

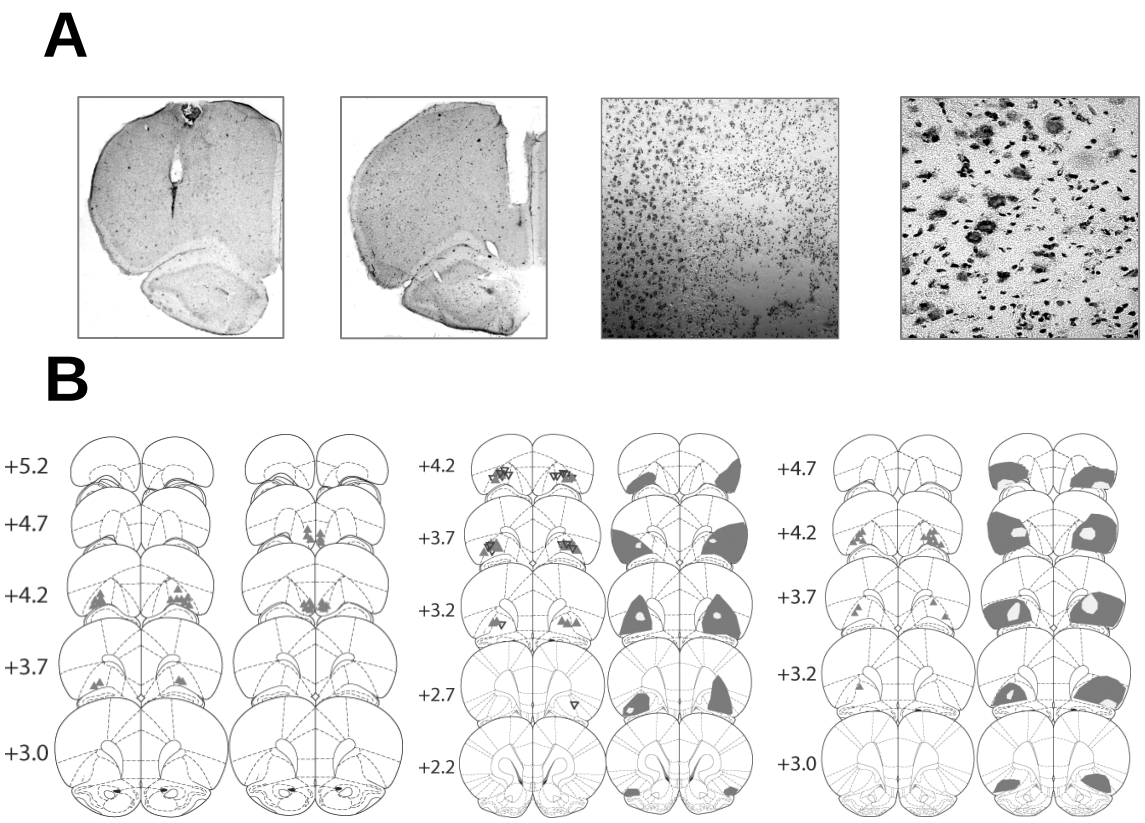

Figure 1. Histological Schematics. A: Photomicrographs of representative brains from rats that received bilateral cannula aimed at the lOFC and mOFC, as well as representative lOFC lesions shown at 10X and 20X magnification B: Schematic representation of cannula placements in the brains of rats from experiments 1-3, as well as the extent of the largest (dark shaded areas) and smallest (light shaded areas) lesions. The triangle symbols represent the most ventral point of the injection cannula tracks. The open and closed triangle symbols represent animals that received saline and cocaine priming injections, respectively, in Experiment 2. The numbers represent the approximate distance (in millimeters) from bregma, based on the atlas of Paxinos and Watson (1997).

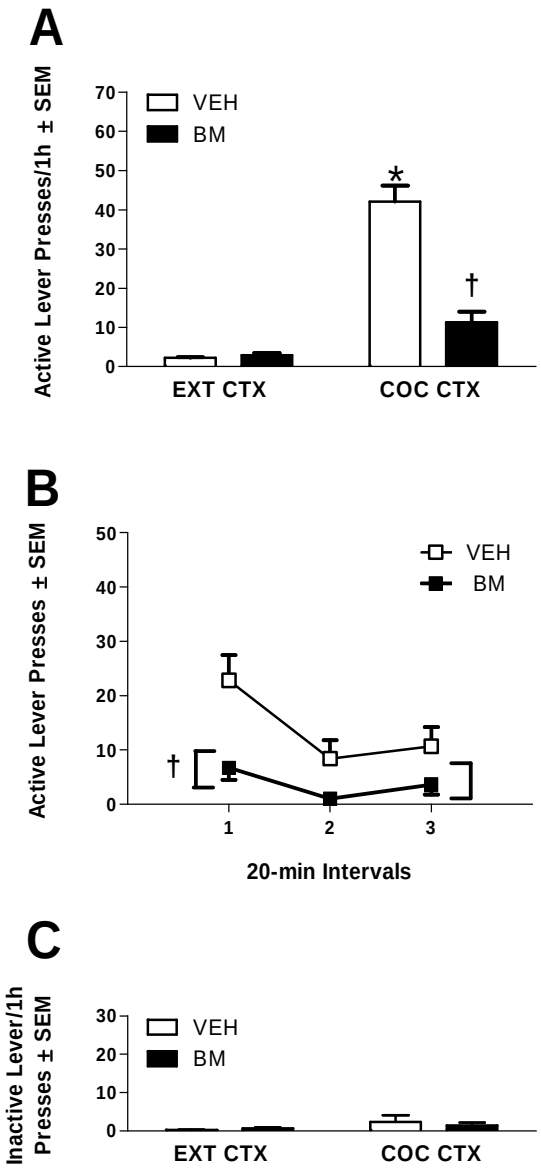

Figure 2. Functional inactivation of the lOFC attenuates context-induced reinstatement of extinguished cocaine-seeking behavior. Rats received intra-lOFC BM or VEH treatment immediately before re-exposure to either the cocaine-paired context (COC CTX) or extinction context (COC CTX). A: Active lever responses (mean/1h $\pm$ SEM) during the extinction and reinstatement test sessions. B: Time course of active lever responses (mean $\pm$ SEM) in the cocaine-paired context during the reinstatement test session. C: Inactive lever responses (mean/1h $\pm$ SEM) during the extinction and reinstatement test sessions. The asterisk represents a significant difference relative to responding in the extinction context (ANOVA context main effect, $p < 0.001$; Tukey, $p < 0.01$). Daggers represent a significant difference relative to VEH treatment in responses during exposure to the cocaine-paired context (Tukey, $p < 0.01$). Sample sizes: lOFC functional inactivation, $n = 10$

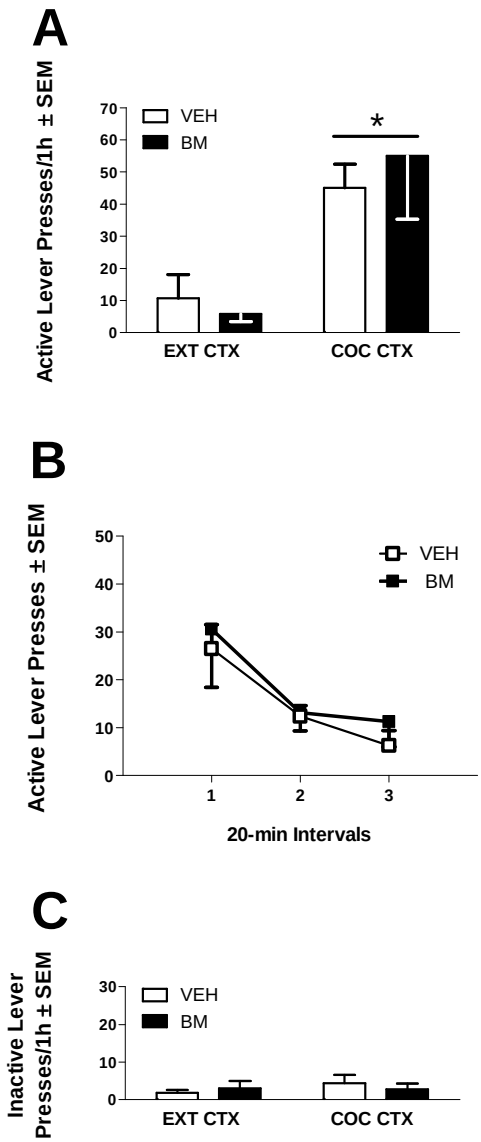

Figure 3. Functional inactivation of the mOFC fails to alter context-induced reinstatement of extinguished cocaine-seeking behavior. Rats received BM or VEH treatment into the mOFC immediately before re-exposure to either the cocaine-paired context (COC CTX) or extinction context (COC CTX). A: Active lever responses (mean/1h $\pm$ SEM) during the extinction and reinstatement test session. B: Time course of active lever responses (mean $\pm$ SEM) in the cocaine-paired context during the reinstatement test session. C: Active lever responses (mean/2h $\pm$ SEM) during the extinction and reinstatement test session. The asterisk represents a significant difference relative to responding in the extinction context (ANOVA context main effect, $p < 0.001$; Tukey, $p < 0.01$). Sample sizes: mOFC functional inactivation, $n = 8$

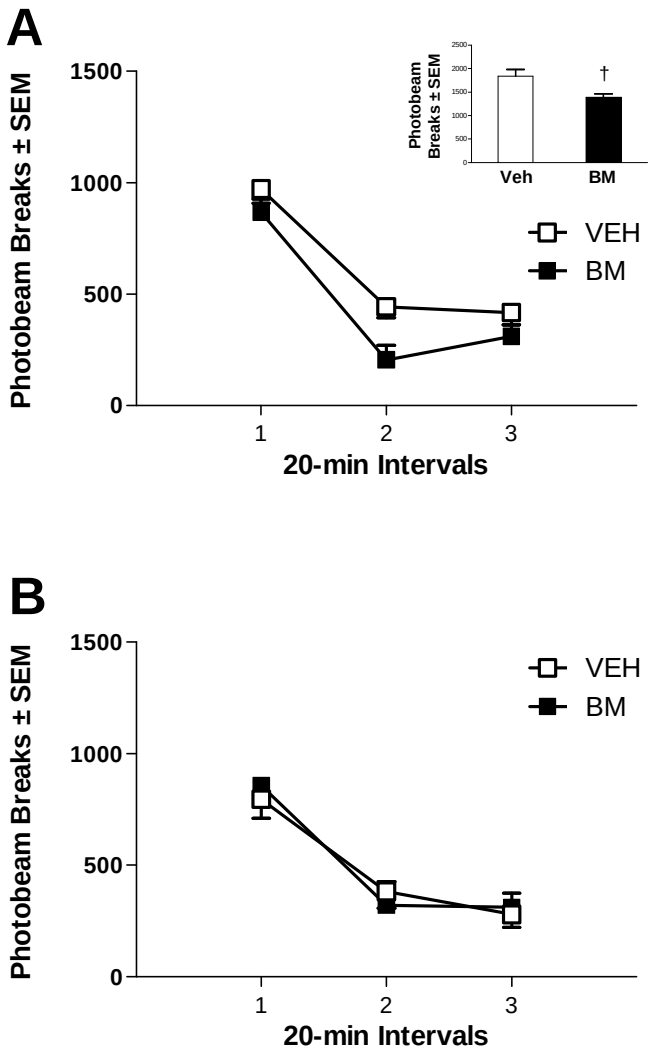

Figure 4. Functional inactivation of the lOFC, but not mOFC, attenuates locomotor activity measured as the number of photobeam breaks (mean ± SEM) triggered by the movement of subjects in a novel context. A: Effects of lOFC functional inactivation on photobeam breaks (mean ± SEM). B: Effects of mOFC functional inactivation on photobeam breaks (mean ± SEM). The dagger represents a significant difference relative to VEH pretreatment (ANOVA treatment main effect, $p = 0.038$). Sample sizes: lOFC, $n = 10$; mOFC, $n = 8$.

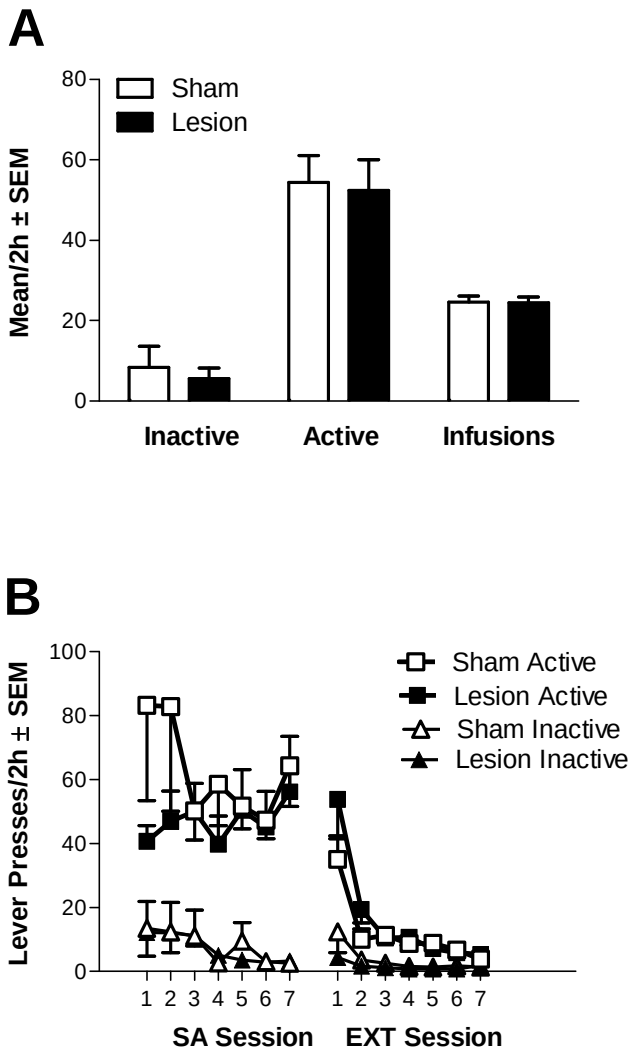

Figure 5. Pre-training lOFC lesions fail to alter responding during self-administration or extinction training. A: Active and inactive lever responses and cocaine intake (mean ± SEM) during the last three days of cocaine self-administration training. B: Active and inactive lever responses (mean/2h ± SEM) during cocaine self-administration (SA) (last 7 days) and extinction training (EXT) (first 7 days). During self-administration training, active lever responses resulted in the delivery of a cocaine infusion (0.2 mg/0.1 ml) and inactive lever responses had no programmed consequences. During extinction training, active and inactive lever responses had no programmed consequences. Sample sizes: lOFC lesion *n* = 22; lOFC sham, *n* = 19.

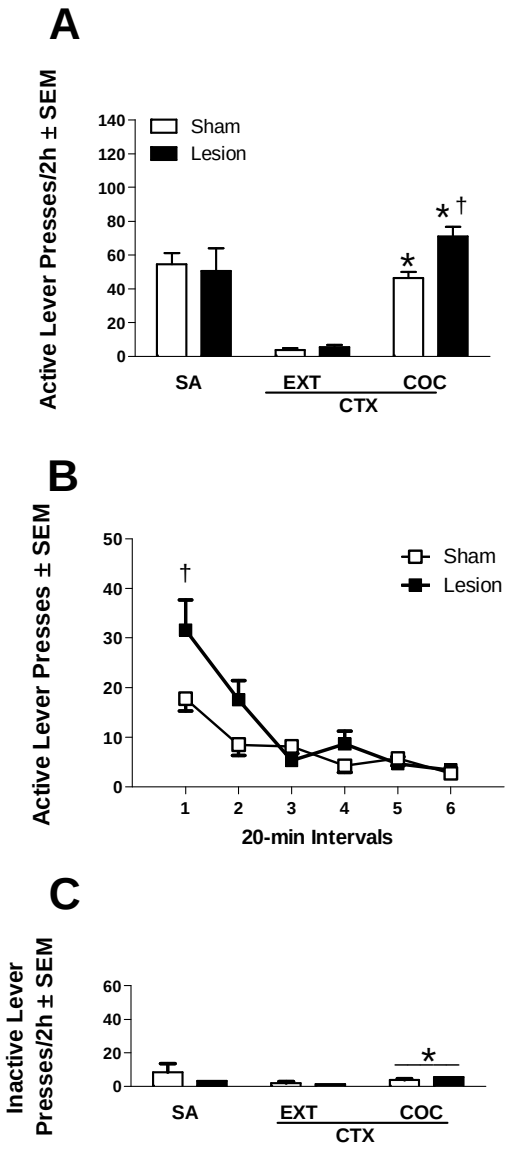

Figure 6. Pre-training lOFC lesions potentiate context-induced reinstatement of extinguished cocaine-seeking behavior. A: Active lever responses (mean/2h ± SEM) during self-administration (SA, last 3 days), extinction (EXT CTX, last day behavioral test) and during the reinstatement test session (COC CTX). B: Time course of active lever responses (mean ± SEM) in the cocaine-paired context during the reinstatement test session. C: Inactive lever responses (mean/2h ± SEM) during self-administration (SA, last 3 days), extinction (EXT CTX, last day behavioral test) and during the reinstatement test session (COC CTX). Asterisks represent significant differences relative to responding in the extinction context (ANOVA context main effect, $p < 0.001$). Daggers represent significant differences relative to the sham group (ANOVA lesion simple main effect, $p < 0.001$; Tukey $p < 0.05$). Sample sizes: lOFC lesions $n = 22$; lOFC sham, $n = 19$.

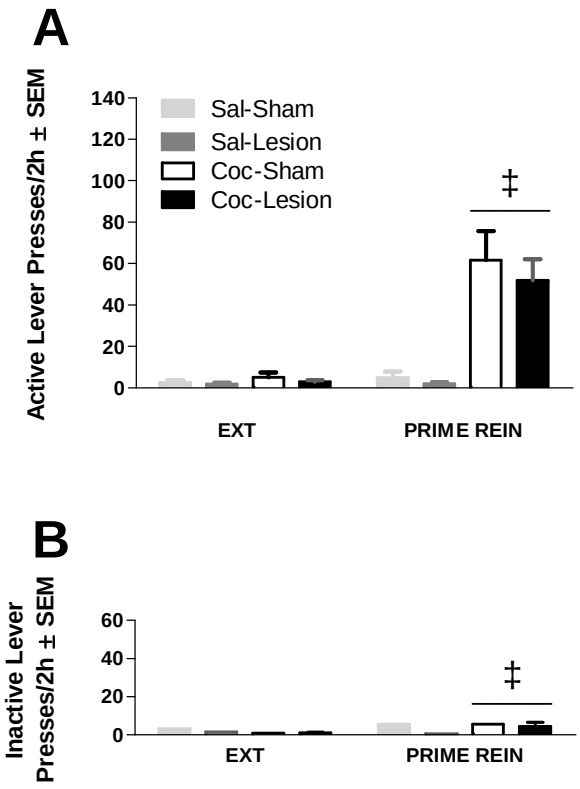

Figure 7. Pre-training lOFC lesions fail to alter cocaine-primed reinstatement of extinguished cocaine-seeking behavior. Intraperitoneal cocaine priming injections (10 mg/kg, *i.p.*) or saline injections were administered prior to placement in the extinction context. A: Active lever responses (mean/2h ± SEM) during the cocaine-primed reinstatement test (PRIME REIN) and the preceding extinction session (EXT). B: Inactive lever responses (mean/2h ± SEM) during the cocaine-primed reinstatement test and the preceding extinction session. Double daggers denote a significant difference relative to saline injections (Tukey, $p < 0.01$). Sample sizes: lOFC lesion-cocaine, $n = 11$; lOFC lesion-saline, $n = 11$; lOFC sham-cocaine, $n = 9$; lOFC sham- saline priming, $n = 10$.

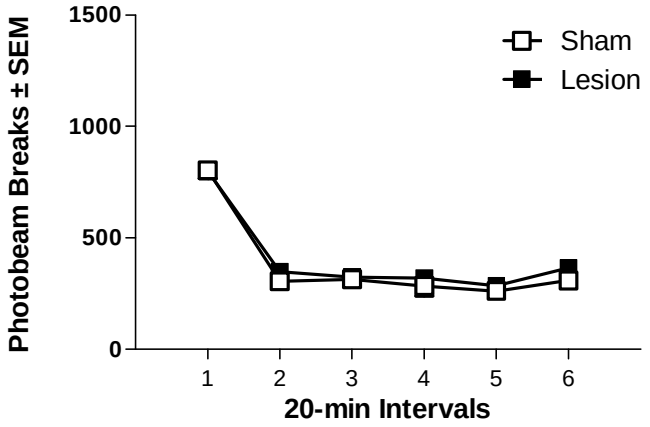

Figure 8. Pre-training lOFC lesions fail to alter locomotor activity measured as photobeam breaks (mean ± SEM) triggered by the movement of subjects in a novel context. Sample sizes: lOFC lesions, *n* = 22; lOFC sham, *n* = 19.

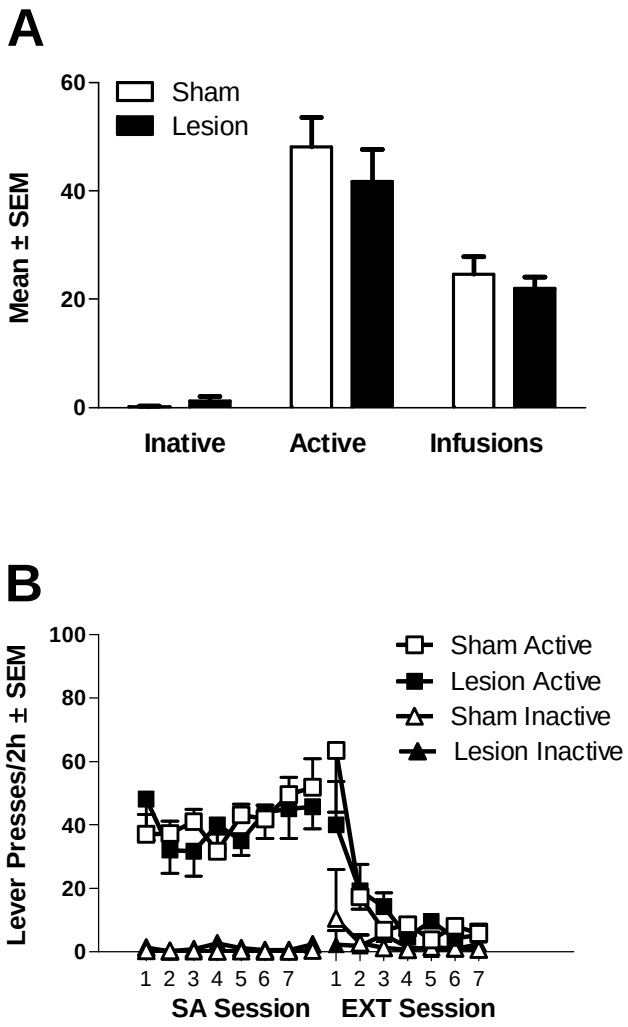

Figure 9. Lack of pre-existing differences during cocaine self-administration and extinction training between subjects that received post-training lOFC lesion or sham manipulation. A: Active and inactive lever responses and cocaine intake (mean ± SEM) during the last three days of cocaine self-administration training. B: Active and inactive lever responses (mean/2h ± SEM) during cocaine self-administration (SA) (last 7 days) and extinction training (EXT) (first 7 days). During self-administration training, active lever responses resulted in the delivery of a cocaine infusion (0.2 mg/0.1 ml) and inactive lever responses had no planned consequences. During extinction training, active and inactive lever responses had no programmed consequences. Sample sizes: lOFC lesions $n = 9$; lOFC sham, $n = 11$.

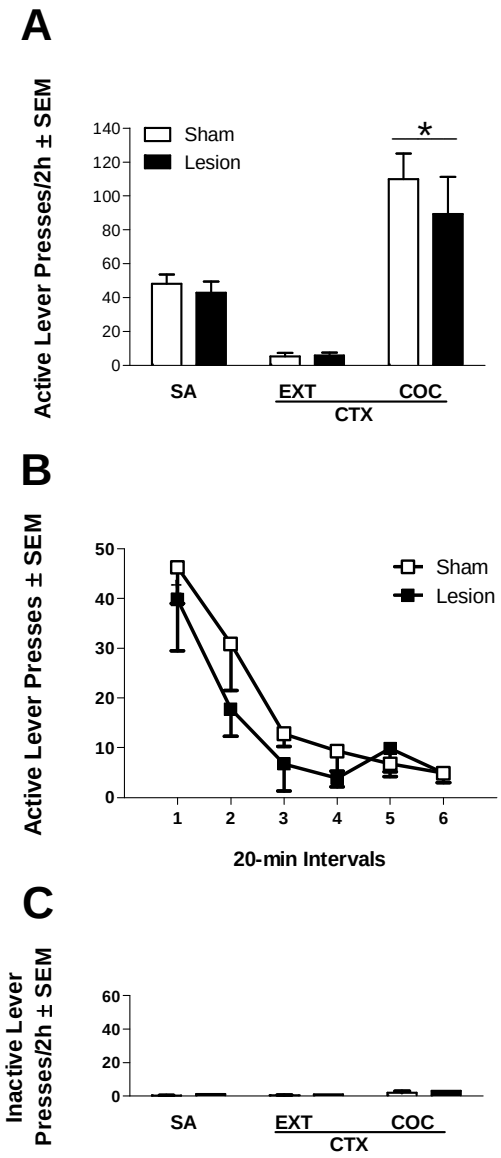

Figure 10. Post-training lOFC lesions fail to alter context-induced reinstatement of extinguished cocaine-seeking behavior. A: Active lever responses (mean/2h ± SEM) during self-administration (SA, last 3 days), extinction (EXT, last day) and during the reinstatement test session (COC CTX). B: Time course of active lever responses (mean ± SEM) in the cocaine-paired context during the reinstatement test day. C: Inactive lever responses (mean/2h ± SEM) during self-administration (SA, last 3 days), extinction (EXT CTX, last day) and during the reinstatement test day (COC CTX). The asterisk represents a significant difference relative to responding during the preceding extinction day (ANOVA context main effect, $p < 0.001$). Sample sizes: lOFC lesions $n = 9$; lOFC sham, $n = 11$.

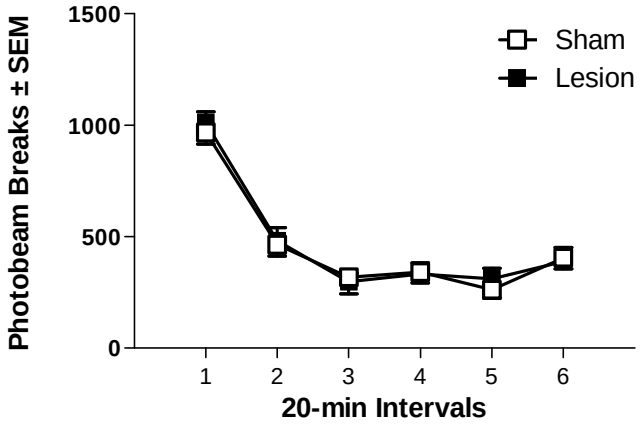

Figure 11. Post-training lOFC lesions failed to alter locomotor activity measured as photobeam breaks (mean ± SEM) triggered by the movement of subjects in a novel context. Sample sizes: lOFC lesions *n* = 9; lOFC sham, *n* = 11.

WORKS CITED

1. American Psychiatric Association. Diagnostic and statistical manual for mental disorders. Washington DC: American Psychiatric Association 2004.

2. Beal MF, Swartz KJ, Finn SF, Mazurek MF, KowallNW (1991) Neurochemical characterization of excitotoxin lesions in the cerebral cortex. J Neurosci 11:147–158

3. Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50(1-3):7-15

4. Bolla KI, Eldreth DA, London ED, Kiehl KA, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, Cadet JL, Kimes AS, Funderburk FR, Ernst M (2003) Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. Neuroimage 19(3):1085-94

5. Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, Kurian V, Ernst M, London ED (2002). Neural systems and cue-induced cocaine craving. Neuropsychopharmacology 26(3):376-86

6. Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y (2007) Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. J Neurosci 27(46):12655-63

7. Bouton, M.E., Bolles RC (1979) Role of conditioned contextual stimuli in reinstatement of extinguished fear.J Exp Psychol Anim Behav Process (4):368-78

8. Camille N, Coricelli G, Sallet J, Pradat-Diehl P, Duhamel JR, Sirigu A (2004) The involvement of the orbitofrontal cortex in the experience of regret. Science 304(5674):1167-70

9. Capriles N, Rodaros D, Sorge RE, Stewart J (2003) A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 168(1-2):66-74

10. Carmichael ST, Price JL (1985) Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. J Comp Neurol 363:615-641

11. Chudasama Y, Robbins TW (2003) Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. J Neurosci 23(25):8771-8780

12. Crombag HS, Shaham Y (2002) Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci 116(1):169-73

13. de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 75(2):134-43

14. Ehrman RN, Robbins SJ, Childress AR, O'Brien CP (1992) Conditioned responses to cocaine-related stimuli in cocaine abuse patients. Psychopharmacology (Berl) 107(4):523-9

15. Elliott R, Dolan RJ, FrithCD (2000) Dissociable functions in the medial and lateral orbitofrontal cortex: evidence from human neuroimaging studies. Cereb Cortex 10:308–317

16. Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. Ann N Y Acad Sci 29;877:412-38

17. Franklin TR, Acton PD, Maldjian JA, Gray JD, Croft JR, Dackis CA, O'Brien CP, Childress AR (2002) Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. Biol Psychiatry 15;51(2):134-42

18. Foltin RW, Haney M. Conditioned effects of environmental stimuli paired with smoked cocaine in humans. Psychopharmacology (Berl) 2000; 149(1):24-33

19. Fuchs RA, Evans KA, Parker MC. Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 2004; 176:459-465

20. Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology 30:296-309

21. Fuchs RA, Eaddy JL, Su ZI, Bell GH (2007) Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats. Eur J Neurosci 26(2):487-98

22. Fuchs RA, Ramirez DR, Bell GH (2008) Nucleus accumbens shell and core involvement in drug context-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 200(4):545-56

23. Gallagher M, McMahan RW, Schoenbaum G (1999) Orbitofrontal cortex and representation of incentive value in associative learning. J Neurosci 19(15):6610-4

24. Goldstein RZ, Tomasi D, Rajaram S, Cottone LA, Zhang L, Maloney T, Telang F, Alia-Klein N, Volkow ND (2007) Role of the anterior cingulate and medial orbitofrontal cortex in processing drug cues in cocaine addiction. Neuroscience 144(4):1153-9

25. Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature 412(6843):141-2

26. Groenewegen HJ (1988) Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain prefrontal cortex topography. Neuroscience 24:379-431

27. Groenewegen HJ, Berendse HW, Wolders JG, Lohman AH (1990) The anatomical relationship of the prefrontal cortex with the striato-pallidal system, the thalamus, and the amygdala: evidence for a parallel organization. Prog Brain Res 85:95-116

28. Haber SN, Kunisho K, Mizobuchi M, Lynd-Balta E (1995) The orbital and medial prefrontal circuit through the primate basal ganglia. J Neurosci 15:4851-4867

29. Hearing MC, Miller SW, See RE, McGinty JF (2008) Relapse to cocaine seeking increases activity-regulated gene expression differentially in the prefrontal cortex of abstinent rats. Psychopharmacology (Berl) 198(1):77-91

30. Holland PC, Gallagher M. Amygdala-frontal interactions and reward expectancy. Curr Opin Neurobiol 2004; 14(2):148-55

31. Hutcheson DM, Everitt BJ (2003) The effects of selective orbitofrontal cortex lesions on the acquisition and performance of cue-controlled cocaine seeking in rats. Ann NY Acad Sci 1003:410-411

32. Izquierdo A, Suda RK, Murray EA (2004) Bilateral orbital prefrontal cortex lesions in rhesus monkeys disrupt choices guided by both reward value and reward contingency. J Neurosci 24(34):7540-8

33. Izquierdo A, Murray EA (2005) Opposing effects of amygdala and orbital prefrontal cortex lesions on the extinction of instrumental responding in macaque monkeys. Eur J Neurosci 22(9): 2341-6

34. Jaffe JH, Cascella NG, Kumor KM, Sherer MA (1989) Cocaine-induced cocaine craving. Psychopharmacology 97(1):59-64

35. Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology (Berl) 146(4):373-390

36. Katz JL, Higgins ST (2003) The validity of the reinstatement model of craving and relapse to drug use. Psychopharmacology 168(1-2):21-30

37. Krettek JE, Price JL (2000) The cortical projectoins of the mediodorsal nucleus and adjacent thalamic nucleu in the rat. J Comp Neurol 1977; 171:157-192

38. London ED, Ernst M, Grant S, Bonson K, Weinstein A (2000) Orbitofrontal cortex and human drug abuse: functional imaging. Cereb Cortex 10(3):334-42

39. Lu L, Grimm JW, Hope BT, Shaham Y (2004) Incubation of cocaine craving after withdrawal: a review of preclinical data. Neuropharmacology 47 Suppl 1:214-26

40. Lynch WJ, Carroll ME (2000) Reinstatement of cocaine self-administration in rats: sex differences. Psychopharmacology (Berl) 148(2):196-200

41. Matsumoto M, Kanno M, Togashi H, Ueno K, Otani H, Mano Y, YoshiokaM (2003) Involvement of GABAA receptors in the regulation of the prefrontal cortex on dopamine release in the rat dorsolateral striatum. Eur J Pharmacol 482:177–184

42. Matochik JA, London ED, Eldreth DA, Cadet JL, Bolla KI (2003) Frontal cortical tissue composition in abstinent cocaine abusers: a magnetic resonance imaging study. Neuroimage 19(3):1095-102

43. McDannald MA, Saddoris MP, Gallagher M, Holland PC (2005) Lesions of orbitofrontal cortex impair rats' differential outcome expectancy learning but not conditioned stimulus-potentiated feeding. J Neurosci 25(18):4626-32

44. McLaulin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking bheavior in rats. Psychopharmacology 168:57-65

45. Oades RD, Halliday GM (1987) Ventral tegmental (A10) system neurobiology. 1. Anatomy and connectivity. Brain Res 434:117-65

46. O'Doherty J, Rolls ET, Francis S, Bowtell R, McGlone F (2001) Representation of pleasant and aversive taste in the human brain. J Neurophysiol 85(3):1315-21

47. Ostlund SB, Balleine BW (2007) Orbitofrontal cortex mediates out-come ending in Pavlovian but not instrumental learning. J of Neurosci 24:4819-4825

48. Pears A, Parkinson JA, Hopewell L, Everitt BJ, Roberts A (2003) Lesions of the orbitofrontal but not medial prefrontal cortex disrupt conditioned reinforcement in primates. J of Neurosci 23(35):11189-11201

49. Paxinos G and Watson C (1997) The rat brain in stereotaxic coordinates. Academic press, New York

50. Pickens CL, Saddoris MP, Setlow B, Gallagher M, Holland PC, Schoenbaum G (2003) Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. J Neurosci 23(35):11078-84

51. Pickens CL, Saddoris MP, Gallagher M, Holland PC (2005) Orbitofrontal lesions impair use of cue-outcome associations in a devaluation task. Behav Neurosci 119(1):317-22

52. Placenza FM, Vaccarino FJ, Fletcher PJ, Erb S (2005) Activation of central neurokinin-1 receptors induces reinstatement of cocaine-seeking behavior. Neurosci Letters 390(1):42-7

53. Price JL (1985) Beyond the primary olfactory cortex: olfactory-related areas in the neocortex, thalamus, and hypothalamus. Chem Senses 10:235-258

54. Price JL (2007) Definition of the orbital cortex in relation to specific connections with limbic and visceral structures and other cortical regions. Ann NY Acad Sci 1121:54-71

55. Ray JP, Price JL (1992) The organization of the thalamocortical connections of the mediodorsal thalamic nucleus in the rat, related to the ventral forebrain-prefrontal cortex topography. J Comp Neuro 323:167-197

56. Ray JP, Price J (1993) The organization of projections from the mediodorsal nucleus of the thalamus to orbital and medial prefrontal cortex in macaque monkeys. J Comp Neuro 320:435-456

57. Rohsenow DJ, Martin RA, Eaton CA, Monti PM (2008) Cocaine craving as a predictor of treatment attrition and outcomes after residential treatment for cocaine dependence. J Stud Alcohol Drugs 68(5):641-8

58. Rose JE, Woolsey CN (1948) The orbitofrontal cortex and its connections with the mediodorsal nucleus in the rabbit, sheep, and cat. Res Publ Ass Nerv Ment Dis 27:210-232

59. Saddoris MP, Gallagher M, Schoenbaum G (2005) Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. Neuron 46(2):321-31

60. Schoenbaum G, Nugent SL, Saddoris MP, Setlow B (2002) Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. Neuroreport 13(6):885-90

61. Schoenbaum G, Shaham Y (2008) The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. Biol Psychiatry 63(3):256-62

62. Stalnaker TA, Roesch MR, Franz TM, Burke KA, Schoenbaum G (2006) Abnormal associative encoding in orbitofrontal neurons in cocaine-experienced rats during decision-making. Eur J Neurosci 24(9):2643-53

63. Stalnaker TA, Roesch MR, Calu DJ, Burke KA, Singh T, Schoenbaum G (2007) Neural correlates of inflexible behavior in the orbitofrontal-amygdalar circuit after cocaine exposure. Ann N Y Acad Sci 1121:598-609

64. Stewart J (1983) Conditioned and unconditioned drug effects in relapse to opiate and stimulant drug self-administration. Prog Neuropsychopharmacol Biol Psychiatry 7: 591–597.

65. Sun W, Rebec GV (2003) Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. J Neurosci 23(32):10258-64

66. Tait SD, Brown VJ (2007) Difficulty in overcoming learned non-reward during reversal learning in rats with ibotenic acid lesions of orbital frontal cortex. Ann NY Acad Sci 1121:407-420

67. Tran-Nguyen LT, Fuchs RA, Coffey GP, Baker DA, O'Dell LE, Neisewander JL (1998) Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19(1):48-59

68. Volkow ND, Fowler JS, Wolf AP, Hitzemann R, Dewey S, Bendriem B, Alpert R, Hoff A (1991) Changes in brain glucose metabolism in cocaine dependence and withdrawal. Am J Psychiatry 148(5):621-6

69. Volkow ND, Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb Cortex 10(3):318-25

70. Volkow ND, Fowler JS, Wang GJ, Goldstein RZ (2002) Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. Neurobiol Learn Mem 78(3):610-24

71. Zavala AR, Osredkar T, Joyce JN, Neisewander JL (2008) Upregulation of Arc mRNA expression in the prefrontal cortex following cue-induced reinstatement of extinguished cocaine-seeking behavior. Synapse 62(6):421-31