EFFECTS OF FUNCTIONAL DISCONNECTION OF THE BASOLATERAL AMYGDALA AND DORSAL HIPPOCAMPUS FOLLOWING COCAINE MEMORY REACTIVATION ON SUBSEQUENT DRUG CONTEXT-INDUCED COCAINE-SEEKING BEHAVIOR IN RATS

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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
2011

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ABSTRACT

AUDREY M. WELLS: Effects of functional disconnection of the basolateral amygdala and dorsal hippocampus following cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior in rats
(Under the direction of Rita A. Fuchs-Lokensgard)

Stimulus control over instrumental drug seeking relies on the reconsolidation of context-response-drug associations into long-term memory following retrieval-induced destabilization. According to previous studies, the basolateral amygdala (BLA) and dorsal hippocampus (DH) regulate cocaine-related memory reconsolidation; however, it is not known whether these brain regions interact or independently control this phenomenon. In the present study, using the contextual rodent extinction-reinstatement paradigm, we demonstrate that disruption of intrahemispheric (disconnection), but not interhemispheric (ipsilateral control), interactions between the BLA and DH following cocaine-related memory reactivation impaired subsequent drug context-induced cocaine-seeking behavior in rats. Furthermore, post-reactivation BLA/DH disconnection inhibited the development of a time-dependent increase, or *incubation*, of drug context-induced cocaine seeking following an extended delay, despite some recovery of cocaine-seeking behavior. Thus, the BLA and DH interact to regulate the reconsolidation of cocaine-related memories, thereby facilitating the ability of drug-paired contexts to trigger cocaine seeking and contributing to the incubation of cocaine seeking.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to and appreciation of my mentor, Dr. Rita Fuchs Lokensgard for the instrumental guidance and knowledge she afforded me with throughout the course of this study. Additionally, I would like to thank Dr. Regina M. Carelli and Dr. Donald T. Lysle for their encouragement as members of my master's thesis committee. Finally, I would like to thank my colleagues, Dr. Xiaohu Xie, Dr. Amy Arguello, and Heather Lasseter, whose technical and intellectual contributions were critical for the completion of these experiments and the associated manuscript.

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CHAPTER I
INTRODUCTION

Significance of the Problem

Cocaine addiction continues to be a serious health and economic problem for the United States, despite a recent trend for a decline in use (Substance Abuse and Mental Health Service Administration 2009). In addition to the obvious health consequences for the estimated 1.9 million cocaine users in the United States, according to the National Survey on Drug Use and Health in 2008 (Substance Abuse and Mental Health Service Administration 2009), cocaine addiction impacts non-users by contributing to the high economic cost of addiction incurred by the United States each year, a figure approaching $193 *billion* dollars (National Drug Intelligence Center 2011). Unfortunately, of the 23.2 million people requiring treatment in 2007, only 2.4 million actually received specialized care (Substance Abuse and Mental Health Service Administration 2009), demonstrating why addiction continues to be so problematic for our society.

In addition to the low percentage of treatment-seeking addicts, efforts to reduce rates of cocaine addiction are further impeded by the high vulnerability to relapse in former users, even after years of abstinence (Gawin and Kleber 1986). The characteristic behavioral pattern associated with cocaine addiction involves alternating cycles of abstinence and drug use, often including periods of excessive and compulsive cocaine consumption (i.e., "binges," Gawin and Kleber 1986). This phenomenon can be partly attributed to the ability of cocaine-associated conditioned stimuli (CS) and environmental contexts to elicit craving and give rise to relapse (Childress et al. 1988; O'Brien et al. 1992; Foltin and Haney 2000). The

ability of cocaine-related stimuli to trigger relapse depends on the formation of robust cocaine-related associative memories over the course of cocaine taking (Alleweirelt et al. 2001; Fuchs et al. 2005; Kearns and Weiss 2007; Crombag et al.2008) as well as the maintenance of cocaine-related associations in long-term memory (LTM; Lee et al. 2005; Miller and Marshall 2005; Milekic et al. 2006; Bernardi et al. 2006, 2009; Valjent et al. 2006; Diergaarde et al. 2008). Preventing the reconsolidation of cocaine-related associative memories may inhibit their maintenance in long-term storage and consequently interfere with relapse, as will be discussed below. Hence, a greater understanding of the neural circuitry and mechanisms underlying cocaine-related memory reconsolidation may inform the development of novel treatments for drug addiction (Taylor et al. 2009; Milton and Everitt 2010).

Memory Reconsolidation: History and Therapeutic Application

According to the memory reconsolidation theory, associative memories are rendered labile during retrieval (i.e., memory reactivation; see Nader and Einarsson 2010). The functional consequence of this memory destabilization is the availability of an accessible, so-called "active," memory trace that can be readily utilized (Lewis 1979). The active trace is akin to short-term memory, which is transiently maintained by the covalent modification of pre-existing proteins (Goelet et al. 1986) and exocytosis of the readily releasable pool of neurotransmitters (Tarnow 2008). This post reactivation short-term memory must then be re-stabilized into LTM storage via processes that are sensitive to anisomycin (ANI), including RNA transcription, the synthesis of new proteins, and/or post-translational modification (Nader et al. 2000b; see Gold 2008 for a review of ANI-sensitive processes), in order to be retained over time and to exert persistent stimulus control over conditioned behaviors

(Tronson and Taylor, 2007). The memory reconsolidation theory suggests that the life cycle of a memory trace is dynamic, which starkly contrasts with the more antiquated assumption that memories are rigidly secured in neuronal networks following initial stabilization, or *consolidation* (for review see McKenzie and Eichenbaum 2011). During memory consolidation, a recently acquired associative memory undergoes stabilization on the cellular level and then again on the systems level. Cellular consolidation involves long-lasting changes in gene expression and synaptic efficacy that support memory trace storage, whereas systems consolidation requires the recurrent activation of hippocampal-cortical neuronal ensembles, which results in the so-called "transfer" of memory from the hippocampus to the cortex (McGaugh, 2000; Dudai 2004). Historically, these processes were hypothesized to result in permanent memory storage, but evidence suggesting that consolidated memories are susceptible to disruption by amnesic agents following retrieval arguably has challenged this view (Misanin et al. 1968; Lewis 1979). In 2000, Nader and colleagues demonstrated that re-exposure to a previously foot shock-paired CS in the absence of the foot shock was sufficient to destabilize the consolidated CS-foot shock aversive associative memory, lending credence to the memory reconsolidation hypothesis. Post-reminder microinfusions of the protein synthesis and post-translational modification inhibitor, ANI, into the basolateral amygdala (BLA) of rats impaired the memory for the tone-foot shock association, and the memory impairment was evident as attenuated conditioned freezing behavior in response to the tone 24 h later in ANI-treated rats, relative to VEH-treated rats (Nader et al. 2000a).

This study provided significant impetus to the study of memory reconsolidation. Since then, memory reconsolidation inhibition has been demonstrated across a number of different learning and memory paradigms and in a number of different species, including

rodents, chicks, zebrafish, and notably, humans (reviewed in Nader and Einarsson 2010). Furthermore, memory reconsolidation inhibition has been proposed as a treatment strategy for disorders characterized by pathological memories, including post-traumatic stress disorder, phobias, and drug addiction (Taylor et al. 2009; Milton and Everitt 2010). With respect to drug addiction, the putative period of memory vulnerability induced by retrieval may represent a therapeutic window during which pharmacological treatment can disrupt the re-stabilization of associative memories and prevent subsequent environmental context- or CS-induced drug relapse.

Modeling the impact of cocaine-related memory reconsolidation on cocaine relapse

Procedural modifications to several animal models of drug relapse, including the contextual variant of the rodent extinction-reinstatement paradigm (Fuchs et al. 2009; Ramirez et al. 2009), have made it possible to test hypotheses about the relationship between drug-related memory reconsolidation and addictive behavior and to explore the putative neural substrates of cocaine-related memory reconsolidation (also see Miller and Marshall 2005; Lee et al. 2006). In the contextual extinction-reinstatement paradigm, rats are trained to self-administer cocaine in a distinct environmental context and undergo extinction training in a different context (see Fuchs et al. 2008 for review). Following extinction training, rats are returned to the previously cocaine-paired context for a test of drug context-induced reinstatement of cocaine-seeking behavior. Re-exposure to the cocaine-paired context reliably reinstates extinguished cocaine-seeking behavior in the absence of cocaine itself, consistent with the retrieval and utilization of cocaine-related associative memories (Fuchs et al. 2008). To adapt this model for the study of drug-related memory reconsolidation, rats are briefly re-exposed to the cocaine-paired context (i.e., cocaine-related memory reactivation)

following extinction training. It is assumed that cocaine memory reactivation triggers the initiation of memory reconsolidation processes (Fuchs et al. 2009). Hence, site-directed pharmacological manipulations following the memory reactivation session (i.e., 0-2 h post session, during the period of putative memory trace lability) allow for the selective manipulations of brain regions, receptors, or molecules that are hypothesized to be critical for cocaine-related memory re-stabilization. The effects of these manipulations on the ability of the drug context to reinstate extinguished cocaine-seeking behavior are tested after additional extinction training (i.e., 72 h later). Furthermore, to examine the longevity of the putative memory reconsolidation impairment, cocaine-seeking behavior may be assessed after an extended drug-free period (e.g. 21 d, present study). Research from our laboratory has utilized this model to elucidate the neural underpinnings of drug memory reconsolidation (e.g., Fuchs et al. 2009; Ramirez et al. 2009).

Neural substrates of drug memory reconsolidation

So far, studies have revealed significant overlap in the neural substrates involved in the expression of drug-seeking behaviors and in the reconsolidation of drug-related memories (Miller and Marshall 2005; Fuchs et al. 2009; Ramirez et al. 2009). One of the most extensively studied brain regions in this respect is the basolateral amygdala (BLA). The BLA is integral to the expression of both CS- and context-induced reinstatement of cocaine seeking (Meil and See 1997; Kantak et al. 2002; Fuchs et al. 2002, 2005, 2007) and is also a site for memory reconsolidation (Nader et al. 2000a; Milekic et al. 2007; Mamiya et al. 2009; Li et al. 2010). The BLA is critically involved in the reconsolidation of conditioned stimulus (CS)-drug associative memories that regulate drug-conditioned place preference (Milekic et

al. 2006; Li et al. 2010; Théberge et al. 2010), conditioned reinforcement, and drug-seeking behavior (Lee et al. 2005, 2006a; Milton et al. 2008; Théberge et al. 2010). Furthermore, research from our laboratory has demonstrated that ANI-sensitive processes in the BLA control the reconsolidation of context-response-cocaine associative memories and the subsequent ability of a drug-paired context to reinstate extinguished cocaine-seeking behavior (Fuchs *et al*., 2009).

Similar to the BLA, the dorsal hippocampus (DH) is required for the expression of drug context-induced cocaine-seeking behavior in rats (Fuchs et al. 2005, 2007), but its exact contribution to the reconsolidation of cocaine-related associative memories remains unclear. Tetrodotoxin-induced neuronal inactivation of, but not ANI treatment in, the DH following re-exposure to a cocaine-paired context inhibits subsequent drug context-induced reinstatement of cocaine-seeking behavior (Ramirez et al. 2009). This effect is cocaine memory reactivation-dependent, an important corollary of a genuine memory reconsolidation deficit (Nader et al. 2000b). This suggests that while the DH is not a critical site for protein synthesis and/or post-translational modification required for memory re-stabilization, *per se*, it is necessary for the utilization of memories that have been reconsolidated elsewhere, perhaps in the BLA.

Intrahemispheric interaction between the BLA and DH is required for the expression of drug context-induced cocaine-seeking behavior (Fuchs et al. 2007). Similar interactions between these brain regions may also be necessary for memory reconsolidation, including the stabilization of cocaine-related associative memories that regulate cocaine-seeking behavior. In support of this idea, neuronal populations within subregions of the BLA and DH exhibit synchronized neural activity concomitant with the reconsolidation of remote fear memories

(Narayanan et al. 2007). However, to date, it has not been investigated whether the BLA and DH – or in fact any two brain regions - interact or independently regulate memory reconsolidation. Hence, the overarching aim of the present study was to examine the role of BLA/DH interactions in cocaine-related memory reconsolidation.

Hypothesis and Predictions

To test the hypothesis that intrahemispheric interaction between the BLA and DH at the time of memory reconsolidation is necessary for the ability of a cocaine-paired context to subsequently elicit cocaine-seeking behavior, the present study utilized a disconnection manipulation to bilaterally impair either intrahemispheric communication (i.e., BLA/DH disconnection) or interhemispheric communication (i.e., ipsilateral control manipulation) between the BLA and DH immediately following re-exposure to a cocaine-paired environmental context (experiment 1). BLA/DH disconnection following cocaine-related memory retrieval was predicted to attenuate subsequent drug context-induced cocaine-seeking behavior to a greater degree than the ipsilateral manipulation, consistent with our hypothesis that intrahemispheric, but not interhemispheric, interactions (Olton et al. 1982; Gaffan et al. 1993) between the BLA and DH regulate cocaine memory reconsolidation. Additionally, consistent with a *bona fide* memory reconsolidation deficit, it was predicted that the effects of BLA/DH disconnection would depend on the retrieval and destabilization of context-response-cocaine associations (Nader and Wang 2006). To this end, BLA/DH disconnection was carried out following exposure to an unpaired context. This manipulation was expected to have no effect on subsequent cocaine-seeking behavior in the cocaine-paired context (experiment 2). Finally, reflecting another characteristic of genuine memory

reconsolidation impairments, BLA/DH disconnection was predicted to produce a long-lasting impairment in drug context-induced cocaine-seeking behavior. Specifically, BLA/DH disconnection was expected to produce similar attenuation in cocaine-seeking behavior following an overnight (i.e., 0-d) or an extended (i.e., 21-d) drug-free period (experiment 3; Alberini et al. 2006).

CHAPTER II
METHODS AND MATERIALS

Subjects

Male Sprague-Dawley rats (Charles-River, Wilmington, MA, USA N= 61) were maintained in a temperature- and humidity-controlled vivarium on a reversed light-dark cycle. Rats weighed between 275-300 g at the start of the experiment and were maintained on 20-25 g of rat chow per day with water available *ad libitum*. The housing and treatment of animals used in the study followed the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996) and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Procedures

Food Training. To accelerate the acquisition of cocaine self-administration, rats were initially trained to press a lever under a continuous schedule of food reinforcement (i.e., each press on the designated active lever resulted in the delivery of a 45-mg pellet: Noyes, Lancaster, NH, USA) during a 16-h session overnight. Food training was conducted in standard sound-attenuated operant conditioning chambers (26 x 27 x 27 cm high; Coulbourn Instruments, Allentown, PA, USA). During the session, lever presses on a second lever, designated as the inactive lever, had no programmed consequences. The contextual stimuli that were used during cocaine self-administration and extinction training were not present in the chambers during food training.

Surgery. Forty-eight hours after food training, rats were fully anesthetized with ketamine hydrochloride and xylazine (66.6 mg/kg and1.33 mg/kg, i.p, respectively). Intravenous catheters were constructed in-house, as described previously (Fuchs et al. 2007). The catheter was inserted into the right jugular vein and ran subcutaneously to the back where it exited between the scapulae. Immediately after the catheterization surgery, rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL, USA). Twenty-six gauge stainless steel guide cannulae (Plastics One) were aimed unilaterally at the right or left BLA (-2.7 mm AP, ±5.2 mm ML, -6.8 mm DV, relative to bregma) and at the contralateral or ipsilateral DH (angled rostrally by 15º to accommodate the BLA cannula, -4.1 mm AP, ±2.1 ML, -2.7 mm DV, relative to bregma). Stainless steel screws and cranioplastic cement secured the guide cannulae to the skull. Stylets (Plastics One) and Tygon caps sealed the guide cannulae and catheter, respectively, in order to prevent occlusion.

Rats were given 5 days of post-operative recovery before the start of the experiment. To maintain catheter patency during this time, the catheters were flushed through 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) followed by 0.1 ml of heparinized saline (70 U/ml). During self-administration training, catheters were flushed through with 0.1 ml of heparinized saline (10 U/ml) before each session and with 0.1 ml of the cefazolin solution followed by 0.1 ml of heparinized saline (70 U/ml) after each session. Catheter patency was assessed before the first self-administration session and periodically during the experiment, using propofol

(1mg/0.1ml, i.v. Eli Abbott Lab, North Chicago, IL, USA), which produces temporary loss of muscle tone when administered intravenously.

Cocaine Self-administration Training. Self-administration training was conducted in standard operant conditioning chambers configured to one of two distinctly different contexts. Context 1 contained a continuous red house light (0.4 fc brightness) opposite to the active lever, intermittent pure tone (80 dB, 1 kHz; 2 s on, 2 s off), pine-scented air freshener strip (4.5 cm x 2 cm, Car Freshener Corp., Watertown, NY, USA), and a wire mesh floor (26 cm x 27 cm). Context 2 contained an intermittent white stimulus light above the inactive lever (1.2 fc brightness, 2 s on, 4 s off), continuous pure tone (75 dB, 2.5 kHz), vanilla-scented air freshener strip (4.5 x 2 cm, Sopus Products, Moorpark, CA, USA), and a slanted ceramic tile wall that bisected a bar floor (19 cm x 27 cm). Rats were randomly assigned to Context 1 or Context 2 and allowed to self-administer cocaine in that context under a fixed-ratio 1 schedule of cocaine reinforcement (cocaine hydrochloride; 0.15 mg/0.05 ml per infusion, i.v.; NIDA, Research Triangle Park, NC, USA). Training took place during daily 2-h sessions during the rats' dark cycle. The rats' catheters were connected to an infusion apparatus (Coulbourn Instruments, Allentown, PA, USA) via polyethylene 20 tubing and liquid swivels (Instech, Plymouth Meeting, PA, USA). Active lever presses activated an infusion pump for 2 s. Each infusion was immediately followed by a 20-s time-out period, during which active lever presses had no programmed consequences. Inactive lever presses were recorded but had no programmed consequences. Training continued until rats reached a criterion of ≥10 cocaine infusions per session during at least 10 sessions. Data collection and reinforcer delivery were controlled using Graphic State Notation software version 2.102

(Coulbourn).

Extinction Training. After reaching the acquisition criterion, rats received 7 daily 2-h extinction training sessions. Rats that had self-administered cocaine in Context 1 were placed into Context 2 for extinction training, and vice versa. During the extinction sessions, active and inactive lever presses were recorded but had no programmed consequences. Immediately after the fourth extinction session, rats were adapted to the intracranial microinfusion procedure. To this end, stainless steel injection cannulae were inserted into the guide cannulae to a depth of either 1mm (DH) or 2mm (BLA) below the tip of the guide cannulae. The injector cannulae remained in place for 4 minutes, but no fluid was infused during the adaptation procedure.

Experiment 1*:* Effects of BLA/DH disconnection on cocaine memory reconsolidation

Experiment 1 was designed to evaluate whether functional disconnection of the BLA and DH following cocaine memory reactivation would impair subsequent drug context-induced cocaine seeking. A schematic representing the experimental timeline is provided in **Fig. 2A.**

Memory Reactivation. After the final day of extinction training, rats were re-exposed to the cocaine-paired context for 15 min in order to destabilize cocaine-related memories (Lewis 1979; Nader et al. 2000b; Tronson and Taylor 2007). This session length was selected because it is sufficient to reactivate cocaine-related associative memories without producing significant behavioral extinction (Fuchs et al. 2009). During the memory reactivation

session, rats were connected to the infusion apparatus, but fluids were not infused and responding on the active and inactive levers had no programmed consequences.

Intracranial manipulations. Immediately after the memory reactivation session, rats received a unilateral microinfusion of anisomycin (ANI; 62.5 µg/0.5 µl) into the left or right BLA plus a unilateral microinfusion of the GABA agonists baclofen/muscimol (B/M; 1.0/0.01 mM/0.5 µl) into the contralateral or ipsilateral DH. The dose of ANI used was selected based on our previous research demonstrating that when microinfused bilaterally into the BLA, but not the overlying posterior caudate-putamen (pCPu), this dose was sufficient to disrupt cocaine memory reconsolidation in our model (Fuchs et al. 2009). While tetrodotoxin was used in our previous study to demonstrate the involvement of the DH, but not the overlying trunk region of the somatosensory cortex (SStr), in cocaine memory reconsolidation (Ramirez et al. 2009), B/M was used in the present disconnection study in order to selectively inhibit neural activity within the DH while sparing fibers of passage (van Duuren et al. 2007). The dose of B/M was selected based on an earlier BLA/DH disconnection study (Fuchs et al. 2007). Vehicle control groups received phosphate buffered saline (VEH; 0.5 µl) unilaterally into the BLA plus the contralateral or ipsilateral DH. Assignment to treatment conditions was counterbalanced based on previous cocaine intake. During the microinjection procedure, the injection cannulae were connected to Hamilton Syringes (Hamilton Co., Reno, NV) that were mounted on a microdrive pump (KD Scientific, Holliston, MA). Microinfusions were delivered over 2 min, and the injection cannulae were left in place for 1 min before and after the microinfusion in order to limit drug diffusion, as described previously (Fuchs *et al*. 2007).

Extinction training and Test of Drug Context-induced Cocaine Seeking. Starting on the day following the memory reactivation session, rats received additional daily 2-h extinction training sessions until they reached an extinction criterion (i.e., ≤25 active lever responses per session on a minimum of 2 consecutive days). Twenty-four hours later, rats were returned to the cocaine-paired context for a 2-h test of cocaine-seeking behavior. During the test session, active and inactive lever presses were recorded, but had no programmed consequences.

General motor activity testing. Intracranial manipulations can alter cocaine-seeking behavior by impairing general motor activity. This was unlikely in the present study since testing occurred at a minimum of 72 hours following the intracranial manipulation. Nonetheless, the possible protracted effects of contralateral and ipsilateral ANI+B/M and VEH treatments on motor activity were assessed 24 hours after the reinstatement test in experiment 1. The general motor activity test took place in novel Plexiglas chambers (42 x 20 x 20 cm) that were equipped with an array of eight photodetectors. A computerized activity system (San Diego Instruments, San Diego, CA) recorded photobeam breaks resulting from the movement of rats in the chamber during a 2-h session.

Experiment 2*:* Effects of BLA/DH disconnection in the absence of explicit cocaine memory reactivation

The memory reconsolidation theory posits that reconsolidation inhibitors selectively target memories that have been rendered labile by reactivation (Nader et al. 2000b; Alberini

et al. 2006; Tronson and Taylor 2007). Experiment 2 was designed to evaluate whether the effect observed in experiment 1 would be similarly observed in the absence of explicit cocaine memory reactivation. The experimental protocol was identical to that in experiment 1 except that the groups were placed into a novel, unpaired context for 15 min prior to receiving ipsilateral or contralateral ANI+B/M or VEH+VEH microinfusions into the BLA and DH, respectively. The unpaired context contained continuous white stimulus lights above each lever, a continuous red house light (0.4 fc brightness) opposite to the active lever, a continuous complex tone (80 dB, alternating between 1, 1.5, and 2.5 kHz at 1 s intervals), a citrus-scented air freshener strip (4.5 x 2 cm, Locasmarts LLC., Ormond Beach, Fl), and ceramic tile flooring (26 cm x 27 cm). A schematic representing the experimental timeline for experiment 2 is provided in **Fig. 4A.**

Experiment 3*:* Time-dependent effects of post-memory reactivation BLA/DH disconnection on drug context-induced cocaine seeking

Genuine memory reconsolidation impairments are characterized by the loss or weakening of the memory trace, and in turn, long-lasting changes in behavior (Nader and Wang 2006). Experiment 3 was designed to evaluate the effects of BLA/DH disconnection, administered following re-exposure to a cocaine-paired context, on the ability of the cocaine-paired context to reinstate cocaine-seeking behavior after an extended drug-free period.

All protocols in this experiment were identical to those used in experiments 1 and 2, except that rats were assigned to stay in their home cages for 21 days following the memory reactivation session and intracranial microinfusions. During the home cage stay, rats were handled regularly. A schematic representing the experimental timeline is provided in **Fig.**

5A. The contralateral BLA/DH-cannulated groups from experiment 1 served as 0-d (overnight) home cage control groups in experiment 3.

Histology. After the last experimental session, rats were overdosed with ketamine hydrochloride and xylazine (66.6 and 1.3 mg/kg, i.v. or 199.8 and 3.9 mg/kg, i.p., respectively, depending on catheter patency). They were then transcardially perfused with 1×-phosphate-buffered saline (Fisher Scientific) and 10% formaldehyde solution (Sigma). Brains were dissected out and stored in 10% formaldehyde solution until they were sectioned in the coronal plane at a thickness of 75 µm using a vibratome. The sections were mounted onto gelatin-coated slides and stained using cresyl violet (Kodak, Rochester, NY, USA). Cannula placements were verified using light microscopy. The most ventral portion of each cannula tract was mapped onto schematics of appropriate plates from the rat brain atlas (Paxinos and Watson 1997).

Data Analysis. Separate ANOVAs were conducted to test for possible pre-existing differences in cocaine intake as well as active and inactive lever responding during cocaine self-administration training (mean of last 3 days), extinction training (day 1, day 7), and during the memory reactivation session for the groups of rats in experiments 1, 2, and 3. In these ANOVAs, group was included as a between-subjects factor and time (extinction day 1, extinction day 7) was included as a within-subjects factor, as appropriate. Pearson *r* correlation coefficients were calculated to examine the relationship between active lever responding during the memory reactivation session and during the test of drug context-induced cocaine-seeking behavior.

Separate mixed-factorial ANOVAs were conducted to examine the effects of post-reactivation manipulations on the number of days required to reach the extinction criterion, on active and inactive lever responses on the test days in the cocaine-paired and extinction contexts (last extinction session before the test in the cocaine-paired context), and on motor activity. In these ANOVAs, treatment (VEH + VEH, ANI + B/M), surgery type (ipsilateral, contralateral), and home cage condition (0 d, 21 d) were included as between-subjects factors, while context (extinction, cocaine-paired) and time (six 20-min intervals) were included as within-subjects factors, as appropriate. Significant main and interaction effects were further probed using post-hoc Tukey tests. In addition, the potential hemispheric laterality of significant effects was examined using t-tests separately because the variables BLA hemisphere and DH hemisphere were not orthogonal. Alpha was set at 0.05

CHAPTER III
RESULTS

Histology. Schematics and photomicrographs representing cannula placements are included in **Fig. 1**. The target brain regions were defined as the lateral and basolateral nuclei of the amygdala (BLA) and the dorsal hippocampus proper (DH). Further inspection of neural tissue using high power microscopy revealed no indication of tissue damage (i.e., extensive cell loss or gliosis). Data from rats with misplaced cannulae were excluded from subsequent statistical analyses. The resulting Ns per vehicle (VEH)- and drug-treated groups were: contralateral VEH (BLA) + VEH (DH) 0 d, n= 8; contralateral VEH (BLA) + VEH (DH) 21 d, n = 7; contralateral ANI (BLA) + B/M (DH) 0 d, n = 10; contralateral ANI (BLA) + B/M (DH) 21 d, n = 7; ipsilateral VEH (BLA) + VEH (DH), n = 8; ipsilateral ANI (BLA) + B/M (DH), n = 7; no reactivation contralateral VEH (BLA) + VEH (DH), n = 7; and no reactivation contralateral ANI (BLA) + B/M (DH), n = 7.

Behavioral History. Analysis of variance (ANOVA) did not indicate any pre-existing differences between the groups in cocaine intake, in active or inactive lever responding during cocaine self-administration training, extinction training, during the memory reactivation session, or in the number of days required to reach the extinction criterion before testing. These data are provided in **Table 1**. Correlational analyses revealed that active lever responding during the memory reactivation session did not significantly predict active lever responding during the test of drug context-induced cocaine seeking for the groups that had received VEH (r = 0.114, p = 0.604) or ANI+B/M treatment following

the memory reactivation session (r = 0.496, p = 0.495). Furthermore, none of the analyses revealed hemisphere-dependent effects (i.e., laterality, data not shown). Only statistically significant effects are reported below. The eta-squared estimates of effect size for all statistically significant effects ranged between 0.015 and 0.770.

Experiment 1

Experiment 1 was designed to evaluate whether intrahemispheric interaction between the BLA and DH is necessary for cocaine-related memory reconsolidation and for the subsequent ability of a cocaine-paired context to reinstate cocaine-seeking behavior (see experimental timeline in **Fig. 2A**). Contralateral BLA/DH treatment with ANI+B/M was expected to bilaterally disrupt putative intrahemispheric interactions between the BLA and DH. Conversely, the ipsilateral manipulation was expected to bilaterally disrupt interhemispheric connections between the BLA and DH while sparing intrahemispheric interactions between these brain regions in the unmanipulated hemisphere (Olton et al. 1982; Gaffan et al. 1993). Thus, it was postulated that requisite intrahemispheric interactions between the BLA and DH would be indicated by greater deficit in cocaine seeking following the contralateral manipulation relative to the ipsilateral manipulation.

BLA/DH disconnection following cocaine memory reactivation attenuated subsequent cocaine-seeking behavior in a context- and lever-dependent manner, while the ipsilateral ANI+B/M manipulation had no effect on responding relative to VEH (see **Fig. 2B**). The 2 x 2 x 2 ANOVA of active lever responses indicated a significant surgery type x treatment x context interaction effect ($F_{(1,29)}$ = 4.679, p = 0.039), as well as significant surgery type x context interaction ($F_{(1,29)}$ = 8.473, p = 0.007), treatment x surgery type

interaction ($F_{(1,29)}$ = 5.209, p = 0.030), context main ($F_{(1,29)}$ = 66.482, p < 0.001), and surgery type main ($F_{(1,29)}$ = 8.463, p = 0.007) effects. Thus, re-exposure to the cocaine-paired context during testing elicited an increase in active lever responding in both VEH control groups and in the ipsilateral ANI+B/M-treated control group, relative to responding in the extinction context (ANOVA context simple main effect, Tukey test, p < 0.05). Conversely, the group that had received BLA/DH disconnection manipulation (i.e., contralateral ANI+B/M treatment) following cocaine memory reactivation subsequently exhibited less active lever responding in the cocaine-paired, but not the extinction context, relative to all other groups (ANOVA treatment and surgery type simple main effects, Tukey test, p < 0.05). As a result, responding in this group was not different in the cocaine-paired and extinction contexts on the test days.

Time course analysis of active lever responding revealed that the effects of contralateral ANI+B/M treatment on drug context-induced cocaine-seeking behavior were independent of time interval (see **Fig. 2D**). The 2 x 2 x 6 ANOVA of active lever responses across the six 20-min intervals of the test session revealed a significant surgery type x treatment interaction effect ($F_{(1,29)}$ = 5.196, p = 0.030), as well as time main ($F_{(5,145)}$ = 10.978, p < 0.001) and surgery type main ($F_{(1,29)}$ = 8.45, p = 0.007) effects. Active lever responding decreased during the test session (ANOVA time main effect, interval 1 > intervals 2-6; Tukey test, p < 0.05). Collapsed across time interval, there was no difference in active lever responding between the VEH control groups and the ipsilateral ANI+B/M-treated group. In contrast, the group that had received BLA/DH disconnection manipulation following cocaine memory reactivation subsequently exhibited less active lever responding

relative to all other groups (ANOVA treatment and surgery type simple main effects, Tukey test, $p < 0.05$).

The 2 x 2 x 2 ANOVA for inactive lever responses indicated that exposure to the cocaine-paired context elicited a slight increase in inactive lever responding in all groups relative to responding in the extinction context (see **Fig. 2C**; ANOVA context main effect only, $F_{(1, 29)} = 6.599$, $p = 0.016$). Time course analysis of inactive lever responses during the test of drug context-induced cocaine seeking revealed a significant treatment x time interaction effect ($F_{(5,145)} = 2.446$, $p = 0.037$) and a time main effect ($F_{(5,145)} = 5.625$, $p < 0.001$). Independent of surgery-type, VEH groups exhibited a decrease in inactive lever responding during the test session (ANOVA time simple main effects, interval 1 > intervals 2-6, Tukey test, $p < 0.05$). Furthermore, the groups that had received ipsilateral or contralateral ANI+B/M treatment following memory reactivation exhibited less inactive lever responding than VEH groups during interval 1 (ANOVA treatment simple main effect, Tukey test, $p < 0.05$).

Motor Activity. The protracted effects of intracranial manipulations on general activity can impact instrumental cocaine-seeking behavior. To examine this possibility, the effect of BLA/DH disconnection and ipsilateral manipulation on locomotor activity was evaluated in a novel Plexiglas chamber 24 h after the test of drug context-induced cocaine seeking.

General motor activity gradually declined as rats habituated to the chamber, and this effect was independent of treatment. These data are provided in **Fig. 3**. The 2 x 2 x 6 ANOVA of photobeam breaks revealed a significant time main effect only ($F_{(5,145)} =$

104.272, $p < 0.001$). Collapsed across surgery type and treatment, rats generated fewer photobeam breaks during intervals 2-6 relative to the first 20-min interval of the session (Tukey test, $p < 0.05$). Importantly, neither BLA/DH disconnection nor the ipsilateral manipulation with ANI+B/M administered following memory reactivation altered subsequent general motor activity relative to VEH treatment.

Experiment 2

Memory reconsolidation deficits are expected to depend on memory reactivation (Nader et al. 2000b; Alberini et al. 2006; Tronson and Taylor 2007). Thus, we evaluated whether the effects of BLA/DH disconnection on cocaine-seeking behavior would depend on re-exposure to the cocaine-paired context immediately prior to the disconnection manipulation. To this end, "no reactivation" control groups were exposed to a novel, unpaired context prior to receiving the BLA/DH disconnection manipulation or VEH treatment (see experimental timeline in **Fig 4A**).

BLA/DH disconnection in the absence of explicit cocaine memory reactivation failed to alter drug context-induced reinstatement of cocaine-seeking behavior, relative to VEH treatment (see **Fig. 4B**). The 2 x 2 ANOVA for active lever responses revealed that exposure to the cocaine-paired context produced an increase in active lever responding in the groups that received ANI+B/M or VEH treatment following exposure to the unpaired context, relative to responding in the extinction context (ANOVA context main effect only, $F_{(1, 12)} = 23.169$, $p < 0.001$). Furthermore, there was no difference between these groups in active lever responding in the extinction or cocaine-paired context on the test days.

The 2 x 2 ANOVA for inactive lever responses revealed that exposure to the cocaine-paired context on the test day elicited a slight increase in inactive lever responding in both groups, relative to responding in the extinction context (see **Fig. 4C**), and BLA/DH disconnection following exposure to the novel context did not subsequently alter inactive lever responding in the extinction or cocaine-paired context, relative to VEH treatment (ANOVA context main effect only, $F_{(1, 12)} = 9.096$, $p < 0.020$).

Experiment 3

Memory reconsolidation inhibitors are expected to impair the target memory trace and, therefore, to exert an enduring effect on conditioned behavior (Alberini et al. 2006; Nader and Wang 2006). Accordingly, we examined whether BLA/DH disconnection following cocaine memory reactivation would disrupt cocaine-seeking behavior after a prolonged drug-free period (i.e., 21-day versus overnight home cage stay, each followed by a minimum of 2 days of extinction training prior to the test of drug context-induced cocaine-seeking behavior; see experimental timeline in **Fig 5A**). During the home cage stay, rats were handled regularly.

BLA/DH disconnection following cocaine memory reactivation attenuated subsequent cocaine-seeking behavior in a context-dependent manner relative to VEH treatment, and this effect was independent of home cage condition (see **Fig. 5B**). The 2 x 2 x 2 ANOVA for active lever responses indicated significant treatment x context interaction ($F_{(1,29)} = 29.881$, $p < 0.001$), home cage condition main ($F_{(1,29)} = 5.631$, $p = 0.010$), context main ($F_{(1,29)} = 136.433$, $p < 0.001$), and treatment main effects ($F_{(1,29)} = 43.263$, $p < 0.001$). Thus, active lever responding increased following the 21-d home cage stay, consistent with

the incubation phenomenon (see **Fig. 5B, inset**; Tran-Nguyen et al., 1998; Grimm et al., 2001; Lu et al. 2004). Collapsed across home cage condition, re-exposure to the cocaine-paired context on the test day elicited increased active lever responding in the VEH groups, relative to responding in the extinction context (ANOVA context simple main effect, Tukey test, $p < 0.05$). Furthermore, the groups that had received BLA/DH disconnection after cocaine memory reactivation subsequently exhibited less active lever responding in the cocaine-paired, but not in the extinction, context relative to the VEH groups (ANOVA treatment simple main effect, Tukey test, $p < 0.05$).

Time course analysis of active lever responses during the test of drug context-induced cocaine seeking indicated that responding depended on treatment, home cage condition, and time (see **Fig. 5D**). The ANOVA of active lever responses during the six 20-min time intervals of the test session indicated a significant treatment x home cage condition x time interaction effect ($F_{(5,140)} = 4.465$, $p = 0.001$), as well as significant treatment x time interaction ($F_{(5,140)} = 3.304$, $p = 0.008$), time main ($F_{(5,140)} = 19.702$, $p < 0.001$), treatment main ($F_{(1,28)} = 39.989$, $p < 0.001$), and home cage condition main effects ($F_{(1,28)} = 4.343$, $p = 0.046$). Active lever responding declined over the course of the test session in the VEH groups; however, the rate of decline differed as a function of home cage condition. Specifically, the 0-d VEH group exhibited less responding during intervals 2-6 relative to interval 1 (ANOVA time simple main effects, Tukey test, $p < 0.05$). Conversely, the 21-d VEH group exhibited stable responding during intervals 1-3, and active lever responding in this group decreased during intervals 4 and 6 relative to intervals 1 and 2 (ANOVA time simple main effect, Tukey test, $p < 0.05$). The 0-d ANI+B/M group exhibited low levels of responding throughout the test session, and responding in this group was significantly lower

than that in the respective 0-d VEH group during interval 1 (ANOVA treatment simple main effect, Tukey test, $p < 0.05$). On the other hand, the 21-d ANI+B/M group demonstrated high levels of responding during interval 1 such that responding in this group was not different than responding in the respective 21-d VEH group, but also did not differ from that in the 0-d VEH or 0-d ANI+B/M groups. Interestingly, however, responding in the 21-d ANI+B/M group rapidly declined such that it was significantly lower than responding in the respective 21-d VEH group during interval 2 (ANOVA treatment simple main effect, Tukey test, $p < 0.05$).

The 2 x 2 x 2 ANOVA for inactive lever responses indicated that exposure to the cocaine-paired context elicited a slight increase in overall inactive lever responding relative to responding in the extinction context (see **Fig. 5C**; ANOVA context main effect only, $F_{(1, 29)} = 7.642$, $p = 0.010$). Neither BLA/DH disconnection nor home cage condition altered inactive lever responding in either context. The time course of inactive lever responses during the test of drug context-induced cocaine-seeking behavior revealed that inactive lever responding declined during the session independent of treatment or home cage condition (**Fig. 5E**; ANOVA time main effect only, $F_{(5,140)} = 9.527$, $p < 0.001$, interval 1 > intervals 2-6, Tukey test, $p < 0.05$).

CHAPTER IV
DISCUSSION

Intrahemispheric interactions between the BLA and DH are critical for the reconsolidation of cocaine-related memories that control drug context-induced cocaine-seeking behavior

To our knowledge, the present study offers the first demonstration that functional interaction between the BLA and DH is required for the reconsolidation of cocaine-related associative memories that underlie the ability of a cocaine-paired environmental context to reinstate extinguished cocaine-seeking behavior. To test for functional interdependence between the BLA and DH, a disconnection procedure was employed. Disconnection of the BLA and DH at the putative time of memory reconsolidation was achieved by administering unilateral microinfusions of ANI into the BLA and B/M into the contralateral DH following cocaine memory reactivation. This manipulation was expected to temporarily inhibit intrahemispheric interaction between the BLA and DH in both hemispheres. Conversely, the ipsilateral ANI+B/M control manipulation was expected to spare intrahemispheric information sharing between these brain regions in one hemisphere while eliminating interhemispheric interactions in both hemispheres. Importantly, ipsilateral and contralateral ANI+B/M treatment affected the same amount of neural tissue (Olton et al. 1982; Gaffan et al. 1993); therefore, unilateral or additive effects of the intra-BLA ANI and intra-DH B/M treatments were expected to manifest similarly following contralateral and ipsilateral administration. In the present study, BLA/DH disconnection with ANI+B/M, but not the ipsilateral manipulation with the same treatment, administered immediately after re-exposure

to the cocaine-paired context, attenuated subsequent drug context-induced reinstatement of cocaine-seeking behavior relative to VEH treatment **(Fig. 2B)**. Following cocaine memory reactivation, bilateral ANI administration into the posterior caudate putamen or bilateral tetrodotoxin-induced neural inactivation of the trunk region of the somatosensory cortex (i.e., reconsolidation inhibitor manipulations in brain regions dorsally adjacent to the BLA and DH, respectively) fails to alter later drug context-induced reinstatement (Fuchs et al. 2009; Ramirez et al. 2009). This suggests that the intracranial manipulations in the present study were anatomically selective to the BLA and DH.

Attenuation in cocaine-seeking behavior observed 48-72 hours following BLA/DH disconnection did not reflect a protracted ANI+B/M-induced motor performance deficit. In strong support of this, in experiment 2, BLA/DH disconnection with ANI+B/M following exposure to an unpaired context did not alter subsequent cocaine-seeking behavior relative to VEH treatment (see **Fig. 4B**). Post-memory reactivation treatment with ANI+B/M administered into the BLA plus the contralateral or ipsilateral DH also failed to suppress general motor activity in a novel context (see **Fig. 3**). Furthermore, ANI+B/M treatment failed to inhibit lever responding in the extinction context or inactive lever responding in either context (see **Fig. 2B** and **2C**). These findings indicate that the attenuation in cocaine-seeking behavior produced by BLA/DH disconnection following cocaine memory reactivation was not due to ANI+B/M-induced hypoactivity or nonspecific impairment in instrumental motor behavior.

An important corollary of the memory reconsolidation hypothesis is that *bona fide* memory reconsolidation deficits depend on memory reactivation (Lewis 1979; Sara 2000; Nader et al. 2000b). Satisfying this requirement, BLA/DH disconnection inhibited

subsequent cocaine-seeking behavior when it was induced following re-exposure to the cocaine-paired context, but not an unpaired context (i.e., in the absence of explicit cocaine-related memory reactivation; see **Fig. 4B**). We have also demonstrated previously that functional disconnection of the BLA and DH at the time of reinstatement testing disrupts the expression of drug context-induced cocaine seeking (Fuchs et al. 2007). Together, these findings suggest that intrahemispheric communication between the BLA and DH critically contributes not only to the reconsolidation of reactivated cocaine-related associative memories into long-term memory storage, but also to the recall or utilization of cocaine-related associative memories in general. Thus, the BLA-DH circuitry tightly regulates the control of environmental stimuli over cocaine-seeking behavior.

While the effects of BLA/DH disconnection were specific to reactivated cocaine-related memories, implying a memory reconsolidation deficit, contralateral ANI+B/M treatment could have directly impaired the labile, post-reactivation short-term memory (PR-STM) that was to be reconsolidated. PR-STM deficits can be determined by measuring conditioned behavior during the period of putative memory lability (i.e., within 4-6 h of memory reactivation; Nader et al. 2000a). The prolonged half-life of B/M (~ 24 h; Martin and Ghez 1993; 1999) prevented such assessment of PR-STM in the present study given that B/M-induced BLA/DH disconnection inhibits the expression of drug context-induced cocaine seeking behavior *per se* (Fuchs et al., 2007). However, somewhat mitigating the possibility that BLA/DH disconnection impaired memory reconsolidation solely by disrupting PR-STM, bilateral intra-BLA ANI treatment fails to disrupt PR-STM in the fear conditioning paradigm (Nader et al. 2000a).

Cocaine-related memory reconsolidation likely involves interactions between the BLA and DH, similar to initial memory consolidation (for review, see Richter-Levin and Akirav 2001). Communication between the BLA and DH facilitates the consolidation of memories that guide hippocampus-dependent behaviors, including maze performance (Packard et al. 1994; Packard and Teather 1998) as well as active and passive avoidance (Roozendaal and McGaugh 1997; Rezayof et al. 2011). Specifically, the BLA appears to play a modulatory role in DH-dependent memory consolidation. Remarkably, however, BLA/DH interactions of a different nature bring about memory reconsolidation in the contextual reinstatement paradigm. The failure of post-reactivation ANI treatment in the DH to impair cocaine-seeking behavior (Ramirez et al. 2009, also see Biedenkapp and Rudy, 2004) suggests that the DH is not the locus of memory re-stabilization *per se,* since ANI-sensitive processes are considered to be necessary for memory reconsolidation (Tronson and Taylor 2007; Nader and Einarsson 2010). Nevertheless, the present study demonstrates that intrahemispheric communication between the DH and the BLA is required for the reconsolidation of context-response-cocaine associative memories that regulate drug context-induced cocaine-seeking behavior. Accordingly, we propose that cocaine-related associative memories undergo ANI-sensitive re-stabilization in the BLA, and the DH may contribute to the maintenance of PR-STM or the establishment of retrieval links in the BLA or elsewhere in the brain during the time of memory reconsolidation. The necessary communication between the BLA and DH may occur via sparse monosynaptic connections between the BLA and DH (Pikkarainen et al. 1999) or via multi-synaptic connections that involve other brain regions. The entorhinal cortex may serve as a relay in this circuit as this brain region has

reciprocal connections with both the BLA and DH (Finch et al. 1986; Witter et al. 1989, van Groen and Wyss 1990; McDonald and Mascagni 1997; Fanselow and Dong 2010).

Time-dependent effects of post-reactivation BLA/DH disconnection on drug context-induced cocaine-seeking behavior and implications for the treatment of drug addiction

Inhibition of memory reconsolidation is predicted to impair the memory trace and, consequently, elicit prolonged interference with conditioned behavior (for review, see Nader 2003; Amaral et al. 2008), and this property is desirable from a treatment perspective. In fact, it has been suggested that disrupting the reconsolidation of maladaptive associative memories may be useful for the treatment of psychiatric disorders, including post-traumatic stress disorder, phobias, obsessive-compulsive disorder, and addictive behavior (Diergaarde et al., 2008; Taylor et al., 2009; Milton and Everitt, 2010). In this respect, it is encouraging that BLA/DH disconnection attenuated overall drug context-induced cocaine-seeking behavior to a similar extent after a 21-day versus overnight home cage stay and approximately 2 extinction training days, relative to VEH (**Fig. 5B**). Thus, memory reconsolidation inhibitors may open a therapeutic window by impairing memory traces that underlie the incentive motivational effects of drug-associated environmental stimuli even though it is unlikely they prevent the resumption of drug-taking behaviors if drug reinforcement contingencies are restored.

Interestingly, the inhibitory effects of BLA/DH disconnection on drug-context-induced cocaine-seeking behavior were apparent only after the first 20-min interval of the test session relative to VEH treatment (**Fig. 5D**). It is unlikely that cocaine-seeking behavior during the first 20-min interval of the test session in the BLA/DH disconnection group reflected

spontaneous recovery, the return of a previously extinguished response with time (Domjan 1998; Eisenberg et al. 2003; Bouton 2004), given that manipulations of the DH (Corcoran et al. 2006b; Bevilaqua et al. 2007) or BLA (Fuchs et al. 2006; McLaughlin and Floresco 2007; Baldi and Bucherelli 2010) impair, rather than enhance, extinction learning. Rather, it probably signifies the maturation – thus delayed availability – of alternate memory traces (McClelland et al. 1995; Frankland and Bontempi 2005; Frankland et al., 2006; Amaral et al. 2008), or the re-strengthening of memory traces weakened by ANI+B/M treatment in the absence of external cue exposure during abstinence. Consistent with the idea of residual memory traces, humans exhibit impaired cue-induced fear memory but intact declarative memory of CS-fear contingency following memory reconsolidation inhibition using beta adrenergic receptor antagonism (Kindt et al. 2009). Similar to declarative memories, the putative residual memories in the present study failed to elicit sustained motivated behavior.

The strengthening of drug-related associative memories may be a mechanism for *incubation*, the well-documented augmentation of drug-seeking behavior after a period of abstinence that has been implicated in the transition from casual drug use to compulsive drug-seeking and drug-taking behaviors (Tran-Nguyen et al., 1998; Grimm et al., 2001; Thomas et al., 2008). In support of this idea, repetitive reflection on drug-related memories predicts future increases in substance abuse symptoms in humans (Nolen-Hoeksema et al. 2007). Consistent with an incubation effect, the groups that remained in their home cages for 21 days following cocaine memory reactivation and were tested on approximately post-cocaine day 32 exhibited more robust context-induced cocaine-seeking behavior than overnight home cage controls (see **Fig. 5B, inset**). This incubation effect was modest relative to that observed in other studies (see Grimm et al. 2001; Lu et al. 2004), likely due to

extensive extinction training (Berglind et al. 2007; Kelamangalath and Wagner 2009). Incubation manifested as impairment in the extinction of drug context-induced cocaine-seeking behavior during the non-reinforced test session (i.e., perseveration) following the 21-day versus overnight home cage stay (see **Fig. 5D**). Remarkably, contralateral ANI+B/M treatment reversed the incubation-related perseveration in cocaine-seeking behavior. These data suggest that, during early withdrawal, the integrity of memory traces that encode context-response-cocaine associations may be necessary for the subsequent development of incubation, consistent with the idea that incubation may involve the strengthening of associative memory traces over time.

In conclusion, BLA/DH disconnection at the time of cocaine memory reconsolidation had a robust inhibitory effect on the motivational effects of a cocaine-paired environmental context when evaluated 18 days after initial memory consolidation and after extensive memory reconsolidation during the 10-day cocaine self-administration training regimen (also see Lee et al. 2006a). Some recovery of cocaine-seeking behavior was observed following an extended drug-free period (i.e., 39 days after initial memory consolidation) likely due to the availability of new or residual cocaine-related associative memories. However, importantly, BLA/DH disconnection attenuated cocaine seeking behavior even after the development of incubation. These findings confirm that the BLA and DH interact to control cocaine-related memory reconsolidation and drug context-induced cocaine-seeking behavior. Furthermore, these findings support the idea that targeting memory reconsolidation to combat environmentally induced relapse may be a worthwhile treatment option for recovering drug addicts.

Treatment Groups	Home cage stay	Cocaine Intake (mg/kg)	Active Lever Responses				Extinction Latency
			Self-administration	Extinction day 1	Extinction day 7	Reactivation	
Contralateral VEH (BLA) + VEH (DH)	0 d	12.93 ± 0.96	61.42 ± 13.49	60.25 ± 12.55	9.00 ± 2.55	28.88 ± 5.58	2.50 ± 0.27
	21 d	10.74 ± 1.12	59.38 ± 17.81	112.9 ± 43.67	5.00 ± 0.98	36.57 ± 5.70	2.43 ± 0.30
Contralateral ANI (BLA) + B/M (DH)	0 d	12.45 ± 1.04	47.47 ± 4.93	69.00 ± 16.91	4.40 ± 0.97	20.40 ± 4.10	2.00 ± 0.00
	21 d	10.36 ± 1.16	55.38 ± 17.71	59.43 ± 13.98	6.43 ± 2.77	13.71 ± 4.35	2.14 ± 0.14
Ipsilateral VEH (BLA) + VEH (DH)	0 d	13.63 ± 1.64	74.79 ± 16.76	39.00 ± 10.35	12.50 ± 3.27	28.13 ± 5.24	2.13 ± 0.13
Ipsilateral ANI (BLA) + B/M (DH)		13.45 ± 1.62	66.29 ± 13.65	99.86 ± 20.88	7.29 ± 2.65	19.71 ± 7.23	2.14 ± 0.14
Contralateral VEH (BLA) + VEH (DH), no reactivation	0 d	11.40 ± 1.41	43.52 ± 4.05	57.71 ± 22.19	6.00 ± 2.51	32.29 ± 12.46	2.29 ± 2.86
Contralateral ANI (BLA) + B/M (DH), no reactivation		12.14 ± 2.54	48.05 ± 11.20	95.71 ± 24.69	6.50 ± 1.91	13.71 ± 5.96	2.00 ± 0.00

Table 1. Cocaine intake (mean mg/kg per session ± SEM), active lever responses (mean ± SEM), and extinction latency (mean number of days needed to reach the extinction criterion ± SEM). Active lever responses are reported for cocaine self-administration training (mean of last 3 days of training), extinction training (the first and last day of training), and for the 15-min memory reactivation or novel context exposure session. The extinction criterion was ≤25 active lever responses on two consecutive sessions following an overnight (0-d) or 21-d home cage stay.

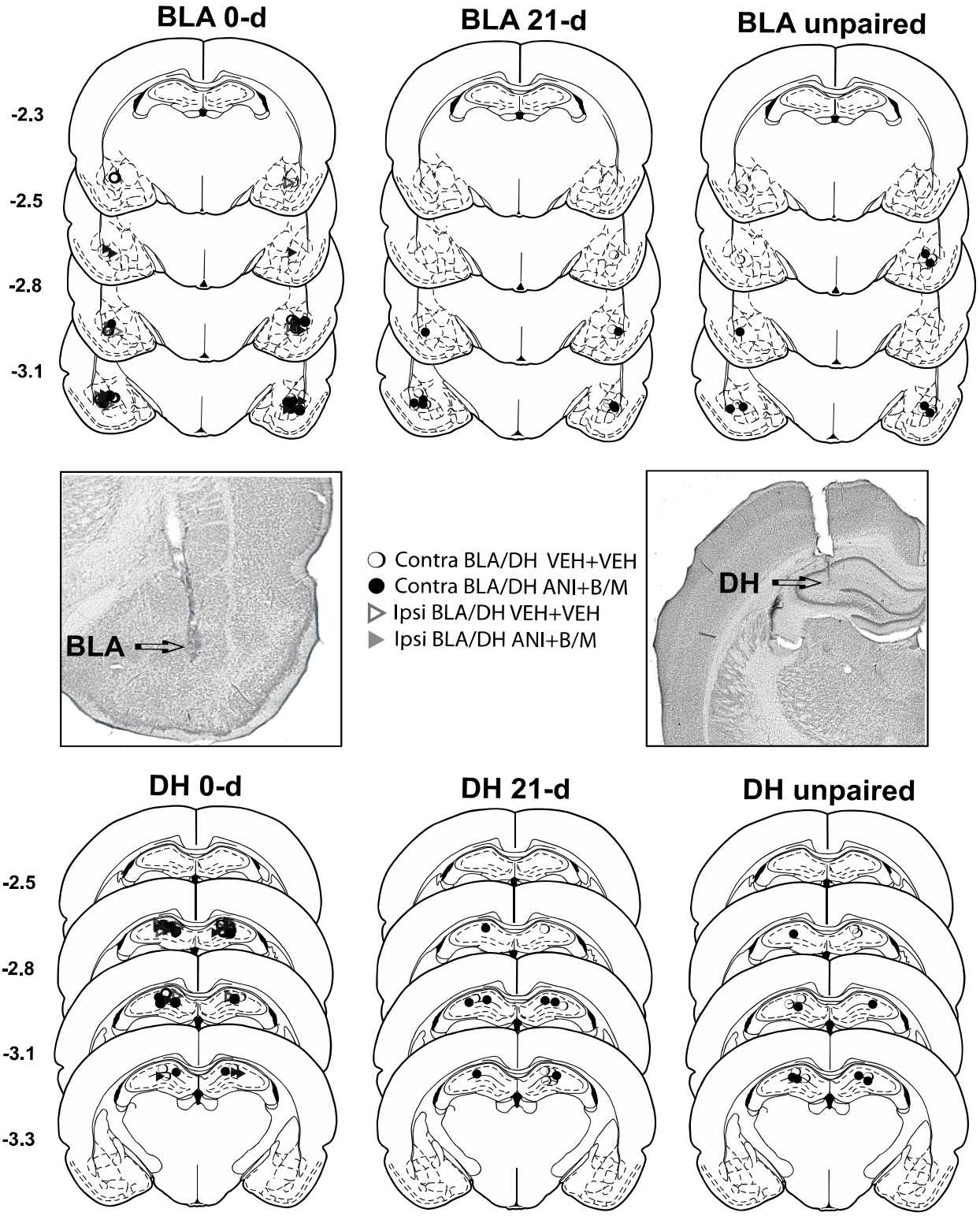

Figure 1. Schematics and photomicrographs depicting cannula placement. Arrows mark the most ventral point of injector cannula tracts for cannulae aimed at the BLA and DH on photomicrographs of representative cresyl violet-stained sections. The symbols on the schematics denote the most ventral point of the injector cannula tracts for rats that received unilateral microinfusions of vehicle (VEH) into the BLA plus VEH into the contralateral DH (*open circles*), anisomycin (ANI) into the BLA plus baclofen/muscimol (B/M) into the

contralateral DH (*filled-in, black circles*), VEH into the BLA plus VEH into the ipsilateral DH (*open triangles*), or ANI into the BLA plus B/M into the ipsilateral DH (*filled-in, grey triangles*). The groups were assigned to remain in their home cages overnight (i.e., *0 d*) or for 21 days following the intracranial manipulations. Additionally, control groups received microinfusions following exposure to an *unpaired* context and remained in their home cages overnight following the intracranial manipulations. Numbers indicate the distance from bregma in mm, according to the rat brain atlas of Paxinos and Watson (1997).

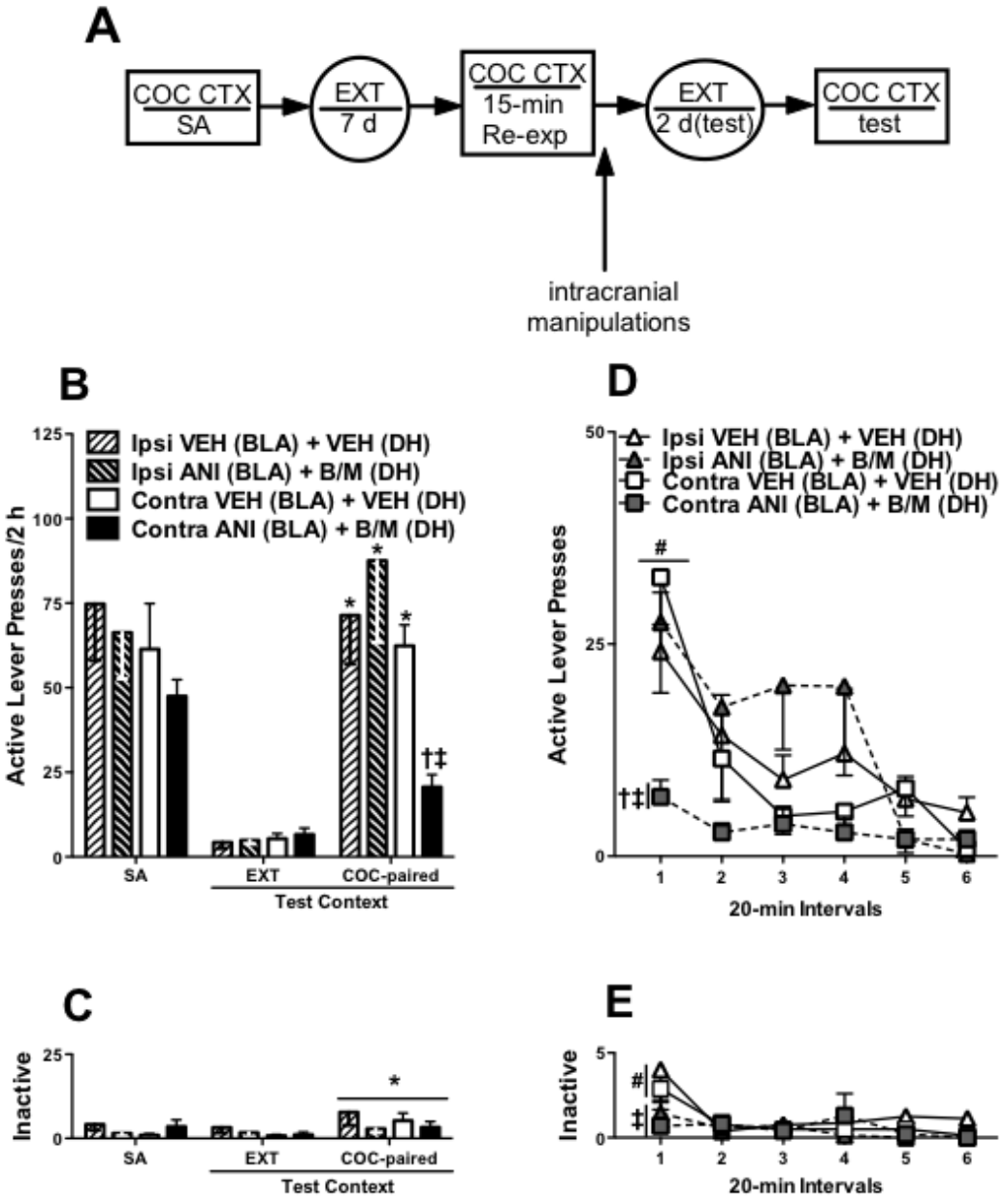

Figure 2. BLA/DH disconnection following cocaine memory reactivation attenuates subsequent drug context-induced cocaine-seeking behavior relative to VEH or ipsilateral ANI+B/M treatment. ***(A)*:** Schematic depicting the timeline for experiment 1. Cocaine self-administration sessions (SA) were conducted in a distinct context followed by extinction (EXT) training in a different context. On post-cocaine day 8, rats were re-exposed to the cocaine-paired context (COC-CTX) for 15 min to reactivate cocaine-related memories and then received unilateral microinfusions of anisomycin (ANI, 62.5 µg/0.5 µl) into the BLA plus baclofen/muscimol (B/M, 1.0/0.01 mM/0.5 µl) into the contralateral or ipsilateral DH.

Control rats received microinfusions of phosphate buffered saline vehicle (VEH, 0.5 µl) into the corresponding brain regions. Groups then remained in their home cages overnight, followed by additional extinction training until they reached the extinction criterion ($\leq$25 active lever responses/session on two consecutive days) and a test of drug context-induced cocaine seeking. ***(B):*** Mean (±SEM) active lever presses during self-administration (SA; mean of the last three training sessions) and during tests of cocaine-seeking behavior in the extinction context (EXT; the last session preceding the test in the cocaine-paired context) and in the cocaine-paired context (COC-paired). ***(C):*** Mean (±SEM) inactive lever presses. ***(D):*** The time course of active lever responses (mean ± SEM) during the test in the cocaine-paired context. ***(E):*** The time course of inactive lever responses (mean ± SEM). *Asterisks* represent significant difference relative to responding in the extinction context (ANOVA context simple main effect, $p < 0.05$). *Dagger* represents significant difference relative to ipsilateral ANI+B/M treatment (ANOVA surgery-type simple main effect, $p < 0.05$). *Double dagger* represents significant difference relative to VEH treatment (ANOVA treatment simple main effect, $p < 0.05$). *Pound sign represents* significant difference relative to all other time intervals (ANOVA time simple main effects, $p < 0.05$).

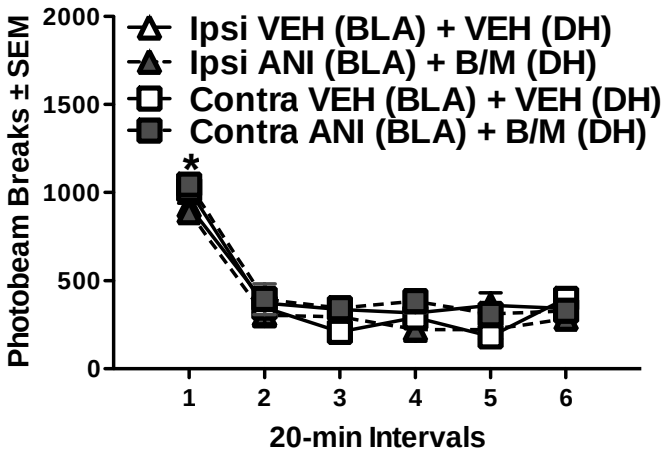

Figure 3. BLA/DH disconnection does not alter general motor activity relative to VEH or ipsilateral ANI+B/M treatment. General motor activity was assessed based on the number of photobeam breaks (±SEM) generated by movement in a novel activity chamber. Motor activity tests were conducted within 24 hours of testing for drug context-induced cocaine-seeking behavior (i.e., approx. 96-h after intracranial manipulation). *Asterisk* represents a significant difference relative to all other 20-min time intervals (ANOVA time simple main effect, $p < 0.05$)

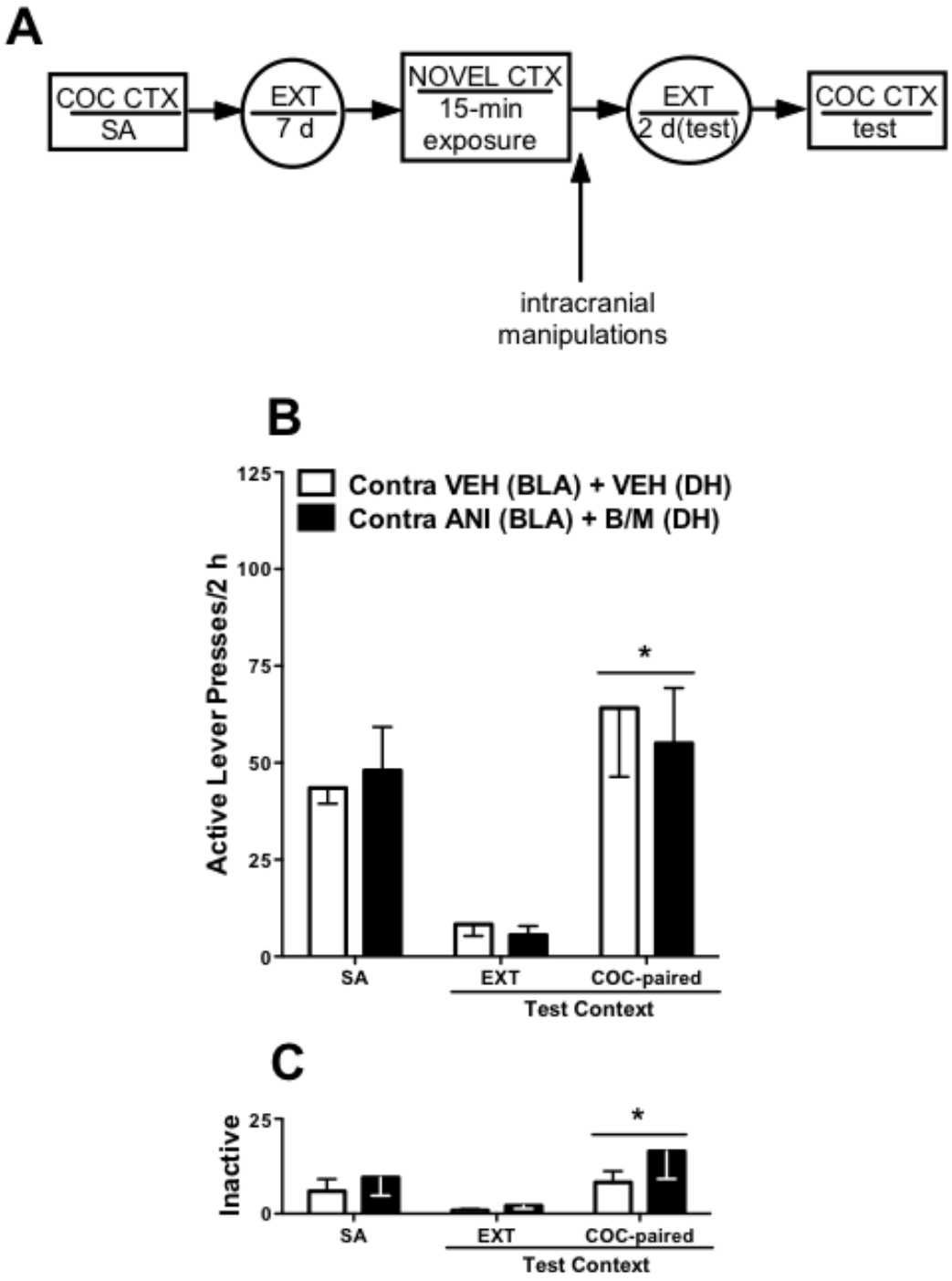

Figure 4. The effects of BLA/DH disconnection on subsequent cocaine seeking are memory reactivation-dependent. ***(A)*:** Schematic depicting the timeline for experiment 2. The procedure was identical to that used in experiment 1 except that rats were exposed to a novel, unpaired context, instead of the cocaine-paired context, before receiving unilateral microinfusions of ANI (62.5 µg/0.5 µl) into the BLA plus B/M (1.0/0.01 mM/0.5 µl) into the

contralateral DH, or VEH microinfusions into both brain regions. As in experiment 1, following the intracranial manipulations, rats received additional extinction training until they reached the extinction criterion ($\leq$25 active lever responses/session on two consecutive days). ***(B):*** Mean (±SEM) active lever presses during self-administration (SA; mean of the last three training sessions) and during tests for cocaine-seeking behavior in the extinction context (EXT; the last session preceding the test in the cocaine-paired context) and in the cocaine-paired context (COC-paired). ***(C):*** Mean (±SEM) inactive lever presses. *Asterisks* represent significant difference relative to responding in the extinction context (ANOVA context main effect, $p < 0.05$).

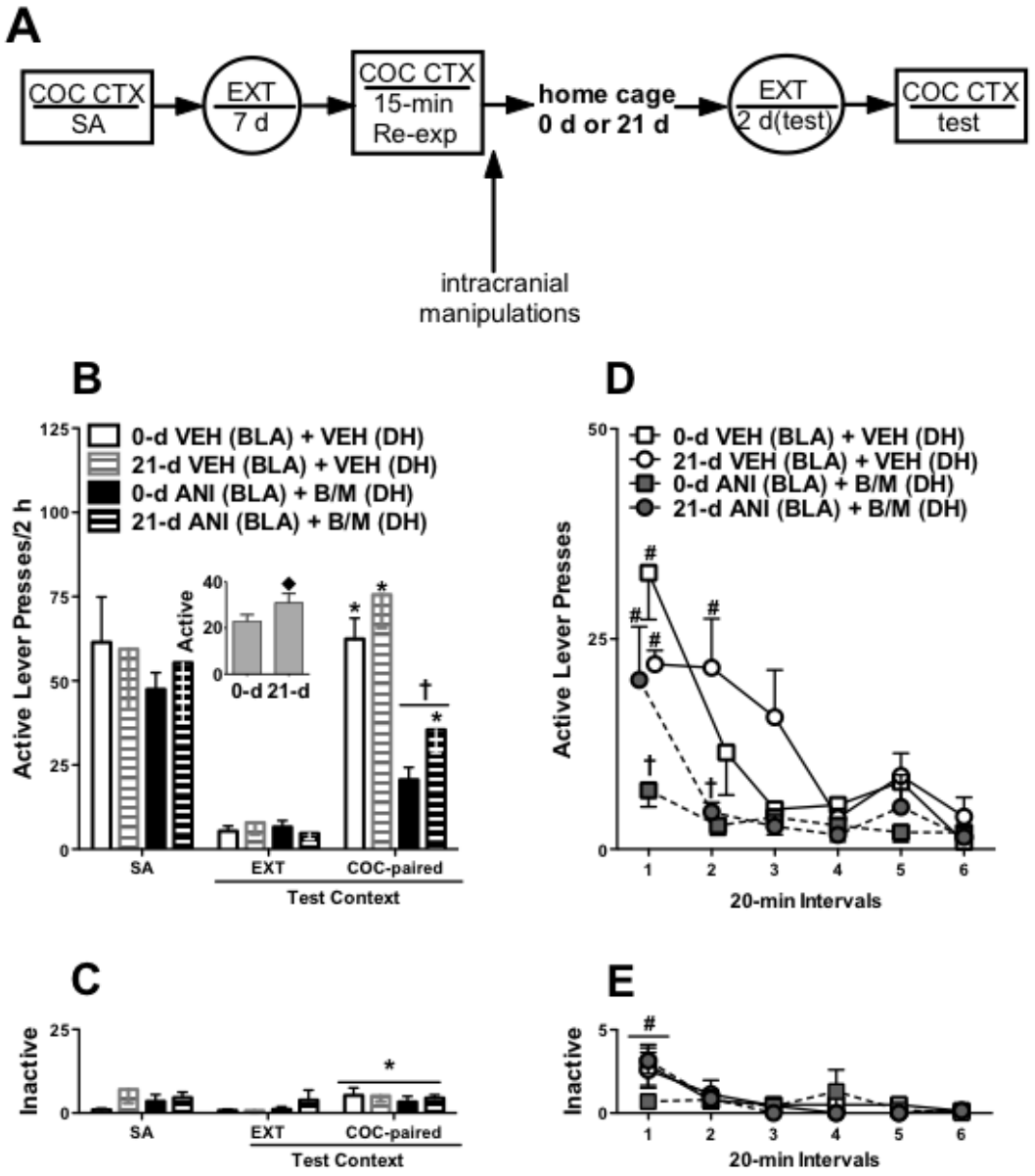

Figure 5. BLA/DH disconnection following cocaine memory reactivation differentially impairs drug context-induced cocaine-seeking behavior after a 0- or 21-d home cage stay. ***(A)*:** Schematic depicting the timeline for experiment 3. The procedure was identical to that used in experiment 1 except that rats remained in their home cages for 0 d (same groups as in experiment 1) or 21 d following unilateral microinfusions of ANI (62.5 µg/0.5 µl) into the BLA plus B/M (1.0/0.01 mM/0.5 µl) into the contralateral DH, or microinfusions of VEH into both brain regions. Following the home cage stay, rats received additional extinction training until they reached the extinction criterion (<25 active lever responses/session on two consecutive days). ***(B):*** Mean (±SEM) active lever presses during self-administration (SA;

mean of the last three training sessions) and during tests of cocaine-seeking behavior in the extinction context (EXT; the last session preceding the test in the cocaine-paired context), and in the cocaine-paired context (COC-paired). **Inset:** Mean active lever presses during testing collapsed across context and treatment. ***(C):*** Mean (±SEM) inactive lever presses. ***(D):*** The time course of active lever responses (mean ± SEM) during the test in the cocaine-paired context. ***(E):*** The time course of inactive lever responses (mean ± SEM). *Diamond* represents significant difference relative to the 0-d condition (ANOVA home cage condition main effect, $p < 0.05$). *Asterisks* represent significant difference in responding relative to that in the extinction context (ANOVA context simple main effect, $p < 0.05$). *Dagger* represents significant difference relative to VEH treatment (ANOVA treatment simple main effect, $p < 0.05$). *Pound sign* represents significant difference relative to all other time intervals (**D:** 0-d VEH and 21-d ANI+B/M groups, ANOVA time simple main effects, $p < 0.05$; **E**, ANOVA time main effect, $p < 0.05$) or relative to intervals 4 and 6 (**D:** 21-d VEH group; ANOVA time simple main effect, $p < 0.05$).

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