FUNCTIONAL INACTIVATION OF THE VENTRAL HIPPOCAMPUS ATTENUATES CONTEXT-INDUCED REINSTATEMENT OF EXTINGUISHED 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
2008

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ABSTRACT

STEPHANIE A. TRAINA: Functional inactivation of the ventral hippocampus attenuates context-induced reinstatement of extinguished cocaine-seeking behavior in rats
(Under the direction of Rita A. Fuchs)

The ventral hippocampus (VH) and the dorsal hippocampus (DH) substantially differ in connectivity, yet both brain regions have been implicated in behaviors that rely on context-US associations. The VH, in particular, has extensive reciprocal connections with several elements of the brain relapse circuitry. Thus, we hypothesized that the VH plays a critical role in context-induced reinstatement of cocaine-seeking behavior. Consistent with this hypothesis, we predicted that selective GABA agonist-induced inactivation of the VH and, in particular, its CA1 or CA3 subregions would attenuate context-induced reinstatement. Male Sprague-Dawley rats were trained to lever press for cocaine reinforcement (0.15 mg/infusion, i.v.) in a distinct multimodal context, consisting of visual, olfactory, auditory, and tactile stimuli. Rats then underwent extinction training in a distinctly different context on a minimum of 7 consecutive days. On the test days, rats received bilateral microinfusions of the $GABA_B$/$GABA_A$ receptor agonists, baclofen/muscimol (1.0/.01mM), or phosphate buffered saline vehicle directly into the VH and cocaine seeking was assessed in the previously cocaine-paired or the extinction context. Inactivation of the VH attenuated drug context-induced reinstatement of cocaine seeking, suggesting that the functional integrity of the VH is necessary for the motivational effects of a cocaine-paired environment on addictive behavior.

ACKNOWLEDGEMENTS

First, and foremost, I must acknowledge with deepest gratitude, my advisor, Dr. Rita A. Fuchs for her guidance, wisdom, mentorship, and motivation. Thank you also to Dr. Todd Thiele and Dr. Mitchell J. Picker for supporting my research endeavors by serving on my thesis committee. I also must express my gratitude to Miss Heather Lasseter, and Dr. Donna Ramirez for the multitude of ways in which they have aided in the collection and discussion of these findings.

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CHAPTER 1
INTRODUCTION

Significance of the Problem

Cocaine addiction and, in particular, cocaine relapse is a prevalent health issue in the United States today. According to the 2006 National Survey on Drug Use and Health (NIDA), approximately 2,700 people per day used cocaine for the first time, and 22.6 million people were classified with substance abuse or dependence. Of these 22.6 million, 3.8 million were dependent only on illicit drugs (including cocaine), and 3.2 million were dependent on both illicit drugs and alcohol. Specifically, 1,671 individuals were classified as dependent on cocaine in the year 2006. Additionally, 928,000 individuals were receiving treatment for cocaine abuse and dependence.

Relapse following periods of abstinence is a major impediment in the treatment of cocaine dependence. Exposure to drug-associated environments (e.g., sight of drug-taking neighborhood) or explicit drug paired stimuli (e.g., drug paraphernalia) can elicit craving and/or relapse in cocaine addicts after detoxification and rehabilitation treatment, even after extended periods of abstinence (Jaffe et al., 1989; Childress et al., 1993; O'Brien et al., 1998; Sinha, 2001). Thus, understanding the neural mechanisms of drug relapse is critical for the development of effective treatments for cocaine dependence.

Modeling Drug Relapse

Several experimental paradigms are used to study drug relapse, the two most common being the conditioned place preference (CPP) and the extinction-reinstatement models. CPP relies on the formation of an association between the motivational effects of a drug and a specific context. This procedure is conducted in an apparatus that contains two distinctly different chambers. Animals are typically injected with drug (i.e. morphine, cocaine) prior to being placed into one conditioning chamber (Meyers et al., 2003; Rezayof et al., 2003; Rademacher et al.2006). The same animals are also injected with vehicle prior to being placed in the other chamber of the conditioning apparatus. On test days, animals are placed in the center and allowed to move freely between both chambers of the apparatus. After conditioning, animals spend more time in the drug-paired context relative to the vehicle-paired chamber, which is considered an index of "drug-seeking behavior." The CPP model has become increasingly popular as a means of studying drug craving and relapse, due to its relative technical ease (Tzschentke, 2007).

A second approach to studying drug-seeking behavior and relapse is the extinction-reinstatement model, which was used in the present study. In this model, animals are trained to perform an operant response for drug infusions in a specific context (or in the presence of drug-paired discrete cues). They are then exposed to a distinctly different context (or tested in the absence of discrete cues), where drug reinforcement is not available upon operant responding (i.e., extinction training). On test days, animals are placed back into the now cocaine-paired context in the absence of drug, or are exposed to the cocaine-paired explicit cues response contingently, and reinstatement of operant responding is assessed. Reinstatement of operant responding serves as a measure

of drug-seeking behavior (Fuchs et al., 2005). It has been established that the extinction-reinstatement model has strong face validity (Katz and Higgins, 2003), indicating that behavior of the animal in the model is similar to the human condition. However, unlike in the extinction-reinstatement model, explicit extinction training does not typically occur in humans prior to relapse. Thus, it is possible that reinstatement following extinction may rely on different mechanisms than relapse following abstinence from a drug. Consequently, the construct validity of the model remains in question. Although these caveats must be kept in mind, both the CPP and extinction-reinstatement models have been widely used and accepted methods for examining drug craving and relapse.

Role of the hippocampus in learning and memory

The hippocampus has long been implicated in both the acquisition and expression of learning and memory (Scoville and Milner, 1957; Andersen et al., 2007). More recently, it has been hypothesized that the hippocampus performs a combination of functions, including the recall of contexts and bridging of contextual gaps (Redish, 2001). Several tasks have been employed to examine the role of the hippocampus in appetitive and aversive learning and memory, such as food or drug self-administration and fear conditioning. Although the hippocampus was known to mediate memory formation and consolidation (Phillips and LeDoux, 1994), it was not until relatively recently that investigators began to investigate its role in memory retrieval. Honey and Good (1993) found that pretraining lesions of the hippocampus inhibit contextual specificity of latent inhibition, which suggests that the hippocampus plays a role in contextual memory formation and retrieval. However, as the lesions were applied prior to training, the

retrieval process was not isolated and manipulated selectively. Holt and Maren (1999) further addressed the issue of hippocampal involvement in contextual memory retrieval by temporarily inactivating the hippocampus prior to fear memory retrieval. Inactivating the hippocampus after training similarly eliminated the expression of contextual fear. Thus, it is clear that the hippocampus as a whole plays a role in the recall and/or utilization of context-specific memories. Furthermore, since similar processes are theorized to underlie context-induced craving and relapse, an examination of the role of the hippocampus with respect to context-induced cocaine-seeking behavior is warranted.

Dorsal versus Ventral Hippocampus

The hippocampus proper is extensively divided in anatomy and function along a dorsal-ventral gradient (Bannerman, 2004). The dorsal (DH) and ventral hippocampus (VH) have different patterns of connections and, therefore, may play differential roles in guiding conditioned behaviors (i.e. retrieval of contextual memory). As far as the DH is concerned, Phillips and LeDoux (1994) reported that lesions of the DH in particular interfered with the acquisition of contextual fear conditioning. In contrast, Maren and Fanselow (1997) showed that neurotoxic lesions of the DH had no effect on the acquisition of contextual fear conditioning, but interrupted the expression of contextual fear. Phillips and LeDoux specifically examined the effects of DH lesion on background vs. foreground conditioning, and found that only the acquisition of background conditioning was interrupted. This distinction between background and foreground contextual conditioning might explain the discrepancy between these two studies.

Relative to the DH, which projects to the nucleus accumbens core (NAc), the VH shares more efferent connections with structures that are implicated in addictive behavior, such as the prefrontal cortex, bed nucleus of the stria terminalis, and NAc (Henke, 1990; Ishikawa & Nakamura, 2006, Kelley and Domesick, 1982; Groenwegen et al., 1987). Additionally, the VH has extensive reciprocal connections with the basolateral amygdala (BLA) (Pitkanen et al., 2000). It has been demonstrated that lesions of the VH, applied at the start of the experiment, can impair acquisition and/or expression of conditioned fear responses (Trivedi et al., 2004). Similarly, infusion of NMDA or MK-801 into the VH prior to conditioning attenuated freezing in response to previously shock-paired discrete cues following delay fear conditioning (Zhang et al., 2001). Furthermore, muscimol-induced VH inactivation disrupts context-specific fear memory retrieval following extinction (Hobin et al., 2006). Therefore, it can be hypothesized that the VH may be critical for both the acquisition and expression of other context-induced behaviors, including cocaine-seeking behavior.

Role of the ventral hippocampus in drug-seeking behavior

Several studies have examined the contributions of the dorsal and ventral hippocampus to drug-seeking behavior. These studies demonstrated that tetrodotoxin-induced functional inactivation of the DH impaired context-induced reinstatement, but had no effect on explicit conditioned stimulus (CS)-induced or cocaine priming-induced reinstatement (Fuchs et al., 2005). Further, muscimol-induced temporary inactivation of the ventral subiculum, the major output region of the VH, attenuated CS-induced and drug priming-induced reinstatement (Sun and Rebec, 2003). Similarly, selective

inactivation of the VH attenuated cue-induced and cocaine priming-induced reinstatement of cocaine seeking (Rogers and See, 2007). Thus, we have hypothesized that the functional integrity of the VH, in particular, is also necessary for context-induced reinstatement of cocaine-seeking behavior.

Contribution of ventral hippocampal subfields

In addition to being divided along the dorsal-ventral gradient, the hippocampus proper is divided into several subfields. Each of these subfields (CA1, CA3, Dentate Gyrus (DG)) has different connection patterns and, therefore, may play differential roles in guiding cocaine-seeking behavior. Initially, it was theorized that hippocampal projections process information across subregions, forming a loop running from the entorhinal cortex to the subiculum, via the DG, CA1 and CA3 subregions, with the subiculum sending output fibers to subcortical structures such as the amygdala (Andersen et al., 2007). However, it has now been established that, in addition to the traditional loop, each of these hippocampal subfields also has a distinct pattern of direct connections with extra-hippocampal structures that have been implicated in cocaine seeking (Andersen et al., 2007). Thus, information transfer via these connections may influence drug-seeking behavior.

The ventral CA1 subregion sends efferents to both the medial prefrontal cortex (mPFC) and the BLA (Ishikawa and Nakamura, 2006), both of which play a role in context-induced reinstatement (Fuchs et al., 2005). It is also well established that the CA1 subregion receives inputs from the amygdala (Andersen et al., 2007. The CA1 subregion also sends direct projections to the entorhinal cortex (Naber et al., 2001) and

subiculum (Andersen et al., 2007), in addition to the CA3 subregion (Ishikawa et al., 1990). Consistent with a possible functional role in context-induced behavior, the CA1 subregion selectively exhibits zif 268-mediated neuronal activation concomitant with the recall of contextual fear memories (Hall et al., 2001), as well as Fos-mediated neuronal activation concomitant with cocaine-seeking behavior in a cocaine-paired context (Neisewander et al., 2000). Furthermore, in a recent lesion study, the ventral, but not dorsal, CA1 subregion was shown to be necessary for the retrieval of contextual fear memories (Hunsaker and Kesner, 2007).

The CA3 subregion has distinctly different patterns of connections relative to the CA1 subregion. The CA3 subregion receives direct afferent connections from the entorhinal cortex (Witter et al., 1993) and amygdala (Pikkarainen et al., 1999; Pitkanen et al., 2000), and has no known efferent connections to the neocortex (Andersen et al., 2007). The CA3 subregion is unique in that the majority of its input and output fibers project within itself; however, it also shares extensive connections with the CA1 subregion (Ishizuka et al., 1990). To date, the majority of research has focused on the dorsal CA3 subregion. This region has been implicated in the formation, but not the recall of associative memories (Daumas, 2004; Daumas 2007).

The ventral CA3 subregion has been consistently implicated in conditioned fear, but not cocaine-seeking behavior. For instance, in a recent study, pretraining ventral CA3 lesions interrupted expression of conditioned fear (Hunsaker and Kesner, 2007). Furthermore, it has been shown that NMDA receptors in the ventral CA3 subregion are necessary for associative memory recall (Nakazawa et al., 2002). The involvement of the ventral CA3 in context-induced reinstatement remains to be investigated in studies that

apply lesions or induce temporary inactivation in the region of interest after conditioning, even though context-elicited Fos expression is not seen in the CA3 subregion of the VH concomitant with cocaine-seeking behavior (Neisewander et al., 2000).

Hypothesis and Predictions

The current experiments aimed to extend the above-mentioned findings, and to test the hypothesis that the ventral hippocampus plays an obligatory role in context-induced reinstatement of cocaine seeking. In order to address this question, muscimol and baclofen, $GABA_B$ and $GABA_A$ agonists, were administered into the VH of rats, to temporarily disrupt neuronal activity immediately prior to testing for context-induced reinstatement of cocaine-seeking behavior. Although cannula placements were not aimed at CA1 or CA3, per se, post-hoc histological analyses revealed that placements could be divided along the medial/lateral axis. Therefore, we analyzed the overall influence of the ventral hippocampus proper, its subregions, and the medial and lateral subareas to context-induced reinstatement. Based on its involvement in memory processing and its connections with brain regions implicated in cocaine seeking (Rogers and See, 2007; Sun and Rebec, 2003; Hobin et al., 2006), functional inactivation of the VH was predicted to disrupt the expression of context-induced reinstatement of cocaine seeking.

CHAPTER 2
METHODS

Subjects: 127 Male Sprague-Dawley rats, weighing 250-300g, were individually housed in a climate-controlled vivarium with a reversed 12-h light-dark cycle. Rats were maintained on 20-25g of rat chow per day with water available *ad libitum*. Animals were acclimated to handling over a 5-day period prior to the start of the experiment. 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).

Food Training: Rats were trained to lever press on a fixed ratio 1 (FR1) schedule of food reinforcement (45 mg pellets; TestDiet, Richmond, IN) in sound-attenuated operant conditioning chambers (26 X 27 X 27cm high; Coulbourn Instruments, Allentown, PA) during a 16-h overnight food training session. During the food training session, stimuli used for contextual cocaine self-administration were not presented. Both an active and inactive lever were presented, and lever presses were recorded. Each press on the active lever resulted in delivery of one food pellet. Lever presses on the inactive lever had no programmed consequences. Rats were required to make a minimum of 100 active lever presses during the overnight session in order to move on to the next phase of the experiment. If the minimum number of active lever presses was not reached, rats were re-trained on the following day.

Surgery: At least 48 h after food training, rats were deeply anesthetized with a mixture of ketamine hydrochloride and xylazine (66 and 1.33 mg/kg, i.p., respectively). Chronic indwelling catheters were constructed 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), Prolite monofilament mesh (Atrium Medical Corp., Hudson, NH) and cranioplastic cement. Incisions were made on the back and the chest to expose the right jugular vein. The silastic end of the catheter was then inserted 32.5 mm into the jugular vein and the catheter exited through the incision in the back.

Immediately after the catheter surgery, the rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL). They were implanted with bilateral stainless-steel guide cannulae (26 gauge, Plastics One) aimed at the ventral hippocampus, using standard stereotaxic procedures (VH: -5.2 mm AP, -5.0 mm DV, 5.4 mm ML). Three small screws and cranioplastic cement secured the guide cannulae to the skull. Stylets (Plastics One) were placed into the guide cannulae and catheter to prevent occlusion.

Immediately after surgery, and on 4 consecutive days thereafter, catheters were flushed with 0.1ml of cefazolin (10.0 mg/ml; Schein Pharmaceuticals, Albuquerque, NM) dissolved in heparinized saline (70 U/ml; Baxter Health Care Corp., Deerfield, IL). Catheter patency was tested before the first self-administration session, and periodically throughout the experiment, by infusing 0.1ml of propofol (10 mg/ml, i.v. Abbot Lab, North Chicago, IL), a fast-acting barbiturate, which produces a loss of muscle tone only when administered intravenously. In order to verify that catheters were not occluded, rats

were flushed with 0.1 ml heparinized saline (10 U/ml) prior to each self-administration session. At the end of each self-administration session, rats were flushed with 0.1 ml of the cefazolin solution and 0.1 ml of heparinized saline (70 U/ml).

Environmental Context: Cocaine self-administration training was conducted in operant conditioning chambers configured to one of two unique environmental contexts that differed in visual, auditory, olfactory and tactile cues. In environment one, cues consisted of a flashing white light above the inactive lever (2 sec on, 4 sec off), a constant pure tone (75 dB, 2.5 kHz), a vanilla scented air freshener (4.5 X 2 cm, Sopus Products, Mooreark, CA), bar floor, and a ceramic tile (9.75 X 11 in) which was angled against the wall opposite to the levers. In environment two, cues consisted of a continuous red house light, a pulsing pure tone (80 dB, 1 kHz, 2 sec on, 2 sec off), pine scented air freshener (4.5 X 2 cm, Car Freshener Corp, Watertown, NY), and wire mesh flooring (11 X 10.5 in.).

Self-Administration: Cocaine self-administration training was conducted during 2-h sessions during the rats' dark cycle. During the session, catheters were connected to liquid swivels (Instech, Plymouth Meeting, PA) via polyethylene 20 tubing that was encased in steel spring leashes (Plastics One). The swivels were suspended above the operant conditioning chamber and were connected to infusion pumps (Coulbourn Instruments, Allentown, PA). Animals were trained to press a lever according to a fixed-ratio 1 schedule of cocaine reinforcement. Each active lever press resulted in the delivery of an infusion of cocaine (0.15 mg/0.05 ml of cocaine hydrochloride, duration 5-s, i.v.;

NIDA, Research Triangle Park, NC) with a 20-s time-out period following each infusion. Responses on the active lever during the time-out period and responses on the inactive lever throughout the session had no programmed consequences, but were recorded. Self-administration training continued until a criterion of ≥ 10 infusions per session was reached on at least 10 days (i.e., acquisition criterion).

Extinction: After meeting the acquisition criterion for self-administration, rats underwent daily 2-h extinction sessions in the environmental context distinctly different from the self-administration context. During the extinction session, active and inactive lever presses were recorded, but had no programmed consequences. Rats received extinction training for a minimum of 7 days. Additional days of training were added if necessary until each rat reached the extinction criterion (≤ 25 active lever presses per session on two consecutive days).

Intracranial Infusion Procedure: In order to acclimate rats to the intracranial infusion procedure, they were given sham infusions on day 4 of extinction. During this session, stainless steel injection cannulae (33 gauge, Plastics One) were inserted to a depth of 2 mm below the tip of the guide cannulae, but no drug was delivered. Injection cannulae were left in place for 4 minutes, to mimic the amount of time necessary for an infusion on the test day. For intracranial infusions on the test days, injection cannulae were inserted into the guide cannulae immediately prior to placement of the rats into the chamber. The injection cannulae were connected to 10-ml Hamilton syringes (Hamilton Co., Reno, NV) that were mounted on an infusion pump (Harvard Apparatus, South Natick, MA). The

$GABA_B/GABA_A$ agonist cocktail baclofen/muscimol (BM – 1.0/0.1, .5µl/hemisphere) or phosphate buffered saline vehicle were infused bilaterally at a volume of 0.5 µl/hemisphere over 2 min. The injection cannulae were left in place for 1 min prior to and after the infusion.

Test Days: On the reinstatement test day, rats received microinfusions of BM or vehicle (VEH), into the VH, immediately prior to being placed into the previously cocaine-paired context. Active and inactive lever presses were recorded during a 120-minute session, in the absence of cocaine reinforcement.

On the extinction test day, rats received microinfusions of BM or VEH into the VH, immediately prior to being placed in the environment in which extinction training occurred previously. Active and inactive lever presses were recorded, but had no programmed consequences.

In between test days, rats underwent a minimum of two additional days of extinction training during which they were required to meet extinction criterion ($\leq$ 25 active lever presses). Approximately 1-3 rats per group underwent testing in the extinction context prior to testing in the cocaine-paired context. The remainder of rats underwent testing in the cocaine-paired context prior to testing in the extinction context.

Locomotor Activity: To assess the general motor effects of BM administration, locomotion was measured during a 2-hr locomotor activity test in novel Plexiglas chambers (42 X 20 X 20 cm) approximately 48 hours after the last test day. Between reinstatement/extinction and locomotor activity tests, rats remained in their home cages.

Immediately prior to testing, rats were infused with BM or Vehicle, consistent with their treatment on reinstatement and extinction test days. The chambers were equipped with an array of eight photodetectors and corresponding light sources that emitted photobeams 8 cm apart and 4.5 cm above the chambers. A computerized activity system (San Diego Instruments, San Diego, CA) recorded the number of times photobeams were broken consecutively by a rat moving in the chamber.

Histological and Data Analysis: After all testing had been conducted, rats were fully anesthetized with pentobarbital (Sigma, 100 mg/kg, i.p.) and perfused with phosphate buffered saline and 10% formaldehyde solution. The brains were dissected out and stored in 10% formaldehyde solution until sectioning. Brains were sectioned in the coronal plane at a thickness of 75µm. Cannula placements were determined on cresyl violet-stained brain sections based on the rat brain atlas (Paxinos & Watson, 1997).

Mixed-factorial analyses of variance (ANOVA) were used to analyze responses on the active and inactive levers separately for each phase of the experiment (self-administration, extinction, testing), with lever (active, inactive), context (extinction, cocaine-paired), and time (day) as within-subjects factors, and treatment as the between subjects factor, when appropriate. Statistically significant interaction effects were further investigated using Tukey HSD post hoc tests or t-tests. Alpha was set at 0.05.

CHAPTER 3
RESULTS

Histological Analysis

A total of 127 rats were used in this study. Of these, 44 rats had bilateral placements within the ventral hippocampus. A schematic diagram illustrating the distribution of injection cannula placements in the brains of rats and photomicrographs of representative cannula tracks are included in Figure 1. The above n's do not include 27 rats that were excluded from the study due to misplaced cannulae, 20 rats that were excluded due to a failure to acquire self-administration, 15 rats that were excluded due to lost headcaps, 9 rats that were excluded due to sickness, 5 control rats that failed to reinstate, and 7 rats that were considered outliers (i.e., > 2 standard deviations from the group mean).

Self-Administration and Extinction History

All rats achieved stable rates of active (main effect of day: $F_{(2,41)} = .422$ $p = .658$) and inactive (main effect of day: $F_{(2,41)} = 1.173$, $p = .319$) lever responding by the last three days of self-administration. There were no pre-existing differences between subsequent treatment groups in active lever presses (main effect of group: $F_{(1,42)} = .264$, $p = .610$; group x day interaction effect: $F_{(2,41)} = .637$, $p = .534$, fig. 2a) or inactive lever presses (main effect of group: $F_{(1,42)} = .675$, $p = .416$; group x day interaction effect: $F_{(2,41)} = .622$, $p = .542$, fig. 2a). Additionally, there were no pre-existing differences in cocaine

intake (main effect of day: $F_{(2,41)} = 1.473$, $p = .241$; main effect of group: $F_{(1,42)} = .222$, $p = .640$; group x day interaction effect: $F_{(2,41)} = .605$, $p = .551$, fig 2b).

Upon removal of cocaine reinforcement, responding decreased in the extinction context on the active (main effect of day: $F_{(6,37)} = 8.011$, $p = .000$), and inactive levers (main effect of day: $F_{(6,37)} = 3.324$, $p = .010$). Specifically, responding on the active and inactive lever was significantly less on days 5-7 of extinction compared to day 1 (Tukey test, $p < .05$). Additionally, there was a significant difference between the subsequent treatment groups in active lever responding (group x day interaction effect: $F_{(6,37)} = 2.376$, $p = .003$, Tukey test, $p < .05$; main effect of group: $F_{(1,42)} = .603$, $p = .442$), only on the first day of extinction. There were no pre-existing differences between the subsequent treatment groups in inactive lever responding (main effect of group: $F_{(1,42)} = .131$, $p = .719$; group x day interaction effect: $F_{(6,37)} = .819$, $p = .562$, fig. 3).

Administration of BM into the Ventral Hippocampus attenuates context-induced reinstatement of cocaine-seeking behavior

Active lever pressing was significantly higher in both groups on the reinstatement test day in the cocaine-paired context than on the extinction test day in the extinction context (main effect of context: $F_{(1,42)} = 46.426$, $p = .000$). There was a significant difference between the BM- and VEH-treated groups in active lever responding on the reinstatement test day ($t_{(42)} = 2.884$, $p = .006$) but not on the extinction test day ($t_{(42)} = -1.561$, $p = .126$; treatment x context interaction effect: $F_{(1,42)} = 10.759$, $p = .002$; main effect of treatment: $F_{(1,42)} = 4.514$, $p = .04$, fig. 4a). Similarly, inactive lever pressing was higher on the reinstatement test day than on the extinction test day (main effect of context: $F_{(1,42)} =$

6.156, p = .017), only for vehicle treated animals ($t_{(1,23)}$= -2.29, p = .031). However, there was no significant difference between the BM- and VEH-treated groups in inactive lever responding on either test day (main effect of treatment: $F_{(1,42)}$ = .322, p = .573; treatment x context interaction effect: $F_{(1,42)}$ = 2.007, p = .164, fig. 4b).

A subsequent time course analysis indicated that active lever responding decreased gradually during the six 20-minute intervals of the 120-minute reinstatement test (main effect of interval: $F_{(5,38)}$ = 13.763, p = .00). Specifically, there was a significant decrease in responding between the first 20-minute interval and intervals 2-6 (Tukey test, p = .05). There was a significant difference in active lever responding between BM- and VEH- treated groups during the reinstatement test (main effect of treatment: $F_{(1,42)}$ = 8.126, p = .006; treatment x interval interaction effect: $F_{(5,38)}$ = .972, p = .436, fig. 5). Specifically, rats treated with BM responded less than rats treated with VEH independent of the specific interval examined. A separate analysis of subregional contributions to this effect failed to identify a single VH subfield (CA1, CA3, DG) or region (medial, lateral) involved in context-induced reinstatement (data not shown).

Administration of BM into the Ventral Hippocampus transiently attenuates general locomotor activity

Locomotor activity declined across the 2-h session, specifically between the first 20-minute interval and intervals 2-6 (main effect of time: $F_{(5,38)}$ = 60.962, p = .000, Tukey Test: p = .01). Administration of BM into the ventral hippocampus decreased locomotor activity relative to VEH, during the first 20-minute interval (treatment x time interaction

effect: $F_{(5,38)} = 5.867$, $p = .000$, Tukey test: p = .01; main effect of treatment: $F_{(1,42)} = .004$, $p = .952$, , fig. 6), but not during intervals 2-6.

CHAPTER 4
DISCUSSION

The present study is the first to indicate a role for the VH in context-induced reinstatement in an animal model of drug relapse. Vehicle treated animals exhibited reinstatement of active lever responding (cocaine-seeking behavior) in the cocaine-paired context following the extinction period. This is consistent with previous context-reinstatement and renewal studies (Fuchs et al., 2007). In support of the role of the VH in context-induced motivation for cocaine, functional inactivation of the VH abolished the ability of a cocaine-paired context to elicit cocaine-seeking behavior (figure 4a). This effect was not due to pre-existing differences in cocaine-reinforced responding or cocaine intake (figure 2). It is also unlikely that BM-induced impairments were due to general motor effects, as BM administration had differential effects across time on reinstatement versus locomotor activity (figure 4b; figure 5). Specifically, BM attenuated reinstatement throughout the session, whereas it decreased locomotor activity only during the first 20 minutes and failed to attenuate inactive lever responding. These results are consistent with previous research indicating that context-elicited Fos expression in the VH seen in animal studies (Neisewander et al., 2000). Furthermore, functional inactivation of the VH attenuates the expression of context-induced fear conditioning (Hobin et al., 2006).

Our findings significantly contribute to the reinstatement literature, which suggests that the VH plays a critical role in mediating explicit cue-induced reinstatement

(Rogers and See, 2007). The present and previous findings together reveal a role for the VH in cue-induced reinstatement regardless of cue type. In apparent contrast with these findings, imaging studies do not indicate cue-induced neural activation in the hippocampus in human subjects (Kilts et al., 2001). Human imaging studies generally utilize video or still images of contexts or drug paraphernalia to elicit the craving response, whereas in reinstatement studies animal subjects are exposed to drug-associated stimuli. This discrepancy in methodology between human and animal studies may suggest that VH recruitment is sensitive to (A) actual context or cue re-exposure or (B) presentation of personally relevant cues. Thus, animal studies may provide novel evidence for involvement of brain regions that are overlooked in human imaging studies.

Analysis of region-specific contributions did not reveal a single VH subregion responsible for the observed effects of BM on cocaine-seeking behavior. This is consistent with previous data indicating that pre-training lesions of the ventral CA1 or CA3 subregions interrupted expression of conditioned fear (Hunsaker and Kesner, 2007). The present findings are novel nevertheless since, to date, no studies have examined the putatively distinct roles of CA1 and CA3 in the expression of *goal directed* behavior based on context-US associations. It is possible that a more selective targeting of the CA1 versus CA3 subregions in the present study would have identified a differential role for these subregions in context-induced reinstatement. Thus, future investigation into the potential functional heterogeneity of the VH with respect to context-induced cocaine-seeking behavior will be essential to further delineate the circuitry underlying context-induced reinstatement.

The critical role of the VH in context-induced reinstatement may be subserved by its extensive connections with the mPFC and amygdala (Ishikawa and Nakamura, 2006), both of which have been implicated in the drug relapse circuitry (Kalivas, McFarland and See, 2003). Relative to the DH, the VH sends more extensive projections to the PFC and shares reciprocal connections with the rostral BLA (Pitkanen et al., 2000). Thus, future disconnection studies will need to examine with which of these brain regions the VH interacts directly within the circuit to contribute to context-elicited reinstatement. Glutamatergic transmission has been implicated directly in both cocaine-seeking behavior (McFarland et al., 2003) and in long-term potentiation (Li et al., 1999). Functional inactivation of the NAc core or shell inhibits context-induced cocaine-seeking behavior (Fuchs et al., in preparation). Glutamate release in the NAc core is necessary for cocaine-seeking behavior (McFarland and Kalivas, 2001) and drug-primed heroin-seeking behavior (LaLumiere and Kalivas, 2008). More relevant to the present study, context-induced heroin seeking is attenuated by inhibition of glutamate transmission in the medial NAc shell and VTA (Bossert et al., 2007). Similarly, activation of group 2 mGluRs, which function as autoreceptors, in the NAc shell attenuates context-induced heroin-seeking behavior (Bossert et al., 2006). Thus, given the involvement of glutamate in other forms of reinstatement and the interactions between the VH, mPFC and NAc, future studies will need to assess the role of VH glutamate release into the mPFC and nucleus accumbens in context-induced cocaine-seeking behavior.

Dopamine activity in both the mPFC and nucleus accumbens has been shown to underlie various cognitive processes. As mentioned earlier, the ventral CA1 subregion projects to the mPFC (Jay and Witter, 1991; Conde et al., 1995) and to the shell of the

nucleus accumbens (Kelley and Domesick, 1982; Groenwegen et al., 1987), which receive DA input from the ventral tegmental area (VTA). It has been hypothesized that the glutamatergic ventral hippocampal input to these regions may play a unique role in modulating the influence of DA on goal-directed behavior. In fact, it has recently been shown that stimulation of the VH leads to prolonged elevation in dopamine in the mPFC and nucleus accumbens (Taepavarapruk et al., 2007). Thus, inhibition of glutamate output from the VH following inactivation may have inhibited DA release in the PFC, which has been shown to be necessary for drug-primed cocaine-seeking behavior (McFarland and Kalivas, 2001).

In summary, previous data and the present study together indicate that the ventral hippocampus is involved in several types of reinstatement, including cue-, context- and cocaine priming-induced reinstatement of cocaine-seeking behavior. Therefore, the ventral hippocampus likely plays a central role in regulating the motivational impact of various triggers on drug craving and relapse in humans. As such, the VH should be a target of intense investigation as this may promote treatment development.

Figure 1a

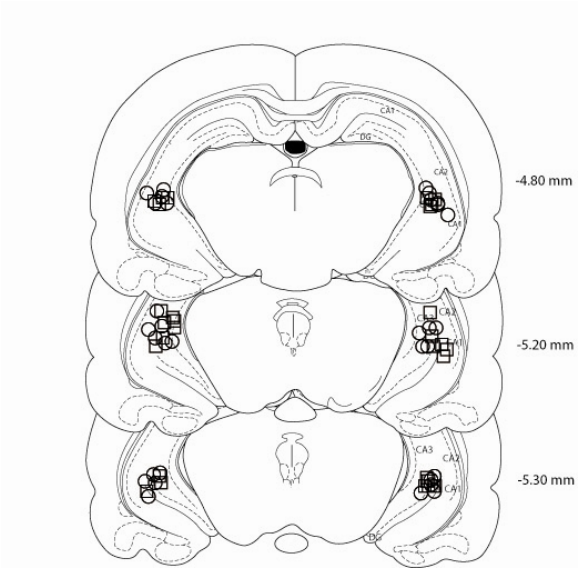

Figure 1b

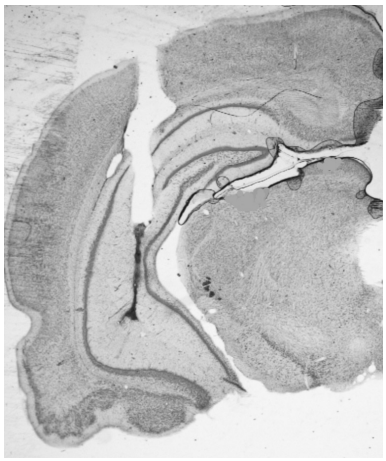

Figure 2a

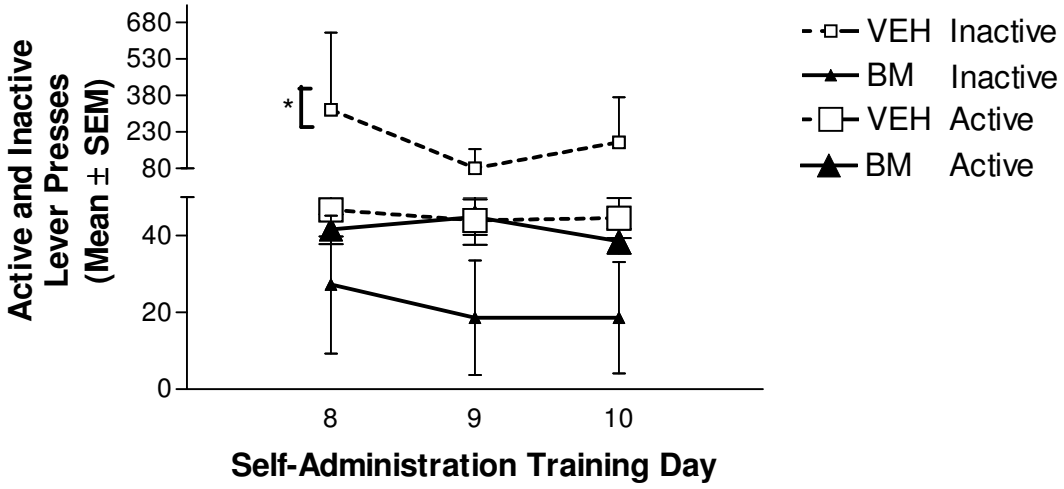

Figure 2b

Figure 3

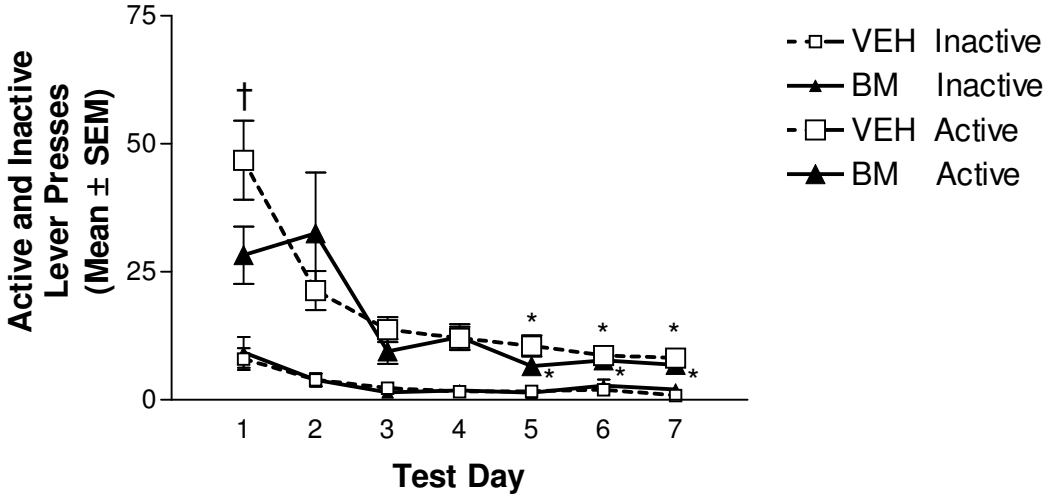
Active and Inactive Lever Presses (Mean ± SEM)
75
50
25
0
1
2
3
4
5
6
7
Test Day
†
*
VEH Inactive
BM Inactive
VEH Active
BM Active

Figure 4a

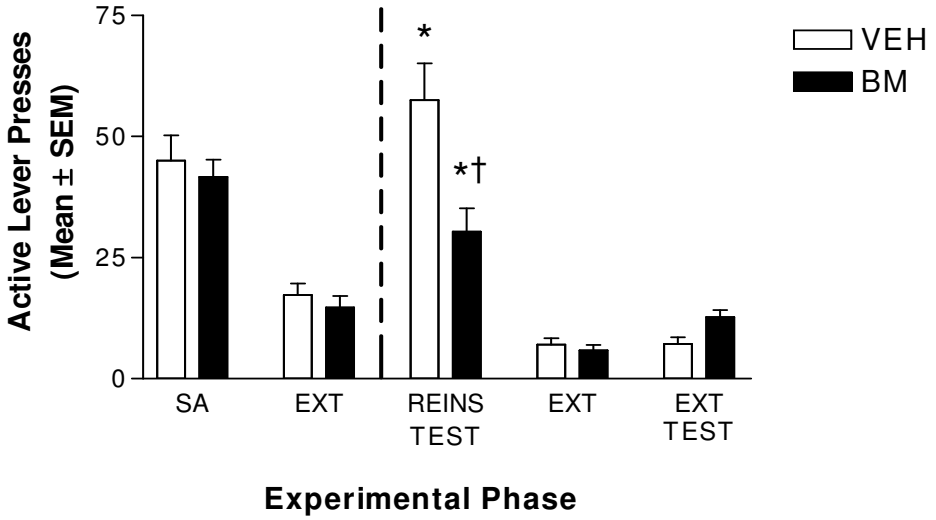

Figure 4b

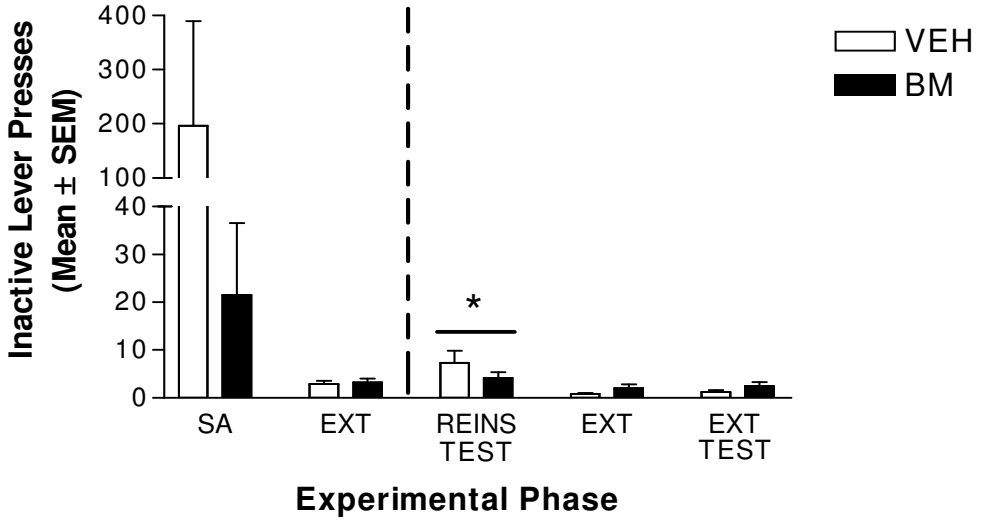

Figure 5

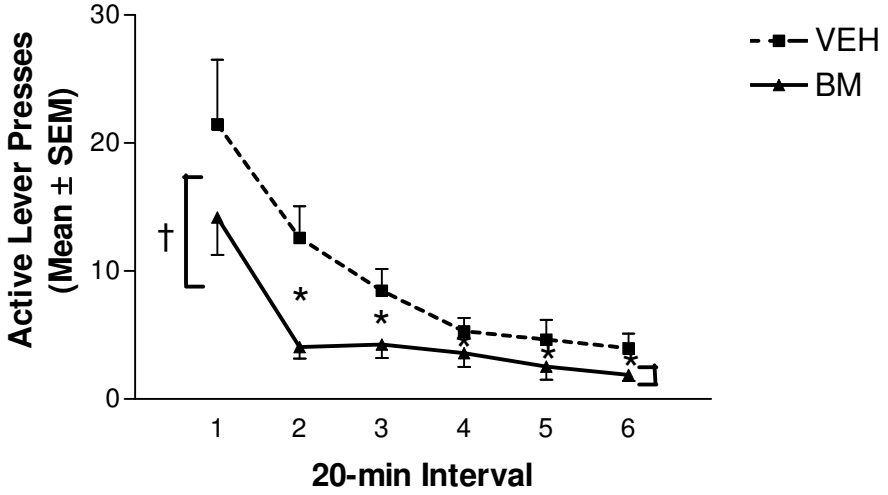
Active Lever Presses
(Mean ± SEM)
30
20
10
0
1
2
3
4
5
6
20-min Interval
VEH
BM
†
*
*
*
*

Figure 6

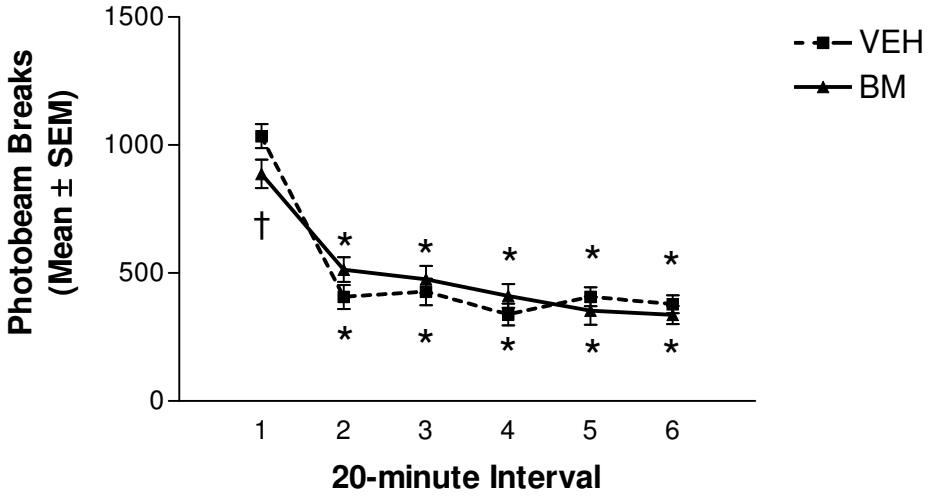
Photobeam Breaks
(Mean ± SEM)
1500
1000
500
0
1
2
3
4
5
6
20-minute Interval
VEH
BM
†
*

FIGURE CAPTIONS

Figure 1: (A) Microinfusion cannula placement as verified on cresyl violet-stained sections. Symbols represent the most ventral point of the cannula tract for each rat on coronal sections based on the atlas of Paxinos and Watson (1997). Open circles and open squares represent cannula placements in the brains of animals treated with BM and VEH, respectively. Numbers represent distance from Bregma in the anterior-posterior plane.

(B) Representative photomicrograph of a microinfusion cannula placement within the ventral hippocampus.

Figure 2: Mean (± SEM) active and inactive lever responding (A) and cocaine intake (B) across the last 3 days of self-administration training in the cocaine-paired context. Cocaine reinforcement (0.15 mg/0.05 ml/infusion) was available at a fixed ratio 1 schedule of reinforcement, with a 20-s time-out period, during 2-h sessions.

Figure 3: Mean (± SEM) number of active and inactive lever presses during the first 7 days of training in the extinction context. Symbols represent significantly less lever pressing compared to day 1 of extinction (ANOVA main effect of day; Tukey test *$p<.05$), and significantly less active lever pressing compared to vehicle (ANOVA group x day interaction effect; Tukey †$p<.05$). Lever presses had no programmed consequences.

Figure 4: (A) Mean (± SEM) number of active lever presses during the 120-minute reinstatement and extinction tests. Self-administration (mean of last 3 days of training ± SEM) and extinction data (mean of last 3 days of training ± SEM) are provided as a reference point. On the reinstatement and extinction test days, rats received BM (1.0/0.1, .5µl/hemisphere) or VEH into the VH immediately prior to re-exposure to the cocaine-paired or extinction context, respectively. Responses were not reinforced. Symbols represent significant difference in responding relative to extinction test (ANOVA treatment x context interaction; ANOVA context simple main effect: *$p = .006$), and relative to vehicle (ANOVA treatment simple main effect: †$p = .000$).

(B) Mean (± SEM) number of inactive lever presses during 120-minute reinstatement and extinction tests. Self-administration and extinction data are provided as a reference point. Symbols represent significant difference in responding relative to extinction test (ANOVA context main effect: $p = .017$).

Figure 5: Mean (± SEM) number of active lever presses during the 120-minute reinstatement test. Symbols represent a significant difference in responding relative to VEH (ANOVA main effect of treatment: †$p < .01$) and a significant difference in responding relative to the first 20-minute interval (ANOVA time main effect; Tukey test, *$p < .05$).

Figure 6: Locomotor activity (mean photobeam breaks/2 h ± SEM) in a novel context. Rats received BM (1.0/0.1, .5µl/hemisphere) or VEH into the VH immediately prior to placement into the locomotor activity chamber. Symbols indicate a significant change in

locomotor activity relative to interval 1 (ANOVA treatment x time interaction; Tukey test, *$p < .01$) or relative to VEH (Tukey test, †$p < .01$).

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