THE ROLE OF DORSAL HIPPOCAMPAL CB1 RECEPTORS IN THE RECONSOLIDATION OF A CONTEXT-RESPONSE-COCAINE MEMORY IN RATS

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ABSTRACT

Mark A. Presker Jr.: The Role of Dorsal Hippocampal CB1 Receptors in the Reconsolidation of a Context-Response-Cocaine Memory in Rats
(Under the direction of Rita A. Fuchs-Lokensgard and Regina M. Carelli)

Re-exposure to a cocaine-associated context induces relapse in humans and the reinstatement of cocaine-seeking in rats. This phenomenon is dependent on learned associations between a context and cocaine availability that persist and trigger recollection of the motivational properties cocaine. The theory of memory reconsolidation posits that, upon retrieval, memory traces become labile and must undergo memory reconsolidation to re-enter long-term memory. Therefore, disruption of memory reconsolidation can be used therapeutically to weaken a maladaptive memory. The reconsolidation of a context-cocaine memory is dependent on dorsal hippocampus (DH), a brain region in which cannabinoid type 1 (CB1) receptors are densely expressed. CB1 receptors have been implicated in memory reconsolidation. Thus, stimulation of DH CB1 receptors may be necessary for context-cocaine memory reconsolidation. To test this hypothesis, rats were trained to self-administer cocaine in a distinct context then received extinction training in an alternate context. Rats were then briefly re-exposed to the cocaine-paired context followed by intra-DH infusions of either the CB1 receptor antagonist AM251, the CB1 receptor agonist CP55940, or DMSO vehicle solution. After additional extinction training, 72 hours following intra-DH drug administration, reinstatement of cocaine-seeking behavior was assessed in the cocaine-paired context. Intra-DH infusion of AM251 or CP55940 at the putative time of memory reconsolidation had no effect on subsequent

context-induced reinstatement of cocaine-seeking behavior. These findings do not support the hypothesis that DH CB1 receptors are involved in the reconsolidation of context-cocaine memories necessary for context-induced cocaine-seeking behavior.

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CHAPTER I: INTRODUCTION

Background

One of the greatest challenges to the successful treatment of cocaine addiction is the high propensity to relapse following extended periods of abstinence (Gawin, 1991). Relapse can be precipitated by numerous factors, such as re-exposure to cocaine-associated cues, periods of stress, or exposure to low doses of the drug itself (Sinha & Li, 2007; Rohsenow et al., 1990; Jaffe et al., 1989; Childress et al., 1993). Cocaine-associated cues, such as drug paraphernalia, are discretely paired with drug administration and predict imminent drug effects. In addition, cocaine-associated cues can be contextual and predict cocaine availability. Following the establishment of either discrete or contextual cue-drug associations, cues can acquire incentive motivational properties which then subsequently drive drug-seeking behavior upon re-exposure to the cue (Robinson & Berridge, 2003; Conklin & Taylor, 2002). Given that over the course of an addict's life numerous cue-cocaine associations form, recovering addicts find it unfeasible to avoid drug-associated cues altogether. Treatments aimed at reducing cocaine-cue reactivity typically focus on discrete cocaine cues even though addicts likely come into contact with contextual cues before explicit cues in situations that result in relapse. Thus, treatments which attenuate or eliminate the ability of cocaine-associated contextual cues to drive drug seeking behavior may prove more valuable for improving treatment outcome for cocaine addicted individuals.

Memory reconsolidation

Following initial acquisition, memories undergo stabilization into long-term memory stores through the process of memory consolidation (McGaugh, 2000). Upon retrieval, previously consolidated memories can enter into a labile state in which the memory can be updated or modified before undergoing memory reconsolidation, a protein synthesis-dependent process that re-stabilizes the memory into long-term storage (Tronson and Taylor 2007). A similar process likely maintains context-response-cocaine memories which encode the association between cocaine availability in a context contingent upon drug-seeking behavior. Thus, it has been hypothesized that one method that may be useful for weakening maladaptive context-response-cocaine memories is to selectively disrupt their reconsolidation (Nader et al., 2000; Walker et al., 2003; Miller & Marshal, 2005; Lee et al., 2005). Thus, targeting neurobiological substrates that are necessary for the reconsolidation of cue-response-cocaine memories may provide an effective method for disrupting learned drug-cue associations, reducing the incentive motivational properties of drug cues, and preventing drug relapse.

Neural substrates of context-cocaine memories

The retrieval and utilization of context-cocaine memories, as measured by context-induced instrumental drug-seeking behaviors and conditioned place preference (CPP), are dependent upon the functional integrity and connectivity of the dorsal hippocampus (DH) and basolateral amygdala (BLA) (Fuchs et al., 2005; Fuchs et al., 2007; Fuchs et al., 2002; Myers et al., 2003). These same regions are also implicated in the *reconsolidation* of context-cocaine memories (Fuchs et al., 2009; Ramirez et al., 2009; Wells et al., 2011). In the BLA, protein synthesis is critical for the reconsolidation of context-response-cocaine memories (Fuchs et al., 2009). Interestingly, in the DH, the reconsolidation of context-response-cocaine memories is not

dependent upon protein synthesis *per se* (Ramirez et al., 2009; Presker et al., unpublished) even though functional disconnection of the BLA and DH inhibits the reconsolidation of context-response-cocaine memories, indicating that functional connectivity between these brain regions is critical for this process (Wells et al., 2011). Thus, further investigation is warranted to identify DH mechanisms that underlie reconsolidation of context-response-cocaine memories in the BLA and possibly elsewhere.

The endocannabinoid system and memory

While numerous neurotransmitter systems have been implicated in memory and reward, only recently has the endocannabinoid system (ECS) received attention for its role in these processes (Mechoulam & Parker, 2013). The ECS is composed primarily of the cannabinoid receptor types 1 and 2 (CB1 and CB2 respectively), the endogenous lipid-based ligands, anandamide and 2-arachodonyl glycerol, and enzymes necessary for the synthesis and degradation of these neurotransmitters (Matsuda et al., 1990; Howlett et al., 1990; Munro et al., 1993; Pazos et al., 2005). CB1 receptors are widely expressed in the central nervous system while CB2 receptors are primarily expressed in peripheral tissue (Herkenham et al., 1990; Munro et al., 1993). CB1 receptors are $G_{i/o}$ coupled G-protein coupled receptors (GPCR) (Devane et al., 1988; Demuth & Molleman, 2006). Activation of CB1 receptors results in inhibition of cyclic-adenosine monophosphate and subsequently decrease in neuronal excitability (Demuth & Molleman, 2006). CB1 receptors are pre-synaptic and have particularly dense expression in the DH in both GABAergic and glutamatergic synapses (Wilson & Nicol, 2002; Davies et al., 2002; Herkenham et al., 1990; Kawamura et al., 2006).

The CB1 receptor has been implicated in the formation, expression, reconsolidation, and extinction of numerous forms of memory (Akirav, 2011; Ruehle et al., 2012). Specifically, CB1

receptor populations within the DH play a critical role in the action for cannabinoids on memory function (Lichtman et al., 1995; Hampson & Deadwyler, 1999; Wise et al., 2009; Mechoulam & Parker, 2013). Consistent with this, intra-DH CB1 agonist administration recapitulates the behavioral deficits observed in a spatial memory task following systemic CB1 agonist administration and systemic CB1 agonist administration produces profound deficits in a short-term memory task that are similar to those observed following selective DH lesions (Lichtman et al., 1995; Hampson & Deadwyler, 1999).

Despite being strongly implicated in all stages of memory processing, the exact role of CB1 receptors in memory reconsolidation *per se* has remained unclear. CB1 agonist and antagonist studies produced inconsistent effects. For example, systemic CB1 antagonist administration during the putative time of reconsolidation failed to alter the reconsolidation of a Pavlovian fear memory but impaired the reconsolidation of a methamphetamine CPP memory (Suzuki et al., 2004; Lin et al., 2006). Intra-BLA CB1 agonist and antagonist administration similarly impaired Pavlovian fear memory reconsolidation (Yu et al., 2009; Ratano et al., 2014). Conversely, intra-DH CB1 agonist administration impaired while antagonist administration enhanced Pavlovian fear memory reconsolidation (de Oliveira Alvares et al., 2008). Taken together these studies indicate a role for CB1 receptors in memory reconsolidation, although the nature of this role remains to be elucidated.

Hypothesis

The experiments in this Master's Thesis were designed to examine whether CB1 receptor blockade by the selective CB1 antagonist, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide (AM251), (Experiment 1) or CB1 receptor stimulation by the selective CB1 agonist, 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-

methyloctan-2-yl)phenol (CP55940), (Experiment 2) in the DH would impair the reconsolidation of instrumental context-response-cocaine memories in rats using a modified version of the contextual reinstatement model (Fuchs et al., 2008). Given the conflicting evidence in the literature regarding the role of ECS and CB1 receptors in memory reconsolidation, CP55940 was initially hypothesized to disrupt memory-reconsolidation and attenuate subsequent context-induced reinstatement of operant responding and, when this hypothesis was not confirmed, we hypothesized that AM251 would impair memory reconsolidation and attenuate context-induced reinstatement.

CHAPTER II: METHODS AND MATERIALS

Subjects

Male Sprague-Dawley rats (Charles Rivers; N=18) weighing 250-300 grams at the start of procedures were used in this experiment. All subjects were individually housed in a temperature and humidity controlled vivarium on a 12-hour reverse light-dark cycle. Subjects were given three days to acclimate prior to the start of experimental procedures. All subjects were maintained on approximately 25 grams of rat chow per day with water available *ad libitum*. All procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee and followed the *Guide for the Care and Use of Laboratory Rats* (Institute of Laboratory Animal Resources, Commission on Life Sciences, 2011).

Procedures

Food training

In order to facilitate the acquisition of drug self-administration, subjects were first trained to lever press for food pellets under a fixed-ratio 1 (FR-1) reinforcement schedule using standard operant conditioning chambers (26 x 27 x 27 cm) (Coulbourn Instruments). Each chamber contained two levers side by side. Each response on one (active) lever resulted in the delivery of a single food pellet (45 mg) while responses on the other (inactive) lever had no scheduled consequence. The acquisition criterion for the task was 100 or more active lever responses during a single training session. The food training session lasted 16 hours overnight. Food training sessions were repeated until subjects reached the acquisition criterion. The distinct

contextual cues used in subsequent drug self-administration and extinction training were not presented during the food training sessions.

Surgery

Twenty-four hours after food training, subjects were anesthetized using a cocktail of ketamine hydrochloride and xylazine (66.6mg/kg and 1.33 mg/kg respectively). Indwelling intravenous catheters, built in house as previously described (Fuchs et al., 2007), were implanted into the right jugular vein. The catheter was positioned subcutaneously and exited between the scapulae. Immediately following catheterization, subjects were placed into a stereotaxic device (Stoelting) and received bilateral stainless steel guide cannula (5 mm; Plastics One) implants aimed at the DH using coordinates previously described (relative to bregma: AP, -3.4 mm; ML, ±3.1 mm; DV -2.15 mm; 15^{o} angle) (Xie et al., 2010). Anchor screws and dental cement were used to secure the guide cannulae to the skull. Subjects received 0.1 ml of oral Naproxen (125 mg/ 5 ml; Roxane Laboratories; a non-narcotic NSAID) immediately following and 24 hours after surgery. Catheters were flushed daily with 0.1 ml of the antibiotic cefazolin (10 mg/ml, Schein Pharmaceuticals, dissolved in 70 U/ml heparinized saline; Baxter Health Care Corp) and 0.1 ml of heparinized saline (10 U/ml) to prevent infection and maintain catheter patency respectively. Catheter patency was tested periodically using 0.1 ml of propofol (1 mg/0.1 ml; Eli Abbott), which rapidly produces transient loss of muscle tone when administered intravenously but not when administered systemically. Following surgery subjects were given seven days of recovery prior to the initiation of behavioral procedures.

Cocaine self-administration training

All subjects were trained to lever press for cocaine infusions (0.15 mg/0.05 ml per infusion) under an FR-1 reinforcement schedule with a 20-second timeout period. A response on

the active lever resulted in a single infusion of cocaine while responses on the inactive lever had no programmed consequence. Subjects were randomly assigned to receive cocaine self-administration training in either Context A or Context B. Context A contained an intermittent white light (2 sec on, 2 sec off) above the inactive lever, a vanilla scented air freshener (Sopus Products), a continuous tone (75 db, 2.5 kHz), and a ceramic tile bisecting horizontal floor bars (19 cm x 27 cm). Context B contained a continuous red house light on the chamber wall opposite the levers, a pine scented air freshener (Car Freshener Corp.), an intermittent tone (80 db, 1 kHz; 2 sec on, 2 sec off), and a wire grid floor (26 cm x 27 cm).

At the start of each self-administration session, subjects were placed in the operant conditioning chambers and connected to the infusion pumps (Coulbourn Instruments) for two hours. The number of lever responses and cocaine infusions were recorded. Data collection and the infusion pumps were controlled using Graphic State Notation version 2.102 (Coulbourn Instruments). Drug self-administration training was discontinued after subjects reached the acquisition criterion of 10 or more infusions of cocaine per day on at least 10 days. Subjects that failed to acquire cocaine self-administration by receiving less than ten infusions per any session over seven consecutive days were excluded from the experiment.

Extinction training

Following drug self-administration training, subjects received 10 daily two-hour extinction training sessions in the context opposite to the one in which they had self-administered cocaine. In the extinction context, responses on either lever resulted in no programmed consequences. Following the fourth extinction session, subjects received "sham" infusions. The sham procedure consisted of inserting injector needles into the guide cannulae and holding the subject stationary for four minutes. This was done to adapt subjects to the stress associated with

the intra-DH drug infusion procedure following the reactivation session, as stress has been shown to affect memory reconsolidation (Akirav & Maroun, 2012).

Memory reactivation

Twenty-four hours after the last extinction session, subjects underwent a reactivation session in the cocaine-paired context in order to destabilize context-response-cocaine memories and initiate their reconsolidation (Nader et al., 2000). During the 15-min reactivation session, lever responding was recorded but had no programmed consequences. Previous work has shown that fifteen minutes is sufficient time to induce memory reactivation without extinguishing lever responding (Fuchs et al., 2009).

Experiment 1: Effects of CB1 antagonism in the DH on context-response-cocaine memory reconsolidation

Intracranial infusions

Immediately following the reactivation session, subjects were given intra-DH infusions of AM251 (n = 6; Sigma Aldrich; 6 ng/0.5µl per hemisphere, dissolved in 100% DMSO) or 100% DMSO vehicle solution (VEH) (n = 6; 0.5µl per hemisphere). This dose of AM251 was selected based on previous research indicating that it disrupted the consolidation and reconsolidation of contextual fear memory when infused into the DH (de Oliveira Alvares et al., 2005; de Oliveira Alvares et al., 2008). The infusions were delivered by a KD scientific microinfusion pump at a rate of 0.25 µl/min. Injectors were inserted into the guide cannulae one minute before infusions began and remained in place for one minute following the infusions in order to allow the drug to diffuse away from the injection site. Injectors extended 1 mm past the tip of the guide cannulae. Subjects were returned to their home cages immediately after the infusions.

Post-reactivation extinction training and reinstatement testing

Twenty-four hours after the reactivation session, daily extinction training sessions resumed and continued until the subjects reached the extinction criterion of two consecutive days with 25 or fewer responses on the active lever per session. Twenty-four hours after reaching the extinction criterion, the subjects were returned to the previously cocaine-paired context for a 2-h test of drug context-induced reinstatement of cocaine-seeking behavior. During the test session responses on both levers were recorded but had no programmed consequences. A schematic representation of the timeline of the experimental timeline can be found in **Fig. 1**.

Experiment 2: Effects of CB1 agonism in the DH on context-response-cocaine memory reconsolidation

A third group of subjects received intra-DH infusions of CP55940 (n = 6; Sigma Aldrich; 15 µg/0.5µl per hemisphere, dissolved in 100% DMSO) following memory reactivation in a manner identical to the procedures in described Experiment 1. The CP55940 dose was selected based on reports that this dose impaired spatial memory when infused into the DH (Lichtman et al., 1995). The subjects that had received intra-DH VEH in Experiment 1 were used for comparison in Experiment 2. All other procedures were identical to those in Experiment 1.

Brain histology

Immediately following the reinstatement test, subjects were decapitated and their brains were rapidly removed, flash frozen in isopentane, and stored at -80º Celsius. The brains were sectioned at a thickness of 40 microns using a cryostat (Leica), mounted on glass slides (Fisher Scientific), and stained using cresyl violet (Sigma). Locations of the most ventral point of the cannula tracks were verified using light microscopy and recorded on schematics adapted from the rat brain atlas (Paxinos & Watson, 1997). The data of subjects with cannula placements outside of the DH were excluded from analysis.

Data analysis

Separate two-way analyses of variance (ANOVA) or t-tests were conducted to test for pre-existing differences between the experimental (AM251 or CP55940) and control groups (VEH) in lever pressing and cocaine intake during self-administration, lever pressing during extinction, or lever pressing during the memory reactivation session. Separate t-tests were performed to test the effects of post-reactivation treatment on extinction responding in the extinction context on the first day post manipulation and in the number of days required to reach extinction criteria post manipulation. Mixed-factorial ANOVAs were performed to assess the effects of post-reactivation treatment on active and inactive lever pressing during the last day of extinction in the extinction context and during the test of reinstatement in the previously cocaine-paired context. For these ANOVAs context (extinction vs. cocaine-paired) was used as the within-subjects factor and treatment (AM251 or CP55940 vs. VEH) as the between subjects factor. Note that the same animals were used for the VEH group in all comparisons in experiment 1 (AM251 vs. VEH) and experiment 2 (CP55940 vs. VEH). Significant main effects or interactions were further probed using Tukey's *post-hoc* tests where appropriate. Alpha was set at 0.05.

CHAPTER III: RESULTS

Brain Histology

Schematic representation and a representative photomicrograph of cannula placements is illustrated **Fig. 2.** Histological verification determined that guide cannulae in all subjects used in all experiments were within the boundaries of the DH, in the absence of gross tissue damage or lesion.

Experiment 1:

Behavioral history

All subjects in Experiment 1 exhibited stable cocaine intake and lever responding during the last 3 days of cocaine self-administration (**Table 1**). There were no pre-existing differences between groups that subsequently received AM251 or VEH in cocaine intake, active lever presses, or inactive lever presses during cocaine self-administration. Cocaine intake increase across the sessions (main effect of day $F_{(9,90)}$=3.66, p<.00, Tukey's test, Day 6 > Day 1-4, Day 8,10 > Day 2, p<0.05) but did not differ between the subsequent treatment groups (day by treatment interaction or main effect of treatment, $F_{(1\text{-}9,10\text{-}90)}$=0.03-0.621, p=0.78-0.86; **Fig. 3a**). Active lever pressing during cocaine self-administration decreased across sessions (day main effect, $F_{(9,90)}$=2.16, p=.03, Tukey's test, Day 1> Day 7,9, p<0.05), likely due to a decrease in timeout responding, but did not differ between the subsequent treatment groups (day by treatment interaction or main effect of treatment, $F_{(1\text{-}9,10\text{-}90)}$=0.08-0.48, p=0.79-0.89; **Fig. 3a**). As expected, active lever pressing during extinction training decreased across sessions (main effect of day $F_{(9,90)}$=8.99, p<.00, Tukey's test Day 1,2> Day 3-10, p<0.05) but did not differ between

the subsequent treatment groups (day by treatment interaction or treatment main effect, $F_{(1\text{-}9,10\text{-}90)}$=0.03-0.16, p=0.87-0.99; **Fig. 3b; Table 1**). Inactive lever responding during cocaine self-administration and extinction training did not differ between the subsequent treatment groups (day main effect, day by treatment interaction, treatment main effect, $F_{(1\text{-}9,10\text{-}90)}$=0.21-2.05, p=0.66-0.16; **Fig. 3a,b**). Additionally, there were no pre-existing differences between the subsequent treatment groups in active or inactive lever presses during the 15-min memory reactivation session ($t_{(10)}$ =0.24-1.23, p=0.25-0.81; **Fig. 4a,b; Table 1**).

Effects of intra-DH AM251 following re-exposure to cocaine-paired context

Active and inactive lever responding during the first extinction session that followed the memory reactivation session and intracranial manipulations did not differ between treatment groups ($t_{(10)}$=0.34-1.75, p=0.11-0.75). There were no group differences in the number of days required to reach the extinction criterion, with all subjects reaching the criterion in two days (t-score was not calculated due to equal means and standard deviations of zero in both groups; **Table 1**). At test, active lever responses increased following re-exposure to the cocaine-paired context compared to responding in the extinction context during the preceding extinction session (main effect of context, $F_{(1,10)}$= 47.919, p < .00). However, intra-DH AM251 administered after the memory reactivation session did not alter subsequent active lever responding in either context compared to VEH (treatment by context interaction and main effect of treatment ($F_{(1,10)}$=1.79-2, p=0.19-0.21; **Fig. 4a**). Inactive lever pressing was unaffected by context or treatment (main effect of context or treatment and treatment by context interaction ($F_{(1,10)}$=0.01-0.29, p=0.6-0.94; **Fig. 4b**).

Experiment 2:

Behavioral history

All subjects in Experiment 2 exhibited stable cocaine intake and lever responding during the last 3 days of cocaine self-administration (**Table 1**). Cocaine intake during the second day of cocaine self-administration was lower than on several other days (main effect of day, $F_{(9-90)}$=2.49, p=0.01, Tukey's test, Day 2< Day1,6,8; **Fig. 5a**). However, there were no differences in cocaine intake between the groups that subsequently received CP55940 or VEH (main effect of treatment and treatment by day interaction, $F_{(1-9,\ 10-90)}$=0.53-1.34, p=0.23-0.48; **Fig. 5a**). Active lever pressing decreased following day 1 of cocaine self-administration training (main effect of day $F_{(9-90)}$=4.79, p< .00, Tukey's test, Day 1> Day 2-10) but did not differ between the subsequent treatment groups (main effect of treatment and treatment by day interaction (main effect of treatment and treatment by day interaction, $F_{(1-9,\ 10-90)}$=0.07-1.04, p=0.41-0.8; **Fig. 5a**). Inactive lever pressing during cocaine self-administration training remained stable across days and did not differ between the subsequent treatment groups (main effects of day and treatment and treatment by day interaction $F_{(1-9,\ 10-90)}$=0.33-1.54, p=0.15-0.94; **Fig. 5a**). As expected, active and inactive lever pressing decreased across extinction training sessions in the extinction context (main effect of day $F_{(9,90)}$=3.02-9.3, p< 0.00, Tukey's test, active lever presses Day 1> Day 3-10, Day 2> Day 6-10; inactive lever presses Day 1> Day 9; **Fig. 5b; Table 1**). there were no pre-existing differences between the subsequent treatment groups in active or inactive lever pressing during the 15-min memory reactivation session ($t_{(10)}$=0.24-0.95, p=0.37-0.82; **Fig. 6a; Table 1**).

Effects of intra-DH CP55940 following re-exposure to cocaine-paired context

Active and inactive lever responding during the first extinction session that followed the memory reactivation session and intra-cranial manipulations did not differ between the treatment

groups ($t_{(10)}$=0.89-1.16, p=0.27-0.39). There were no group differences in the number of days required to reach the extinction criterion with all subjects reaching the criterion in two days (t-score was not calculated due to equal means and standard deviations of zero in both groups; **Table 1**). Active lever responses increased following re-exposure to the cocaine-paired context during the test of reinstatement compared to responding in the extinction context during the preceding extinction session (main effect of context, $F_{(1,10)}$= 21.04, p< .00). However, intra-DH CP55940 administered after the memory reactivation session did not alter subsequent active lever responding in either context compared to VEH (treatment by context interaction and main effect of treatment ($F_{(1,10)}$=0.01-0.26, p=0.62-0.93; **Fig. 6a**). Inactive lever pressing was unaffected by context or treatment (main effect of context or treatment and treatment by context interaction ($F_{(1,10)}$=0.48-0.71, p=0.42-0.51; **Fig. 6b**).

CHAPTER IV: DISCUSSION

Summary

The DH is critical for the reconsolidation of contextual memories, particularly those encoding cue-drug associations; however, the neurochemical mechanisms of this phenomenon remain poorly understood. CB1 receptors contribute to the reconsolidation of different types of Pavlovian memories. In support of this, intra-BLA CB1 receptor agonist or antagonist administration during memory reconsolidation impairs subsequent expression of Pavlovian fear memories, intra-insular cortex CB1 receptor agonist administration disrupts the reconsolidation of conditioned taste aversion memories, and intra-DH CB1 receptor agonist and antagonist administration inhibits and facilitates the reconsolidation of contextual fear memories (Lin et al., 2006; Ratano et al., 2014; Kobilo et al., 2007; de Oliveira Alvares et al., 2008). However, no studies have investigated whether CB1 receptors are involved in the reconsolidation of instrumental memories in general. Furthermore, it is unclear whether the stimulation of DH CB1 receptors facilitates or inhibits the reconsolidation of *contextual-drug memories per se*. The experiments in this Master's Thesis investigated the effects of both DH CB1 receptor stimulation and blockade on the reconsolidation of an instrumental context-response-cocaine memory. Interestingly, neither stimulation nor inhibition of DH CB1 receptors altered the reconsolidation of a context-response-cocaine memory in these experiments. Several factors have to be considered in the evaluation of these negative findings.

Technical considerations

One possible explanation for the lack of statistically significant findings in Experiment 1 is that the dose of AM251 used was too low to produce an effect. In order to increase our confidence in these results, a dose-response curve needs to be generated. The dose selected for this experiment was based on that used previously in a fear-conditioning paradigm which employed only one conditioning session (de Oliveira Alvares et al., 2005; de Oliveira Alvares et al., 2008). In contrast, subjects underwent a minimum of 100 context-cocaine pairings in the current study (based on the acquisition criterion). Extensive learning, repeated memory retrieval, and daily memory reconsolidation in the course of instrumental drug self-administration of memory traces may have resulted in memory traces that were more resistant to disruption than the memory trace generated by de Oliveira Alvares et al. (2005; 2008), rendering the selected 6ng dose of AM251 ineffectual. (Tronson & Taylor, 2007). If this is the case, higher doses of AM251 would be expected to produce impairments in context-response-cocaine memory reconsolidation. Insufficient dosing could also explain the lack of statistically significant findings in Experiment 2. Overall, it will be prudent to generate both AM251 and CP55940 dose-response curves before making conclusions about the involvement of CB1 receptors in context-response-cocaine memory reconsolidation. Notably, while both CB1 receptor-selective drugs used in these experiments have high affinity for CB1 receptors, they may also produce effects through non-CB1 mediated mechanism at higher doses (Barann et al., 2002; Baur et al., 2012). It will be important to consider these putative off-target effects in the interpretation of what will likely be non-linear dose-response curves.

Other considerations

In addition to basic technical issues, null effects in Experiments 1 and 2 may have resulted from differential involvement of CB1 receptors in memory reconsolidation as a function of memory valence, memory type, and anatomical location. These factors are discussed next.

First, the valence of the manipulated memory may determine the recruitment of CB1 receptors to memory reconsolidation. The growing body of literature supports the hypothesis that *stimulation* of CB1 receptors impairs the reconsolidation of *aversive* memories while *blockade* of CB1 receptors impairs the reconsolidation of *appetitive* memories. Specifically, CB1 receptor stimulation in the DH, BLA, and insular cortex impairs the reconsolidation of contextual fear memories, explicit cue-induced fear memories, and taste-aversion memories, respectively (de Oliveira Alvares et al., 2008; Lin et al., 2006; Kobilo et al., 2007). Conversely, systemic CB1 receptor blockade impairs the reconsolidation of CPP memories for morphine, methamphetamine, and nicotine (Yu et al., 2009; Fang et al., 2011; De Carvalho et al., 2014). Together these findings suggest that, remarkably, CB1 receptors inhibit aversive and facilitate appetitive memory reconsolidation. If memory valence indeed determines the contribution of CB1 receptors to memory reconsolidation in this fashion, CP55940 is not expected to impair context-response-cocaine memory in our model and enhancement may not be detectable due to a ceiling effect. However, one would expect AM251 to impair memory reconsolidation in the current study which was not the case. Thus, the idea that DH CB1 receptors facilitate context-response-cocaine memory reconsolidation is not supported by the present findings.

Second, it is possible that CB1 receptors in the DH are necessary for the reconsolidation of *Pavlovian* context-drug memories but not *instrumental* context-response-drug memories. As noted above, systemic CB1 antagonist administration impairs the reconsolidation of Pavlovian

context-drug memories that underlie CPP (Yu et la., 2009; Fang et al., 2011; De Carvalho et al., 2014). The present study represents the first attempt to test the role of CB1 receptors in the reconsolidation of an instrumental drug memory. Differential involvement of neural mechanisms in memory reconsolidation based on memory type is not unprecedented. Previous work from this and other labs has shown that the functional integrity of certain brain regions, including the nucleus accumbens and agranular cortex, is necessary for the reconsolidation of a Pavlovian context-cocaine memories, but it is not necessary for the reconsolidation of instrumental context-response-cocaine memories (Miller & Marshal, 2005; Wells et al., 2013; Arguello et al., in preparation). Accordingly, CB1 receptors in the DH may be involved in the reconsolidation of Pavlovian drug memories. To examine this, we can test the effects of an intra-DH CB1 antagonist administered after context re-exposure on the subsequent expression of cocaine CPP.

Third, CB1 receptor populations in the DH may not control the reconsolidation of drug-associated contextual memories while CB1 receptors in other brain regions may. Consistent with this, it has been established that systemic CB1 antagonist administration impairs the reconsolidation of drug-associated contextual memories (Yu et al., 2009; Fang et al., 2011; De Carvalho et al., 2014). Evidence that CB1 antagonism in the BLA impairs the reconsolidation of fear memories suggests that the BLA may be one site of action where CB1 receptors mediate drug-associated contextual memory reconsolidation, especially since the BLA, as a whole, is critically involved in the reconsolidation of context-response-drug memories in our paradigm (Lin et al., 2006; Ratano et al., 2014; Fuchs et al., 2009; Wells et al., 2013). However, given that systemic administration of a CB1 antagonist has not yet been shown to impair the

reconsolidation of an instrumental drug-associated contextual memory per se, it will be prudent to first conduct a systemic AM251 study before proceeding with a BLA localization experiment.

Additional control experiments

Significant effects of any post-memory reactivation manipulation on cocaine-seeking behavior are typically followed up with a "no reactivation" control experiment. This control experiment assesses whether the effects of the manipulation are memory reactivation dependent as is expected in the case of a genuine memory reconsolidation deficit (Nader et al. 2000; Alberini et al. 2006; Tronson and Taylor 2007). To this end, control groups undergo self-administration and extinction, as described above. However, on the memory reactivation day, control subjects are exposed to a novel context for 15 min. The novel context is distinctly different from the cocaine-paired or extinction contexts in order to avoid explicit cocaine or extinction memory reactivation (Fuchs et al., 2009). Alternatively, control subjects receive the manipulation 4-6 hours after exposure to the cocaine-paired context (Higginbotham et al., unpublished). This time period is outside of the known time window of memory reactivation. Therefore, long-term memories of context-response-cocaine associations are expected to be invulnerable to a memory reconsolidation inhibitor (Tronson and Taylor, 2006). No reactivation experiments are also sensitive to possible response rate altering effect of manipulation and are a better alternative to locomotor activity testing. If the experiments in this Master's Thesis had resulted in statistically significant findings, no reactivation control experiments would have been performed. Finally, as with all localization studies, the functional significance of possible, unintended spread of AM251 or CP55940 from the DH would have been explored in the course of anatomical control experiments.

Concluding remarks

CB1 receptors are critically involved in memory as well as reward processes thus may be a valuable target for addiction treatments aimed at reducing the motivational salience of drug cues by altering or abolishing learned drug-cue associations. Disruption of memory reconsolidation has shown promise in animal models of drug seeking and is currently investigated in clinical settings (Pachas et al., 2014; Saladin et al., 2013). Several brain regions, including the DH and BLA, have been implicated in memory cocaine memory reconsolidation although the critical neurochemical systems underlying this process in these brain regions are still being actively explored. CB1 receptors are richly expressed in the DH and BLA and reportedly regulate memory reconsolidation in some animal models. While the present study failed to find a role for DH CB1 receptors in the reconsolidation of a contextual drug memory in the context-induced reinstatement paradigm, the existing conditioned fear and CPP literature strongly suggest a yet undetermined role for these receptors. Future studies will undoubtedly clarify the role of the ECS and CB1 receptors in drug memory maintenance and inform our understanding of the memory processes underlying addiction and improve our ability to combat addiction.

Treatment Groups (ns)	Cocaine Intake (mg/kg/session)	Active Lever Responses				Extinction Latency
		Self-administration	Extinction day 1	Extinction day 10	Reactivation	
AM251 (n = 6)	14.28 ± 1.23	61.22 ± 8.93	30.33 ± 12.39	5.5 ± 2.81	30.67 ± 7.02	2 ± 0
CP55940 (n = 6)	12.11 ± 1.23	44.33 ± 5.53	45.83 ± 12.36	3.33 ± 0.95	25.83 ± 5.46	2 ± 0
VEH (n = 6)	14.5 ± 1.29	55.11 ± 8.16	31.17 ± 6.53	5.5 ± 1.52	32.67 ± 4.72	2 ± 0

Table 1

Table indicating average cocaine intake (mg/kg) during the last three days of self-administration, active lever pressing during the Last three days of self-administration, extinction day 1, extinction day 10, and memory reactivation, and extinction latency (i.e., the number of days required to reach the extinction criterion of $\leq$ 25 active lever presses per session on two consecutive days) in subjects that received intra-DH AM251, CP55940, or VEH immediately after the memory reactivation session.

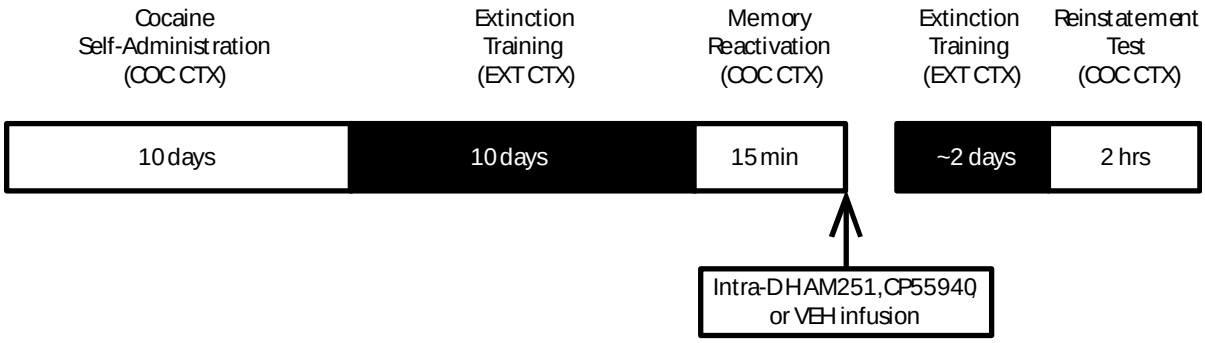

Figure 1

Timeline of behavioral procedures used in Experiments 1 & 2: Cocaine self-administration in the cocaine context (COC CTX), extinction training in the extinction context (EXT CTX), memory reactivation session in the cocaine context, and test for reinstatement of cocaine seeking in the cocaine context.

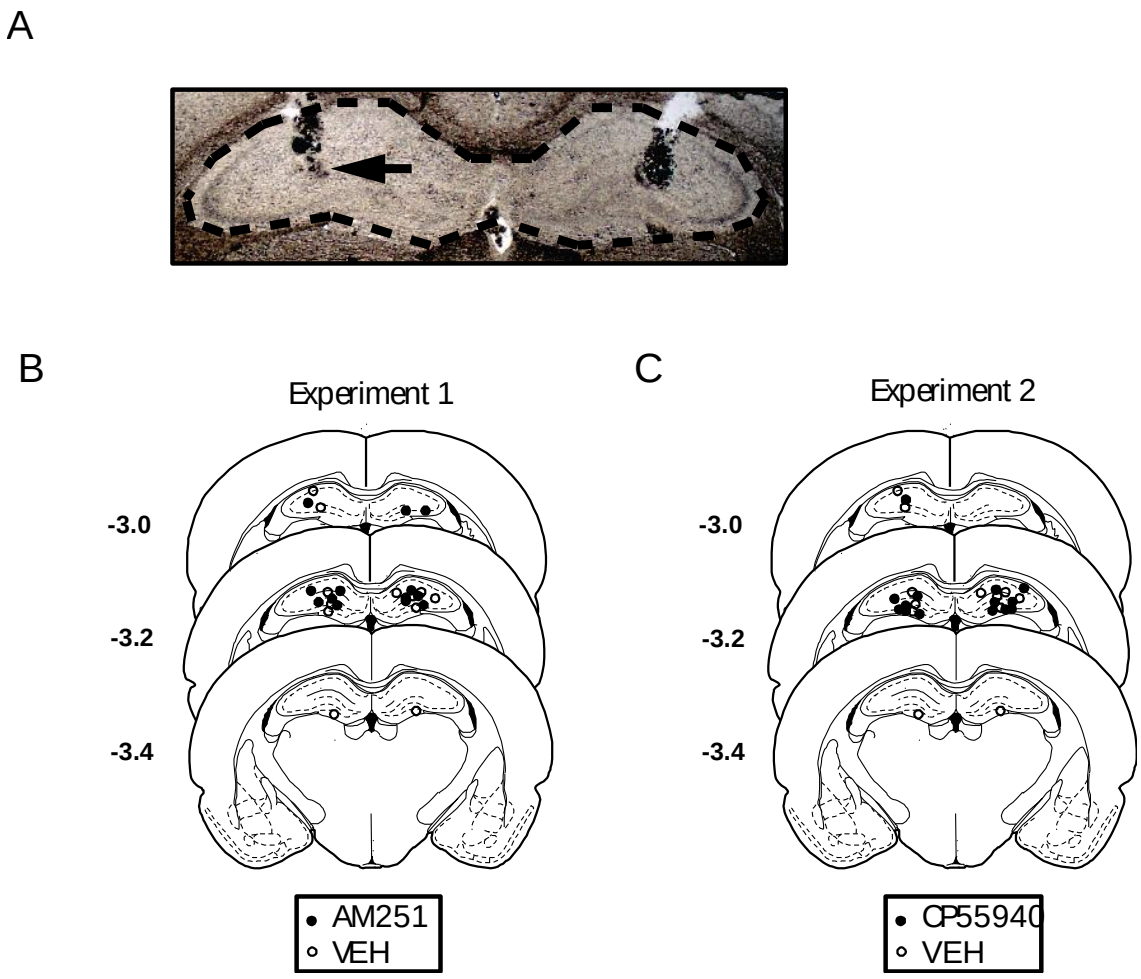

Figure 2

A) Representative photomicrograph of cannula tracks following bilateral microinfusions administered into the DH. B) Schematic representation of the most ventral point of the cannula tracks in subjects that received AM251 (filled circles) or VEH (open circles) in Experiment 1. C) Schematic representation of the most ventral point of the cannula tracks in subjects that received CP55940 (filled circles) or VEH (open circles) in Experiment 2. Note that the VEH group was made up of the same subjects for Experiments 1 & 2. Numbers indicate distance from bregma in millimeters based on the Paxinos and Watson rat brain atlas (1997).

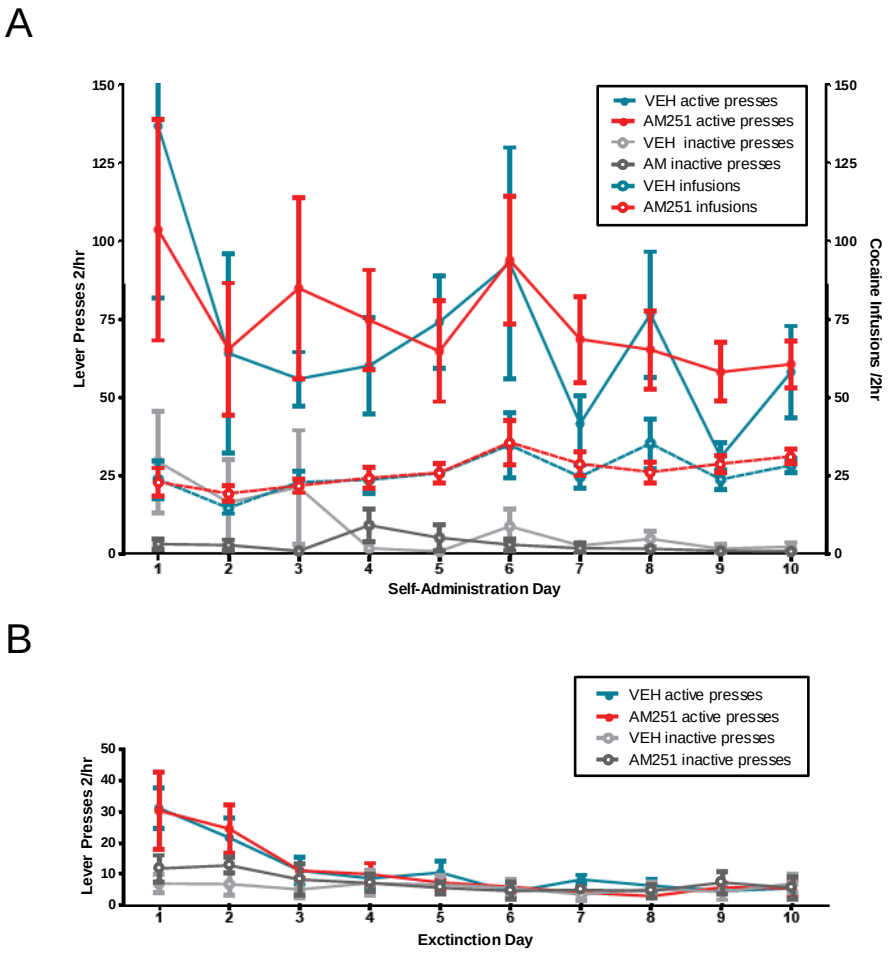

Figure 3

Lever responding during cocaine self-administration and extinction training in Experiment 1. A) Active lever presses (closed red and blue symbols), inactive lever presses (closed grey symbols), and cocaine infusions (open symbols) over 10 days of cocaine self-administration training in the groups that subsequently received intra-DH AM251 or VEH. B) Inactive lever presses over 10 days of extinction training in the groups that subsequently received intra-DH AM251 or VEH.

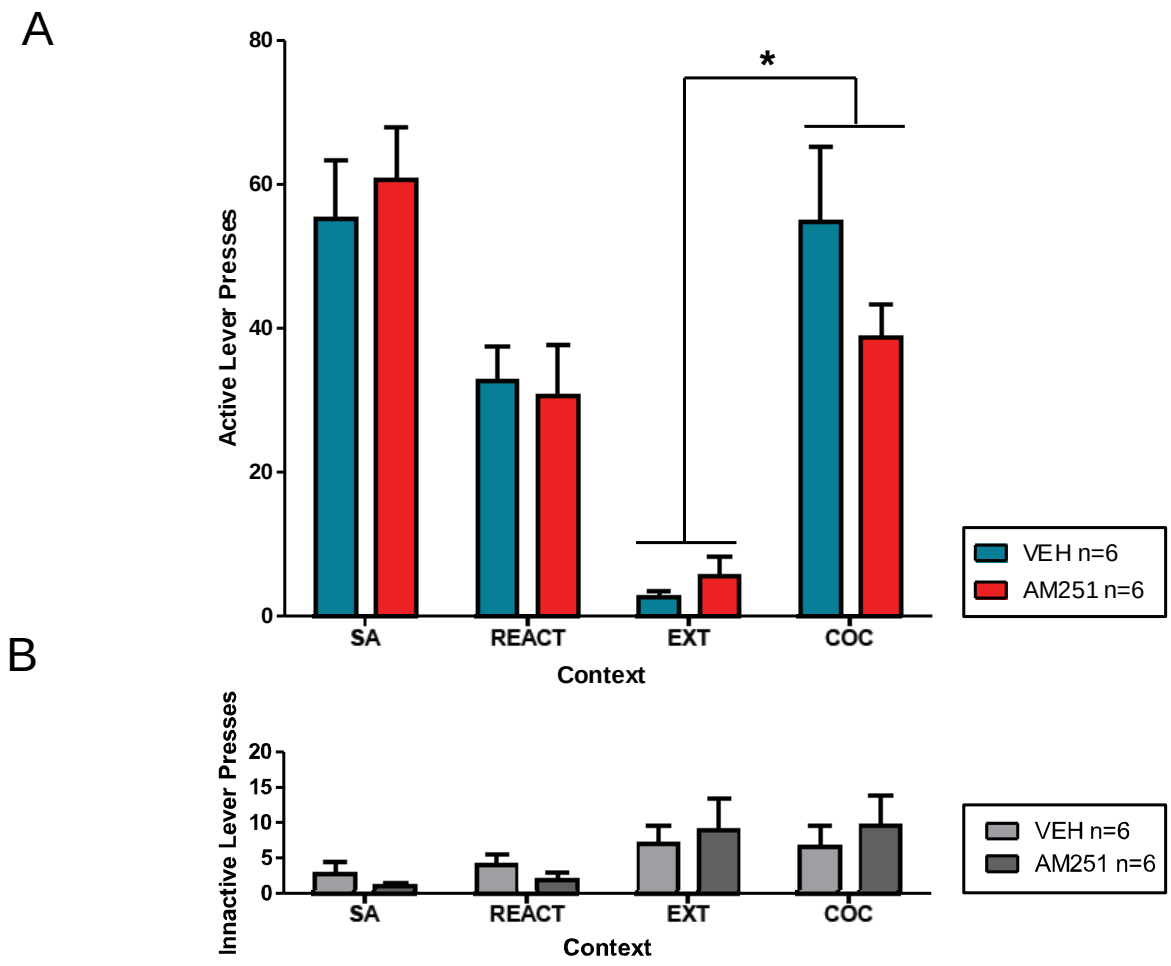

Figure 4

Effects of intra-DH AM251 following cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior A) Active lever presses during self-administration training (SA, mean of last 3 days + SEM/2h), during the memory reactivation session (REACT, mean + SEM/15min), in the extinction context (EXT, mean of last day + SEM/2h), and during the reinstatement test in the previously cocaine-paired context (COC, mean + SEM/2h) in subjects that received intra-DH AM251 (red bars) or VEH (blue bars) after memory reactivation. B) Inactive lever presses during each phase of the experiment in subjects that received intra-DH AM251 (dark grey bars) or VEH (light grey bars). Asterisk indicates a statistically significant difference relative to the EXT context ($P < 0.05$).

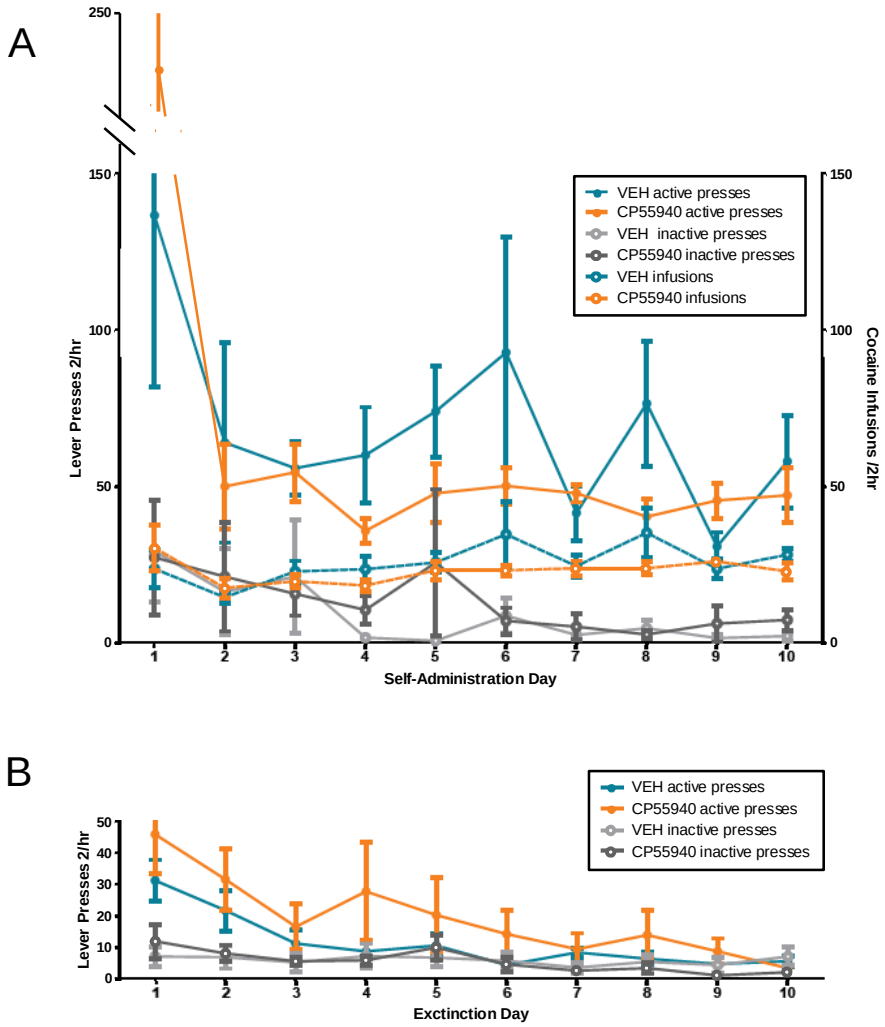

Figure 5

Lever responding during cocaine self-administration and extinction training in Experiment 1. A) Active lever presses (closed orange and blue symbols), inactive lever presses (closed grey symbols), and cocaine infusions (open symbols) over 10 days of cocaine self-administration training in the groups that subsequently received intra-DH CP55940 or VEH. B) Inactive lever presses over 10 days of extinction training in the groups that subsequently received intra-DH CP55940 or VEH.

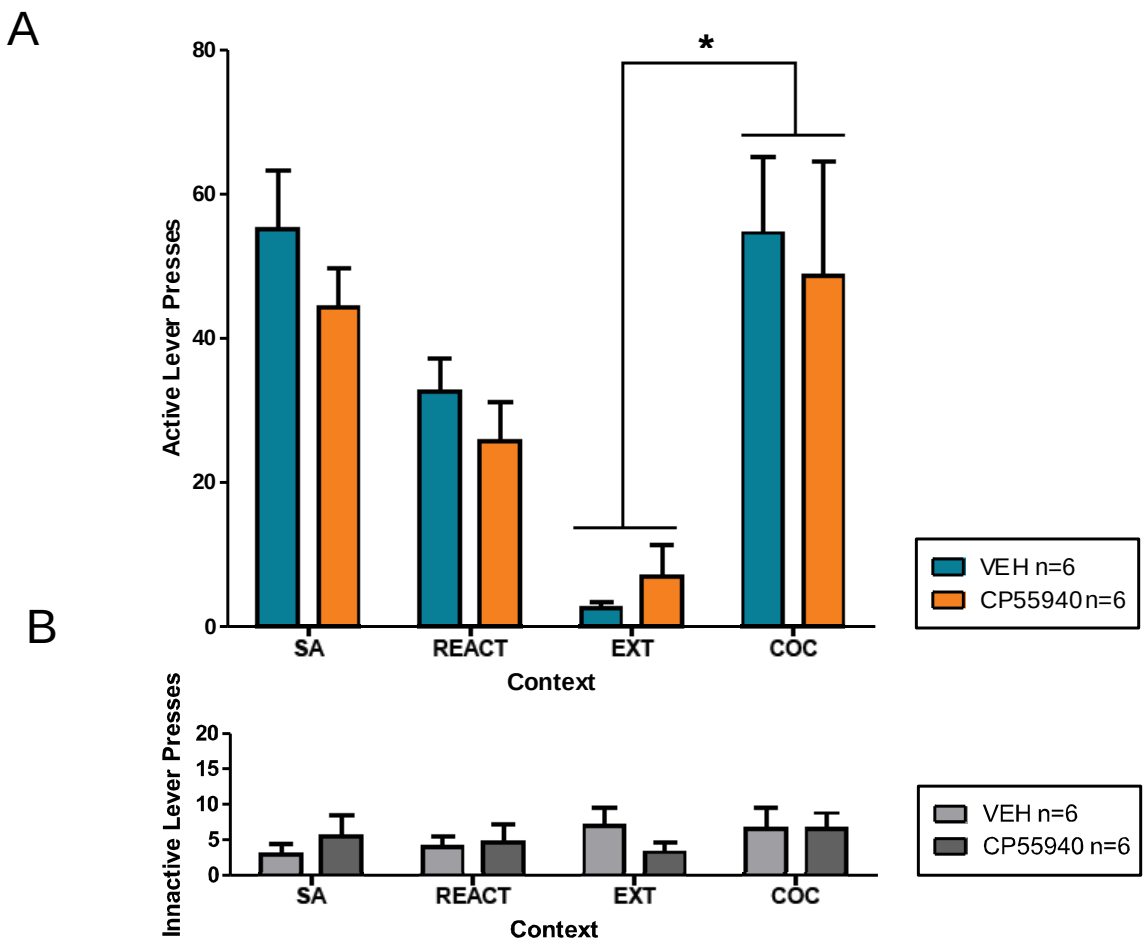

Figure 6

Effects of intra-DH CP55940 following cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior. A) Active lever presses during self-administration (SA, mean of last 3 days + SEM/2h), during the memory reactivation session (REACT, mean + SEM/15min), in the extinction context (EXT, mean of last day + SEM/2h), and during the reinstatement test in the previously cocaine-paired context (COC, mean + SEM/2h) in subjects that received intra-DH CP55940 (red bars) or VEH (blue bars) after memory reactivation. B) Inactive lever presses during each phase of the experiment in subjects that received intra-DH CP55940 (dark grey bars) or VEH (light grey bars). Asterisk indicates a statistically significant difference relative to the EXT context ($P < 0.05$).

WORKS CITED

Akirav, I. (2011). "The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus." *Front Behav Neurosci 5*: 34.

Akirav, I. and M. Maroun (2012). "Stress modulation of reconsolidation." *Psychopharmacology (Berl) 226*(4): 747-61.

Alberini, C. M., M. H. Milekic, et al. (2006). "Mechanisms of memory stabilization and de-stabilization." *Cell Mol Life Sci 63*(9): 999-1008.

Arguello, A. A., M. A. Hodges, et al. (2014). "Involvement of amygdalar protein kinase A, but not calcium/calmodulin-dependent protein kinase II, in the reconsolidation of cocaine-related contextual memories in rats." *Psychopharmacology (Berl) 231*(1): 55-65.

Barann, M., G. Molderings, et al. (2002). "Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site." *Br J Pharmacol 137*(5): 589-96.

Baur, R., J. Gertsch, et al. (2012). "The cannabinoid CB1 receptor antagonists rimonabant (SR141716) and AM251 directly potentiate GABA(A) receptors." *Br J Pharmacol 165*(8): 2479-84.

Childress, A. R., A. V. Hole, et al. (1993). "Cue reactivity and cue reactivity interventions in drug dependence." *NIDA Res Monogr 137*: 73-95.

Conklin, C. A. and S. T. Tiffany (2002). "Applying extinction research and theory to cue-exposure addiction treatments." *Addiction 97*(2): 155-67.

Corbille, A. G., E. Valjent, et al. (2007). "Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants." *J Neurosci 27*(26): 6937-47.

Davies, S. N., R. G. Pertwee, et al. (2002). "Functions of cannabinoid receptors in the hippocampus." *Neuropharmacology 42*(8): 993-1007.

De Carvalho, C. R., F. A. Pamplona, et al. (2014). "Endocannabinoids underlie reconsolidation of hedonic memories in Wistar rats." *Psychopharmacology (Berl) 231*(7): 1417-25.

de Oliveira Alvares, L., L. F. de Oliveira, et al. (2005). "Amnestic effect of intrahippocampal AM251, a CB1-selective blocker, in the inhibitory avoidance, but not in the open field habituation task, in rats." *Neurobiol Learn Mem 83*(2): 119-24.

de Oliveira Alvares, L., B. P. Genro, et al. (2008). "Differential role of the hippocampal endocannabinoid system in the memory consolidation and retrieval mechanisms." *Neurobiol Learn Mem 90*(1): 1-9.

De Vries, T. J., Y. Shaham, et al. (2001). "A cannabinoid mechanism in relapse to cocaine seeking." *Nat Med 7*(10): 1151-4.

Demuth, D. G. and A. Molleman (2006). "Cannabinoid signalling." *Life Sci 78*(6): 549-63.

Devane, W. A., F. A. Dysarz, 3rd, et al. (1988). "Determination and characterization of a cannabinoid receptor in rat brain." *Mol Pharmacol 34*(5): 605-13.

Fuchs, R. A., G. H. Bell, et al. (2009). "Basolateral amygdala involvement in memory reconsolidation processes that facilitate drug context-induced cocaine seeking." *Eur J Neurosci 30*(5): 889-900.

Fuchs, R. A., J. L. Eaddy, et al. (2007). "Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats." *Eur J Neurosci 26*(2): 487-98.

Fuchs, R. A., K. A. Evans, et al. (2005). "The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats." *Neuropsychopharmacology 30*(2): 296-309.

Fuchs, R. A., H. C. Lasseter, et al. (2008). "Relapse to drug seeking following prolonged abstinence: the role of environmental stimuli." *Drug Discov Today Dis Models 5*(4): 251-258.

Fuchs, R. A., S. M. Weber, et al. (2002). "Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats." *Brain Res 929*(1): 15-25.

Gawin, F. H. (1991). "Cocaine addiction: psychology and neurophysiology." Science 251(5001): 1580-6.

Hampson, R. E. and S. A. Deadwyler (1999). "Cannabinoids, hippocampal function and memory." *Life Sci 65*(6-7): 715-23.

Herkenham, M., A. B. Lynn, et al. (1990). "Cannabinoid receptor localization in brain." *Proc Natl Acad Sci U S A 87*(5): 1932-6.

Howlett, A. C., M. Bidaut-Russell, et al. (1990). "The cannabinoid receptor: biochemical, anatomical and behavioral characterization." *Trends Neurosci 13*(10): 420-3.

Jaffe, J. H., N. G. Cascella, et al. (1989). "Cocaine-induced cocaine craving." *Psychopharmacology (Berl) 97*(1): 59-64.

Kawamura, Y., M. Fukaya, et al. (2006). "The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum." *J Neurosci 26*(11): 2991-3001.

Kobilo, T., S. Hazvi, et al. (2007). "Role of cortical cannabinoid CB1 receptor in conditioned taste aversion memory." *Eur J Neurosci 25*(11): 3417-21.

Le Foll, B. and S. R. Goldberg (2005). "Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence." *J Pharmacol Exp Ther 312*(3): 875-83.

Lee, J. L., P. Di Ciano, et al. (2005). "Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior." *Neuron 47*(6): 795-801.

Lichtman, A. H., K. R. Dimen, et al. (1995). "Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats." *Psychopharmacology (Berl) 119*(3): 282-90.

Lin, H. C., S. C. Mao, et al. (2006). "Effects of intra-amygdala infusion of CB1 receptor agonists on the reconsolidation of fear-potentiated startle." *Learn Mem 13*(3): 316-21.

Matsuda, L. A., S. J. Lolait, et al. (1990). "Structure of a cannabinoid receptor and functional expression of the cloned cDNA." *Nature 346*(6284): 561-4.

McGaugh, J. L. (2000). "Memory--a century of consolidation." *Science 287*(5451): 248-51.

Mechoulam, R. and L. A. Parker (2013). "The endocannabinoid system and the brain." *Annu Rev Psychol 64*: 21-47.

Mereu, M., V. Tronci, et al. (2015). "Cocaine-induced endocannabinoid release modulates behavioral and neurochemical sensitization in mice." *Addict Biol 20*(1): 91-103.

Meyers, R. A., A. R. Zavala, et al. (2003). "Dorsal, but not ventral, hippocampal lesions disrupt cocaine place conditioning." *Neuroreport 14*(16): 2127-31.

Miller, C. A. and J. F. Marshall (2005). "Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory." *Neuron 47*(6): 873-84.

Munro, S., K. L. Thomas, et al. (1993). "Molecular characterization of a peripheral receptor for cannabinoids." *Nature 365*(6441): 61-5.

Nader, K., G. E. Schafe, et al. (2000). "The labile nature of consolidation theory." *Nat Rev Neurosci 1*(3): 216-9.

Pachas, G. N., J. Gilman, et al. (2014). "Single dose propranolol does not affect physiologic or emotional reactivity to smoking cues." *Psychopharmacology (Berl).*

Pazos, M. R., E. Nunez, et al. (2005). "Functional neuroanatomy of the endocannabinoid system." *Pharmacol Biochem Behav 81*(2): 239-47.

Ramirez, D. R., G. H. Bell, et al. (2009). "Dorsal hippocampal regulation of memory reconsolidation processes that facilitate drug context-induced cocaine-seeking behavior in rats." *Eur J Neurosci 30*(5): 901-12.

Ratano, P., B. J. Everitt, et al. (2014). "The CB1 receptor antagonist AM251 impairs reconsolidation of pavlovian fear memory in the rat basolateral amygdala." *Neuropsychopharmacology 39*(11): 2529-37.

Robinson, T. E. and K. C. Berridge (2003). "Addiction." *Annu Rev Psychol 54*: 25-53.

Rohsenow, D. J., R. S. Niaura, et al. (1990). "Cue reactivity in addictive behaviors: theoretical and treatment implications." *Int J Addict 25*(7A-8A): 957-93.

Ruehle, S., A. A. Rey, et al. (2011). "The endocannabinoid system in anxiety, fear memory and habituation." *J Psychopharmacol 26*(1): 23-39.

Saladin, M. E., K. M. Gray, et al. (2013). "A double blind, placebo-controlled study of the effects of post-retrieval propranolol on reconsolidation of memory for craving and cue reactivity in cocaine dependent humans." *Psychopharmacology (Berl) 226*(4): 721-37.

Sinha, R. and C. S. Li (2007). "Imaging stress- and cue-induced drug and alcohol craving: association with relapse and clinical implications." *Drug Alcohol Rev 26*(1): 25-31.

Solinas, M., S. Yasar, et al. (2007). "Endocannabinoid system involvement in brain reward processes related to drug abuse." *Pharmacol Res 56*(5): 393-405.

Suzuki, A., S. A. Josselyn, et al. (2004). "Memory reconsolidation and extinction have distinct temporal and biochemical signatures." *J Neurosci 24*(20): 4787-95.

Tronson, N. C. and J. R. Taylor (2007). "Molecular mechanisms of memory reconsolidation." *Nat Rev Neurosci 8*(4): 262-75.

Walker, M. P., T. Brakefield, et al. (2003). "Dissociable stages of human memory consolidation and reconsolidation." *Nature 425*(6958): 616-20.

Wells, A. M., A. A. Arguello, et al. (2013). "Extracellular signal-regulated kinase in the basolateral amygdala, but not the nucleus accumbens core, is critical for context-response-cocaine memory reconsolidation in rats." *Neuropsychopharmacology 38*(5): 753-62.

Wells, A. M., H. C. Lasseter, et al. (2011). "Interaction between the basolateral amygdala and dorsal hippocampus is critical for cocaine memory reconsolidation and subsequent drug context-induced cocaine-seeking behavior in rats." *Learn Mem 18*(11): 693-702.

Wilson, R. I. and R. A. Nicoll (2002). "Endocannabinoid signaling in the brain." *Science 296*(5568): 678-82.

Wise, L. E., A. J. Thorpe, et al. (2009). "Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol." *Neuropsychopharmacology 34*(9): 2072-80.

Wiskerke, J., T. Pattij, et al. (2008). "The role of CB1 receptors in psychostimulant addiction." *Addict Biol 13*(2): 225-38.

Xie, X., D. R. Ramirez, et al. (2010). "Effects of mGluR1 antagonism in the dorsal hippocampus on drug context-induced reinstatement of cocaine-seeking behavior in rats." *Psychopharmacology (Berl) 208*(1): 1-11.