THE ROLE OF NMDA RECEPTORS IN THE DORSAL HIPPOCAMPUS IN COCAINE-PAIRED CONTEXTUAL MEMORY RECONSOLIDATION

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ABSTRACT

KATI L. HEALEY: The Role of NDMA Receptors in the Dorsal Hippocampus in Cocaine-Paired Contextual Memory Reconsolidation
(Under the direction of Kathryn Reissner and Rita A. Fuchs-Lokensgard)

Relapse to drug-seeking behaviors is thought to enduring in addiction because of the resilient association between the rewarding properties of the drug and the context in which the drug is used. Exposure to a cocaine-paired context increases the probability of relapse in humans and leads to cocaine-seeking behavior in rats. The theory of memory reconsolidation postulates that memory traces, when recalled, become labile and need to be re-stabilized into long-term memory. This process is referred to as memory reconsolidation. If memory restabilization is disrupted, the memory can be weakened. Previous research indicates that the dorsal hippocampus (DH) is integral for the reconsolidation of context-drug associated memories. The DH is abundant in N-methyl-D-aspartate (NMDA) receptors that are important for learning and memory and may play a role in cocaine-related contextual memory reconsolidation. To test this hypothesis, rats were trained to self-administer cocaine in a distinct environmental context followed by extinction training in a different context. Rats were then re-exposed to the cocaine-paired context to reactivate the cocaine-paired contextual memory and received intra-DH administration of the NMDA receptor antagonist, D-APV, or vehicle, immediately after this session. After two additional days of extinction training, 72 hours after intracranial manipulation, reinstatement of cocaine-seeking behaviors was assessed in the previously

cocaine-paired context. APV administered into the DH inhibited subsequent cocaine-seeking behavior in a memory reactivation-dependent manner. These findings indicate that NMDA receptor stimulation in the DH is integral for the reconsolidation of associative memories that are required for context-induced cocaine-seeking behavior.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANI	Anisomycin
ANOVA	Analysis of variance
APV	(2*R*)-amino-5-phoshonovaleric acid
BLA	Basolateral amygdala
DH	Dorsal hippocampus
IKK	IκB kinase
NMDA	*N*-methyl-d-aspartate
SSTR	Somatosensory trunk region
VEH	Vehicle
*	Significant difference relative to extinction context
#	Significant difference relative to all other time intervals
†	Significant difference relative to vehicle

CHAPTER 1: INTRODUCTION

Context-induced reinstatement of drug seeking

Drug addiction is a disorder that is chronic and characterized by high rates of relapse. Exposure to environmental stimuli that have previously been paired with a drug of abuse can lead to drug craving (Foltin & Haney, 2000) and relapse (Everitt, Dickinson, & Robbins, 2001), indicating the importance of contextual drug cues. Because substance abuse disorders are a growing problem, it is important to investigate new treatment options that disrupt the cue-drug associations that lead to craving and subsequent relapse. It is theorized that, when a memory is retrieved, it becomes labile and must be re-stabilized to return to long-term memory. This process is referred to as memory reconsolidation (Nader, Schafe, & LeDoux, 2000a; Tronson & Taylor, 2007). If an associative memory is not reconsolidated properly, the associations can weaken. Recent research indicates that reactivated memories undergo processes that result in their reconsolidation into long-term memory that are distinctly different from those involved in initial memory consolidation (Lee & Hynds, 2012; Tronson & Taylor, 2007). Thus, understanding the unique mechanisms of drug memory reconsolidation could lead to methods for disrupting maladaptive associations between contextual cues and drugs of abuse (Milton & Everitt, 2010), and this could be a powerful treatment tool for addiction.

Our laboratory has studied the mechanisms of instrumental drug memory reconsolidation using an ABA design. In the ABA model, animals are placed in a distinct

context (A) to be trained to self-administer drug rewards over multiple sessions. After context-response-drug associations are established, the animals are placed in another, distinctly different context (B) where drug is not available and the instrumental behavior learned in context A extinguishes. To assess whether the drug-associated environment acquired motivational effects, the animals are returned to context A where context-induced goal-seeking behavior is assessed in the absence of drug reinforcement during a reinstatement test. Our modified version of this ABA model to study drug memory reconsolidation includes a brief re-exposure to the drug-paired context prior to the test of reinstatement. This brief session is designed to trigger the retrieval and reconsolidation of context-drug memories and creates an opportunity for experimental manipulation of memory reconsolidation.

Using the ABA model, our laboratory has shown that interference with protein synthesis, post-translational modifications, or neuronal excitability in specific brain regions after a brief re-exposure to the drug-paired environment subsequently attenuates reinstatement of cocaine-context induced drug-seeking behaviors relative to vehicle (Fuchs et al. 2009; Ramirez et al., 2009, Wells et al., 2012, 2014; Arguello et al., 2014). Importantly, the same manipulations failed to alter drug seeking behaviors in animals that had not been re-exposed to the drug-paired environment demonstrating that the effects were dependent on memory retrieval. Such, memory reactivation-dependent impairments in cocaine-seeking behavior likely reflect impairment in cocaine memory reconsolidation. This indicates that the ABA model provides a reliable model to study the underpinnings of drug-memory reconsolidation.

The neural circuitry of drug-memory reconsolidation

The neural circuitry of drug-memory reconsolidation is poorly understood, with most studies thus far indicating involvement of two brain regions, the basolateral amygdala (BLA) and DH, in this phenomenon. The BLA is integral for the reconsolidation of cue-induced (Lee et al., 2006a; Sanchez, Quinn, Torregrossa, & Taylor, 2010) and context-induced (Lasseter, et al., 2011; Wells et al., 2011) cocaine-associated memories. However, the involvement of the dorsal hippocampus (DH) in memory reconsolidation is less well understood. Although the DH has been found to be involved in memory reconsolidation, it may not be the location that these memories are actually stored (Ramirez et al., 2009). Instead, these memories are likely stored in the BLA (Fuchs et al., 2009; Wells et al., 2011). In support of this idea, Ramirez et al. (2009) has shown that neuronal inactivation of the DH using a sodium channel blocker disrupted, but inhibition of anisomycin (ANI)-sensitive processes (i.e., protein synthesis and/or post-translational modification) within the DH did not disrupt, cocaine-paired context memory reconsolidation (Ramirez et al., 2009). Given that memory reconsolidation requires *de novo* protein synthesis, these findings suggest the DH is not the site of storage for reconsolidated memories. However, the DH appears to be integral to the restabilization of memory traces via interaction with the BLA (Wells et al., 2011). Consistent with this, functional disconnection of the DH and BLA immediately after cocaine-paired context memory reactivation disrupted subsequent cocaine-seeking behavior (Wells et al., 2011). Overall, these findings indicate that the DH plays a critical, albeit poorly understood, role in the reconsolidation of a destabilized memory trace.

N-methyl-d-aspartate receptors and memory processes

N-methyl-d-aspartate (NMDA) receptors play a central role in learning and memory processes in the brain (Collingridge, 1987). The NMDA receptor is a ligand- and voltage-gated ion channel (Johnson & Ascher, 1987;Mayer, Westbrook & Guthrie, 1984) composed of four subunits; two GluN1 subunits and one or two GluN2 subunits GluN2A-D (Furukawa et al., 2005). The DH is very rich in NMDA receptors (Olverman, Jones and Wakins, 1984), specifically those that contain the GluN2 subunits GluN2A or GluN2B (Monyer et al., 1994).

A number of studies have implicated NMDA receptors in Pavlovian memory reconsolidation in various brain regions (Alaghband et al., 2014; Liddie & Itzhak, 2014; Chia & Otto, 2013; Alaghband et al., 2013; Wu et al., 2011) and in instrumental memory reconsolidation, such as food-reward memory (Garcia-Delatorre et al., 2014; Lee and Everitt, 2008), odor-reward memory (Rorras-Garcia et al., 2005), and drug-reward memory (Tedesco et al., 2014; Milton et al., 2008; Sadler et al., 2007).

The role of NMDARs in the DH in Pavlovian memory reconsolidation is well documented. A study by Lee et al. (2012) showed that NMDA receptor antagonism using (2*R*)-amino-5-phoshonovaleric acid (APV), in the DH disrupted Pavlovian fear memory reconsolidation. In this study, APV was injected into the DH of rats prior to re-exposure to a previously shock-paired context. Twenty-four hours after memory reactivation, freezing behavior was assessed in the shock-associated context. Animals that had been treated with APV exhibited reduced freezing response to the context compared to control groups, including those that received APV without fear memory reactivation. This is strong evidence for NMDA receptor involvement in aversive memory reconsolidation in the DH; however,

the role of NMDA receptors in appetitive instrumental memory reconsolidation in the DH has not been assessed.

Further support for the role of NMDA receptors in memory reconsolidation comes from studies that provide evidence that downstream elements of NDMA receptor-mediated signaling pathways are involved in context-drug memory reconsolidation in the DH. Specifically, recent studies implicated the role of the IκB kinase (IKK) pathway in aversive memory reconsolidation (Lee & Hynd, 2013; Boccia et al., 2007). Consistent with this, IKK inhibition in the DH disrupted a fear memory reconsolidation (Lee & Hynd, 2013) and IKK or nuclear factor κB (a downstream effector of IKK) inhibition disrupted the reconsolidation of an inhibitory avoidance task (Boccia et al., 2007). Furthermore, systemic NMDA receptor antagonism, systemic NMDA receptor subunit GluN2B inhibition, and systemic inhibition of downstream signaling pathways of the NMDA receptor neuronal nitric oxide synthase (nNOS) and mitogen-activating extracellular kinase attenuated cocaine-associated CPP memory (Liddie and Itzhak, 2014). These findings point to the possibility that DH NMDA receptors and their downstream targets may be involved in appetitive instrumental memory reconsolidation as well.

Hypotheses and predictions

The experiments in this master's thesis examined the involvement of NMDA receptors in the DH in cocaine-memory reactivation. To this end, NMDA receptor antagonist was administered into the DH following cocaine-memory reactivation or no memory reactivation, and the effects of this manipulation were assessed on subsequent cocaine-paired context-induced reinstatement of cocaine seeking behavior. We predicted that NMDA

antagonism in the DH would impair subsequent cocaine-seeking behavior in a memory reactivation-dependent manner. The effects of NMDA antagonism in an anatomical control region, the somatosensory cortex (SSTR), were also assessed to examine whether the effects of the NMDA antagonist were due to diffusion away from the DH in the most likely, dorsal direction. We predicted that if NMDA receptor stimulation in the DH is necessary for the reconsolidation of context-response-cocaine memories then NMDA antagonism following cocaine memory reactivation (thus destabilization) would impair subsequent cocaine-paired context-induced reinstatement of cocaine seeking behavior, consistent with impairment in the memory trace. Conversely, we predicted that the same manipulation would be without effect in the absence of memory reactivation or if the NMDA antagonist was infused into the SSTR.

CHAPTER 2: METHODS AND MATERIALS

Subjects

Male Sprague-Dawley rats (Harlan: 200-250 grams) were single-housed in a vivarium under reversed 12-hr light/dark cycle (lights off at 7pm). Rats were habituated to the vivarium for 5 days. Rats were fed *ad libitum*, kept under temperature controlled conditions (68-70°), and had unlimited access to water throughout the experiment. After the habituation period, rats were placed on a diet of 20g of rat chow per day for the remainder of the study. The housing and treatment of animals followed the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996) and were approved by the University of North Carolina Institutional Animal Care and Use Committee.

Food Training

Rats were first trained to lever press under an FR1 schedule of food reinforcement (45 mg pellets; Purina, Richmond, IN) to expedite the subsequent acquisition of cocaine self-administration. Twenty-four hours prior to food training, the rats were food restricted (8g). Operant chambers (26 x 27 x27 cm high; Coulbourn Instruments, Allentown, PA) were equipped with two retractable levers and a food pellet dispenser. During the food training session, each lever press on the right lever (active lever) resulted in the delivery of one grain-based food pellet into the food hopper. Lever presses on the left lever (inactive) had no

programmed consequences. Food training sessions were at least 16 hours overnight, until rats met a criterion of 100 presses on the active lever per session.

Surgery

Prior to surgery, rats were anesthetized with ketamine hydrochloride and xylazine (66.6 mg/kg and 1.33 mg/kg, intraperitoneal, *i.p*, respectively). Chronic indwelling catheters were made in house and implanted into the right jugular vein for the administration of intravenous (IV) cocaine, as described previously (Fuchs et al., 2006). The catheter ran subcutaneously and exited the back between the shoulder blades where it was capped by sealed Tygon tubing and a protective screw cap (PlasticsOne). After catheter surgery, the rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL) and 26-gauge stainless steel guide cannulae (Plastics One) were aimed bilaterally at the DH (angled by 15° laterally; -3.4 mm AP, ±3.1 mm ML, -2.15 mm DV, relative to bregma) or the anatomical control region, the trunk region of the somatosensory cortex (SSTR; angles by 15° laterally; -3.4 mm AP, ±3.1 mm ML, -0.8 mm DV, relative to bregma). Guide cannulae were sealed with screw caps (PlasticsOne) to prevent occlusion and infection.

Rats were given at least 5 days of post-operative recovery before the start of the experiment. Catheters were flushed daily during recovery with an antibiotic solution of cefazolin (0.1 mL, 10.0 mg/mL cefazolin; Schein Pharmaceuticals) dissolved in heparinized saline (70 U/mL; Baxter Health Care Corp.) followed by heparinized saline (0.1 mL; 70 U/mL) to extend catheter patency and prevent infection. During self-administration training, catheters were flushed before the session with heparinized saline (0.1 mL; 10U/mL) and after the session with the cefezolin solution (0.1 mL; 10.0 mg/mL) followed by 0.1 mL of

heparinized saline (70 U/mL). Tests of catheter patency was performed periodically with propofol (1 mg/0.1 mL, i.v.), a short acting barbiturate that causes a rapid loss of muscle tone when administered intravenously.

Cocaine self-administration

Cocaine self-administration training was conducted in standard operant-conditioning chambers (Med Associates Inc.) set up to form two distinct contexts. Context A contained a continuous red house light on the wall across from the levers, intermittent pure tone (80 dB, 1kHZ; *2s* on, 2*s* off), pine scent, a wire mesh floor (26 cm x 27 cm), and a stimulus light above the inactive lever (5*s* on). Context B contained an intermittent white stimulus light above the inactive lever (2*s* on, 4*s* off), a continuous pure tone (75 dB, 2.5 kHz, vanilla scent, a bar floor, a slanted ceramic tile wall (19 cm x 27 cm). These stimuli were not presented during food training.

Rats were randomly assigned to a context and allowed to lever press for cocaine reinforcement. Active lever presses resulted in cocaine infusions (cocaine hydrochloride; 0.15 mg/0.05 mL per infusion, i.v.; NIDA) under a fixed ratio 1 (FR1) reinforcement schedule with a 20s time out after each infusion. Inactive lever presses had no programmed consequences but they were recorded to provide an index of inadvertent. Rats were trained to self-administer cocaine in the cocaine-paired context until they reached the acquisition criterion (a total of 10 days with greater than 10 infusions per session). Assignment to treatment groups was balanced based on average active lever responding and cocaine intake during the last three days of training.

Extinction training

Rats received seven daily 2-h extinction training sessions in a distinctly different context than the self-administration training context. During the extinction sessions, active and inactive lever presses were recorded but did not have any programmed consequences. After the fourth extinction session, rats were adapted to the intracranial microinfusion procedure. To do so, stainless steel injection cannulae were inserted into the guide cannulae. The injection cannulae extended 1mm below the tip of the guide cannulae. The rats were held by the experimenter for 4 minutes; however, no fluid was infused.

Experiment 1a. Effects of NMDA antagonism in the DH following cocaine memory reactivation

Experiment 1a examined whether intra-DH administration of the NMDA receptor antagonist, APV, following cocaine memory reactivation would impair context-response-cocaine memory reconsolidation. On the day after extinction training, animals were placed in the previously cocaine-paired context for a 15 minute memory reactivation session to initiate destabilization and memory reconsolidation (Fuchs et al. 2009). During the session, the levers were extended and responses were recorded; however, lever presses had no programmed consequences.

Intracranial microinfusion and additional extinction training

Rats received bilateral microinfusions of either the NMDA receptor antagonist, APV (2.5μg/0.5μL per hemisphere), or vehicle (VEH; phosphate-buffered saline; 0.5μL/hemisphere) into the DH or SSTR. This dose of APV was selected because it had been

shown to disrupts the expression of drug context-induced reinstatement of cocaine-seeking behavior after infusion into the DH (Xie et al., 2013). Following the infusion, rats received additional daily 2-h extinction training sessions in the extinction context until they reached the extinction criterion (a minimum of two consecutive days with ≤ 25 active lever responses per session).

Test of drug context-induced cocaine-seeking behavior

Twenty-four hours after the extinction criterion was reached, rats received a 1-h test session. During the test session, lever responses were assessed in the previously cocaine-paired context without cocaine reinforcement. Active and inactive levers were recorded but had no programmed consequences. Reinstatement was defined as a significant increase in active lever responding in the cocaine-paired context on the test day relative to responding during the first hour of the last extinction session in the extinction context.

Experiment 1b. Effects of NMDA antagonism in the DH following cocaine memory reactivation

Experiment 1b explored whether the effects of intra-DH NMDAR antagonism were memory reactivation dependent, as memory reconsolidation is theorized to be dependent on memory destabilization (Lewis, 1979; Nader et al., 2000b). Experimental procedures were identical to those in Experiment 1a except that instead of placing animals into their cocaine-paired context for memory reactivation, animals were placed in a novel-unpaired context (context C) that distinctly differed from contexts A and B. Context C contained a continuous white house light on the wall opposite the active lever, continuous white stimulus lights

above both the active and inactive levers, a continuous complex tone (80) d B; alternating between 1, 1.5 and 2.5 kHz at 1 s intervals), citrus-scented air freshener strip (4.5 x 2 cm, Locasmarts LLC., Ormond Beach, FL), and a ceramic tile floor (26 cm x 27 cm).

Experiment 1c. Effects of NMDA antagonism in the SSTR following cocaine memory reactivation

Experiment 1c investigated whether the effects of NMDAR antagonism observed in Experiment 1a were dependent on the DH. Experimental procedures were identical to those in Experiment 1a except that the guide cannulae were directed at the SSTR. The SSTR is the brain region most likely to receive off target effects of APV, due to possible diffusion of the drug from the DH dorsally, along the cannula shaft.

Experiment 2. Effects of NMDA antagonism in the DH on locomotor activity

Intracranial manipulations can have an effect on instrumental cocaine-seeking due to a general effect on motor activity. To assess this, animals were microinjected with either VEH or APV into the DH. Seventy-two hours later (to match the treatment to testing interval in Experiment 1), the rats were placed in a novel Plexiglas chamber (42 x 20 x20 cm high) where locomotor activity was assessed for 2h. Locomotor activity was evaluated by a computerized activity system (San Diego Instruments, San Diego, CA) monitoring photobeam break, as described previously (Fuchs et al., 2007). Five animals received both APV and VEH treatment in a counter balanced design, with at least 3 days between locomotor test and the second microinjection. 9 additional animals only received one test of locomotion.

Data analysis

Two-way analyses of variance (ANOVA) were conducted to assess possible pre-existing group differences in cocaine intake as well as active and inactive lever responding during cocaine self-administration training. T-tests were conducted to examine possible pre-existing group effects in active and inactive lever responding during the 15-minute memory reactivation/no-reactivation session. Mixed-factorial ANOVAs were conducted to examine the effects of post-reactivation manipulations on (a) active and (b) inactive lever responses on the test day in the cocaine-paired context and during the last extinction session in the extinction context and on (c) locomotor activity counts across time. In these ANOVAs, treatment (VEH/APV) was included as a between subjects factor; context (cocaine-paired, extinction) and time (20-minute intervals) were included as a within-subjects factors when appropriate. Significant main and interaction effects were further analyzed using Tukey's test. Alpha was set at 0.05.

CHAPTER 3: RESULTS

Histology

Schematics illustrating cannula placements are included in **Fig. 1**. The target brain regions were defined as the dorsal hippocampus (DH) and the somatosensory cortex trunk region (SSTR). Only data from rats with the correct placements were included in the statistical analyses. The resulting Ns per group for vehicle (VEH) and drug-treated (APV) groups were as follows, experiment 1a and 2 – VEH (n = 7), APV (n = 10), experiment 1b – VEH (n = 10), APV (n = 10), and experiment 1c – VEH (n = 4) and APV (n = 3).

Behavioral history

There were no pre-existing differences between the groups that subsequently received APV or VEH into the DH or SSTR in cocaine intake, active lever responding or inactive lever responding during cocaine self-administration training (day 8-10), active lever responding during extinction training (day 5-7), or extinction latency (see results for each experiment below and descriptive statistics in **Table 1**). Active lever responding during reactivation did not correlate with active lever responding during reinstatement for groups that received VEH (r=-0.19, p=0.41) or APV (r= 0.34, p=0.15) after the memory reactivation session, suggesting that it did not result in behavioral extinction.

NMDAR antagonism in the DH following cocaine memory reactivation blocks subsequent drug context-induced cocaine-seeking behavior

There was no pre-existing difference between the subsequent APV- and VEH-treated groups in active or inactive lever responding during cocaine self-administration training (day x treatment interaction, $F_{(9,135)}$=0.58-0.89, p=0.54-0.89; treatment main effect, $F_{(1,15)}$=0.15-2.75, p=0.12-0.70; day main effect, $F_{(9,135)}$=1.58-1.64, p=0.11-0.15). Cocaine intake gradually increased across time (day main effect, $F_{(9.135)}$=5.41, p<0.001), but there was no difference between the groups in cocaine intake (day x treatment interaction or main effect of treatment ($F_{(1\text{-}9,15\text{-}135)}$=0.19-1.57, p=0.13-0.89; **Fig. 2C**). Responding gradually declined when reinforcement was removed during extinction training (day main effects, F(6,90)=7.46-11.041, p<0.05, Tukey's test, Day 1> Day 2-7, p<0.05), but there was no pre-existing difference between the groups in active or inactive lever responding during extinction training (treatment by day interaction, $F_{(6,90)}$=0.183-1.825, p=0.103-0.981; treatment main effects, $F_{(1,15)}$= 1.11-4.16, p=0.06-0.31; **Fig. 2A-B**). Finally, there was no pre-existing difference between VEH/APV-treated groups in active lever responding during the 15-min cocaine memory reactivation session ($t_{(15)}$=-0.182, p=0.86; **Fig. 2B**).

APV administration into the DH following the cocaine-memory reactivation session altered subsequent drug context-dependent cocaine-seeking behavior (ANOVA context x treatment interaction, $F_{(1,15)}$=16.73, p<0.001; treatment main effect, $F_{(1,15)}$=4.09, p=0.06; context main effect, $F_{(1,15)}$=39.18, p<0.001; **Fig. 2D**). The group that received VEH injection into the DH following cocaine memory reactivation showed enhanced active lever responding in the cocaine-paired context compared to responding in the extinction context (Tukey's test, p<0.05). The APV-treated group responded less than the VEH-treated group in

the cocaine-paired context (Tukey's test, $p<0.05$), but not in the extinction context. Furthermore, the APV-treated group failed to show increased active lever responding in the cocaine-paired context relative to the extinction context (Tukey's test, $p>0.05$). There was not difference between the groups in inactive lever responding in either context (all treatment and context main effects and interaction, $F_{(1,15)}=2.55\text{-}3.84$, $p=0.07\text{-}0.13$; **Fig. 2E**).

NMDAR antagonism in the DH in the absence of cocaine memory reactivation fails to alter subsequent drug context-induced cocaine-seeking behavior

As in Experiment 1a, active and inactive lever responding was stabile during cocaine self-administration training (day main effect, $F_{(19,235)}=0.87\text{-}1.44$, $p=0.18\text{-}0.56$), while active and inactive lever responding decreased when drug reinforcement was removed during extinction training (active lever main effect of day: $F_{(6,90)}=5.64\text{-}8.42$, $p<0.01\text{-}0.001$, Tukey's test, Extinction Day 1> Day2-7, $p<0.05$; **Fig. 3B**). Importantly, there was no pre-existing difference between the treatment groups that later received APV or VEH in active lever responding during cocaine self-administration training or extinction training (day x treatment interaction, $F_{(6\text{-}9,90\text{-}135)}=1.00\text{-}1.16$, $p=0.334\text{-}1.00$; treatment main effect, $F_{(1,15)}=0.006\text{-}0.39$, $p=0.54\text{-}0.94$). Similarly there was no difference between the groups in cocaine intake (day x treatment interaction and main effect of treatment, $F_{(1\text{-}9,15\text{-}135)}=0.59\text{-}0.98$, $p=0.20\text{-}0.46$). Lastly, there was no difference in responding between the groups during the 15-minute reactivation session ($t_{(15)}=-1.37$, $p=0.19$).

Unlike in Experiment 1a, APV administration into the DH following exposure to a novel, unpaired context failed to alter subsequent cocaine seeking in a context-dependent manner (Fig. 3D). Active lever responding in both groups increased in the cocaine-paired

context at test relative to responding in the extinction context (ANOVA context main effect $F_{(1,15)}$=17.23, p<0.001; Fig. 3D). However, the groups did not differ in active lever responding in either context (treatment x context interaction, $F_{(1,15)}$=0.70, p=0.416; treatment main effect, $F_{(1,15)}$=1.47, p=0.161). Inactive lever increased upon exposure to the cocaine-paired context relative to the extinction context (context main effect, $F_{(1,15)}$=7.03, p<0.02), but the groups did not differ in inactive lever responding in either context (treatment x context interaction and treatment main effect, ($F_{(1,15)}$=0.71-1.47, p=0.24-0.71; Fig. 3E).

NMDAR antagonism in the SSTR following cocaine memory reactivation fails to alter subsequent drug context-induced cocaine-seeking behavior

As in Experiments 1a and 1b, there was no pre-existing difference between the groups that later received APV or VEH into the SSTR in active or inactive lever responding during cocaine self-administration training (day x treatment, $F_{(9,45)}$ =1.21-1.79, *p*=0.10-0.31; treatment main effect $F_{(1,5)}$=0.20-1.15, *p*=0.33-0.68; day main effect, $F_{(9,45)}$=0.98-0.46, p=0.47-0.90; **Fig. 4B**). Similarly, there was no difference between the groups in cocaine intake (treatment x day interaction and main effects, $F_{(1\text{-}9.5\text{-}45)}$=0.09-1.57, *p*=0.16-.77; **Fig. 4C**). Active lever responding declined when drug reinforcement was removed (day main effect, $F_{(6,30)}$=3.29, *p*<0.02; **Fig. 4B**); however, there was no difference between the groups in active lever responding during extinction training (treatment x day interaction and treatment main effect, $F_{(1\text{-}6,5\text{-}30)}$=0.96-2.27, *p*=0.19-0.47; **Fig. 4B**). Furthermore, inactive lever responding did not change and there was no pre-existing group difference in inactive lever responding during extinction training (treatment x day interaction and main effects, $F_{91\text{-}6,5\text{-}}$

$_{30)}$=0,19-1.76, *p*=0.14-0.68, **Fig. 4B**). There was no difference in responding during the 15 minute reactivation session between VEH/APV-treated animals ($t_{(5)}$=2.03, *p*=0.10).

Unlike in Experiment 1a, APV administration into the SSTR following re-exposure to the cocaine-paired context failed to alter subsequent context-dependent cocaine seeking at test (**Fig. 4D**). Exposure to the cocaine-paired context failed to increase active or inactive lever responding in either group relative to responding in the extinction context (context main effect ($F_{(1,5)}$=2.04-3.79, *p*=0.11-0.21; **Fig. 4D-E**). Furthermore, there was no difference between the groups in responding on the active or inactive lever in either context (treatment x context interaction, $F_{(1,5)}$=0.44-5.04, *p*=0.08-0.54; treatment main effects, $F_{(1,5)}$=0.02-0.98, *p*=0.77-0.89; **Fig. 4D-E**).

NMDA antagonism did not have a protracted effect on motor activity

General motor activity was assessed 72 hours after infusion with either VEH or APV into the DH of a subset of rats (VEH, n = 9, APV, n = 10). Locomotor activity decreased as the animals habituated to the chamber (time main effect, $F_{(5,85)}$=80.41, p<0.001, Tukey's test Interval 1>Interval 2-6; **Fig. 5C**). However, there was no difference in locomotor activity between the treatment groups (treatment x time interaction, $F_{(5,85)}$=1.17, *p*=0.33; treatment main effect, $F_{(1,17)}$=0.21, *p*=0.65).

CHAPTER 4: DISCUSSION

Results from this study indicate that NMDA receptor antagonism in the DH following contextual cocaine-memory reactivation attenuates subsequent cocaine-seeking behavior compared to VEH. This is consistent with the established importance of the DH in memory reconsolidation, and expands on the current literature by demonstrating that the NMDA receptor in the DH is an integral component of the signaling mechanisms of memory reconsolidation in a memory reactivation dependent manner.

An important aspect of these findings is that NMDA receptor-dependent reconsolidation in the DH is memory reactivation-dependent. Specifically, APV infusion immediately after re-exposure to the cocaine-paired context resulted in impaired reinstatement behavior, consistent with a weakened context-response-cocaine memory trace. In contrast, APV infusion immediately after exposure to a novel, unpaired context failed to alter reinstatement behavior in the cocaine-paired context. This indicates that pharmacological antagonism of NMDA receptors during memory reconsolidation does not cause a general amnesic effect. Instead, it only affects the reactivated target memory, the cocaine-paired contextual memory trace. Notably, the present study utilized a single dose of the NMDA antagonist. Linear dose-effect curves and the absence of off-target effects cannot be ruled out. Thus, it will important to examine the effects of a range of APV doses and those of multiple NMDAR antagonist with differing off-target effects to more fully understand the role of NMDAR in cocaine memory reconsolidation.

In the anatomical control experiment, APV was infused into the SSTR directly after re-exposure to the cocaine-paired context. This manipulation failed to attenuate subsequent cocaine-seeking behavior, suggesting that impairment in cocaine seeking in the group that received APV into the DH following cocaine memory reactivation was not due to APV diffusion dorsally. However, the sample sizes were small in the anatomical control experiment, which limits confidence in these findings. Furthermore, reinstatement responding was overall very low (i.e., below the extinction criterion) and may have precluded the observation of attenuation in reinstatement due to a floor effect. Weak responding was in part due to a bacterial infection that affected the colony. Overtly sick animals were eliminated from the study, but the abnormally low reinstatement responding suggests that some of the remaining animals were also sick.

The disruption in operant drug-reward memory reconsolidation shown in this study expands upon previous literature indicating that NMDA receptors are an important component of signaling pathways responsible for Pavlovian fear and drug-reward memory reconsolidation (Garcia-Delatorre et al., 2014; Liddie & Izthak, 2014; Alaghband et al., 2011; Wu et al., 2011; Milton, 2008). Further, these results establish that NMDA receptor activation within the DH specifically is important for operant drug-reward memory reconsolidation, corroborating results from previous studies on Pavlovian fear and drug-reward memory reconsolidation (Lee & Hynd, 2012).

The results of this study provide evidence for the involvement of NMDA receptors in memory reconsolidation without the confounding contribution of retrieval or destabilization processes because APV was administered directly after memory reactivation session when memory reconsolidation is postulated to occur (Tronson and Taylor, 2006). Conversely, in

past memory reactivation studies, manipulations were administered prior to the memory reactivation session (Lee & Hynd, 2013; Hellemans, Everitt & Lee, 2006; Lee, Milton & Everitt, 2006). While in those studies it is unclear whether the manipulation affected memory retrieval, destabilization, or reconsolidation, the effects were ascribed to impairment in memory reconsolidation. For example, it has been shown that pharmacological NMDAR blockade in the DH has to be applied prior to (but not after) retrieval in order to disrupt fear memories (Lee & Hynd, 2013). Similarly, NMDAR antagonism in the nucleus accumbens has to be induced before memory retrieval (but not after) in order to block subsequent morphine conditioned place preference (Wu et al., 2012). These results are not consistent with an NMDA antagonist-induced impairment in memory reconsolidation per se. Thus, it appears that the contribution of NMDA receptors to memory reconsolidation depends on memory type and brain region.

Future studies will need to assess the possible contributions of NMDA receptors in the DH to memory retrieval or destabilization. Some evidence has suggested that these contributions are NMDA receptor subunit specific at least in the BLA. Consistent with this, a recent study has shown that selective antagonism of GluN2A- and GluN2B- containing NMDA receptors disrupts memory reconsolidation and memory destabilization, respectively (Milton et al., 2013). Administration of a GluN2A-preferring NMDA antagonist prior to auditory fear cue re-exposure attenuated auditory fear memory 24 hours later without having an acute effect on auditory freezing behavior during the memory reactivation session. Conversely, intra-BLA injection of GluN2B-preferring antagonist prior to memory reactivation failed to have an acute effect on retrieval or on subsequent responding 24 hours later. However, remarkably, GluN2B antagonism administered prior to memory reactivation

prevented anisomycin-induced impairment in memory reconsolidation. These findings suggested that GluN2B subunit containing NMDA receptors play a role in the destabilization of fear memories in the BLA during after memory retrieval. Similar differences in the contribution of GluN2A- and GluN2B –containing NMDA receptors in the DH may also be present, but this remains an empirical question given that the DH is distinctly different in many respects from the BLA (Ramirez et al., 2009).

The present study leads to questions about downstream mechanisms by which DH NMDA receptors regulate contextual memory reconsolidation. A potential downstream target of NMDA receptors in the hippocampus is the IκB kinase (IKK-NF-κB) pathway, one of two major kinase cascades associated with the NMDA receptor, the other being the MEK-ERK cascade. There is evidence that receptors containing GluN2A-containing NMDARs and GluN2B-containing NMDARs have different downstream signaling mechanisms (Chen et al., 2007) and this leads to the possibility of specific NMDA receptor subtypes having distinct contributions to memory reconsolidation. A recent study by Lee & Hynd (2013) found that the IKK pathway but not the MEK-ERK pathway was involved in the reconsolidation of a conditioned fear memory in the DH. The contribution of the IKK pathway in the DH extends to other forms of aversive memory reconsolidation, as another study has demonstrated that IKK and nuclear factor-κB inhibition in the DH similarly disrupted the reconsolidation of inhibitory avoidance memory (Boccia et al., 2007). However, it remains to be investigated whether appetitive memory reconsolidation in the DH is mediated by the IKK pathway.

Previous research in this lab has implicated MEK-ERK pathway in the BLA in the reconsolidation of instrumental cocaine-context memories (Wells et al, 2011), and systemic

studies have also implicated ERK in memory reconsolidation of both Pavlovian CPP memory (Liddie & Itzhak, 2014; Valjent et al., 2006) and hippocampal recognition memory (Kelly, Laroceh & Davis, 2003). GluN2B subunit containing NMDA receptors are linked to the MEK-ERK pathway (Krapivinsky et al., 2003), and systemic GluN2B subunit antagonism disrupted cocaine CPP memory reconsolidation (Liddie & Itzhak, 2014). However, the contribution of the MEK-ERK pathway to memory reconsolidation may be limited to the BLA, as intra-hippocampal MEK inhibitor UO126 did not attenuate fear response in a fear conditioning memory reconsolidation experiment (Lee & Hynds, 2013). Thus, future studies will need to determine whether the MEK/ERK signaling pathway in the DH plays a role in appetitive instrumental contextual memory reconsolidation.

In conclusion, NMDA receptors in the DH are integral for the reconsolidation of cocaine-paired contextual memories after these are destabilized upon retrieval. The cellular mechanism involved in this phenomenon will be an area of future research. These studies, as well as those that explore the potential contribution of the DH to memory retrieval and destabilization, may generate information that is relevant for the therapeutic management of maladaptive memories that promote drug relapse.

Treatment Group	Cocaine Intake	Active Lever Responses				Extinction Latency
		Self-administration	Extinction Day 1	Extinction Day 7	Reactivation	
APV (DH + SSTR)	26.15 ±2.47	62.08 ± 6.36	50.08 ± 13.43	7.46 ± 1.60	15.39 ±3.72	2.00 ± 0.0
Vehicle (DH + SSTR)	26.94±2.23	53.95 ± 4.70	40.73 ± 12.99	6.09 ± 2.18	19.18 ± 4.05	2.18 ± 0.18
APV (DH), no reactivation	26.58±4.15	67.05 ± 15.84	50.71 ± 7.69	12.71 ± 4.77	15.29 ± 4.17	2.86 ± 0.70
Vehicle (DH), no reactivation	30.28±5.58	54.83 ± 8.93	475.70 ± 394.41	12.9 ± 4.18	9.10 ± 2.43	2.00 ± 0.0

Table 1. Cocaine intake (mean mg/kg per session ± SEM), active lever responses (mean ± SEM), and extinction latency (mean number of days needed to reach the extinction criterion ± SEM). Active lever responses are reported for cocaine self-administration training (mean of last 3 days of training), extinction training (the first and last day of training), and for the 15-minute memory reactivation session. The extinction criterion was ≤25 active lever responses on two consecutive extinction training

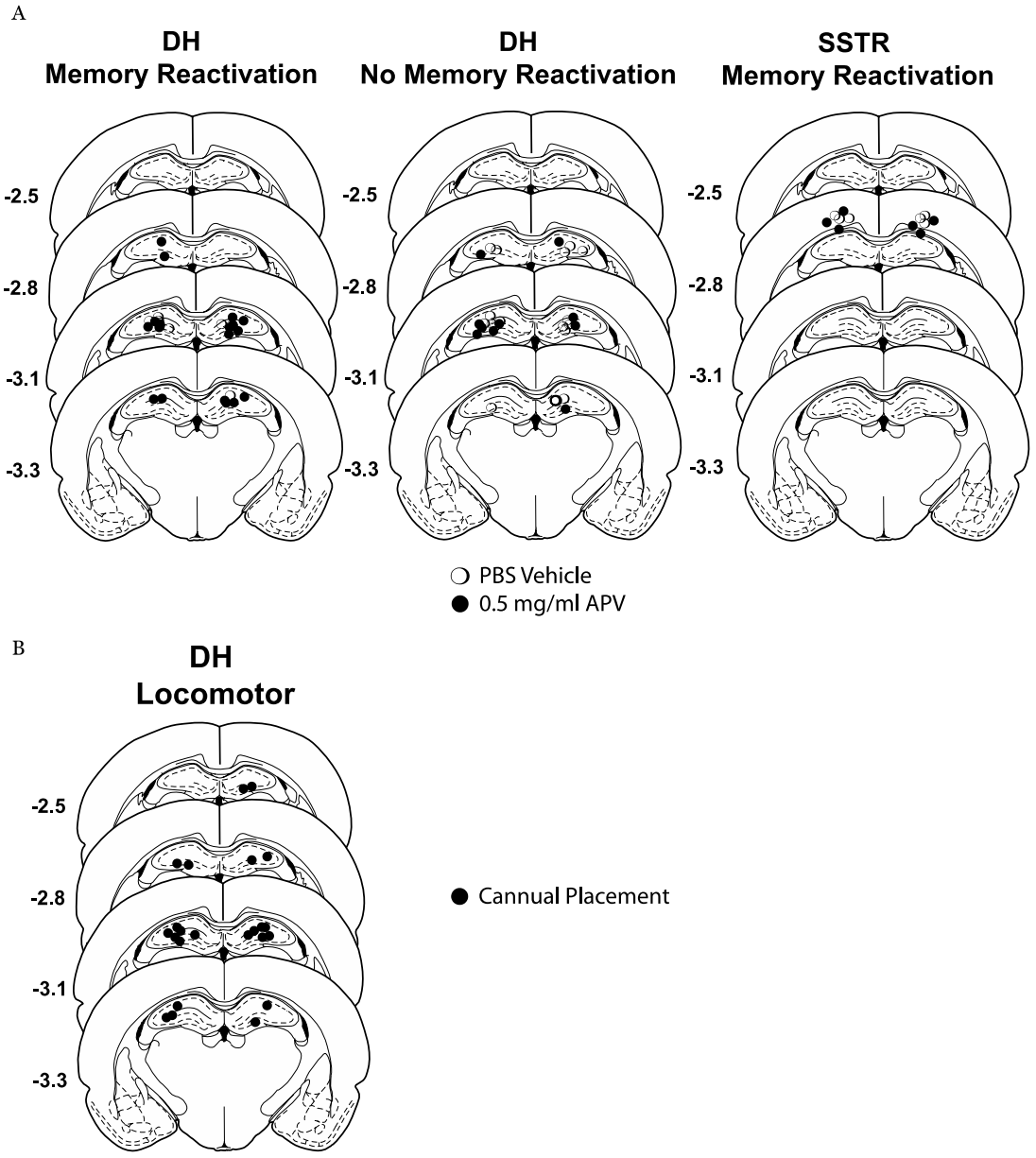

Figure 1. Schematics depicting cannula placement into the dorsal hippocampus (DH) and somatosensory cortex trunk region (SSTR). A. Cannula placements for Experiment 1a-c. The symbols denote the most ventral point of the injection cannulae tracks for rats that received bilateral microinfusions of vehicle (VEH; *open circles*) or (2*R*)-amino-5-phoshonovaleric acid (APV; *closed circles*). B. Cannula placements for Experiment 2. The symbols denote the most ventral portion of the cannulae tracts for rats that received bilateral microinfusions of VEH or APV. Numbers indicate the distance from bregma in mm, as specified in the rat brain atlas of Paxinos and Watson (1997).

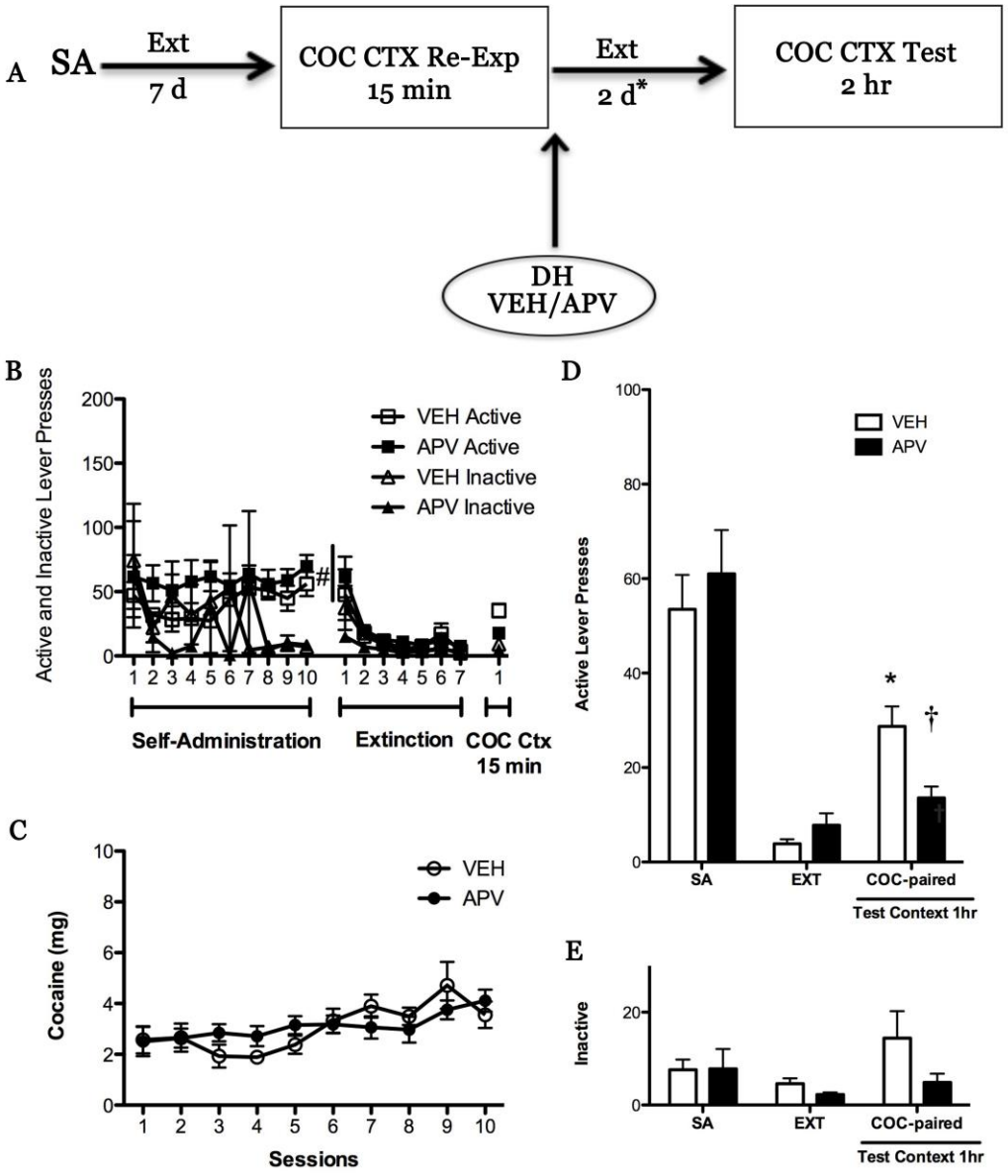

Figure 2. NMDA receptor antagonism following memory retrieval of a context-drug associative memory alters subsequent cocaine seeking behaviors. A. Schematic depicting timeline for experiment 1a. Abbreviations: self-administration training (SA), extinction training (Ext), preciously cocaine-paired context (COC CTX). B. Mean (± SEM) lever responses (active and inactive) over SA, Ext and reactivation sessions. C. Mean (± SEM) cocaine (mg) intake across sessions. D. Mean (± SEM) active lever responses during SA (mean of last 3 sessions of training) and during tests of cocaine seeking behavior in the extinction context (last day before test in the cocaine-paired context) and test of cocaine seeking in the cocaine context (COC-paired). E. Mean (± SEM) inactive lever presses. *Asterisks* represent significant difference relative to responding in the extinction context (ANOVA context simple main effect, $p < 0.05$). *Dagger* represents significant difference relative to VEH treatment (ANOVA treatment simple main effect, $p < 0.05$). *Pound sign*

represents significant difference relative to all other time intervals (ANOVA time simple main effects, $p < 0.05$).

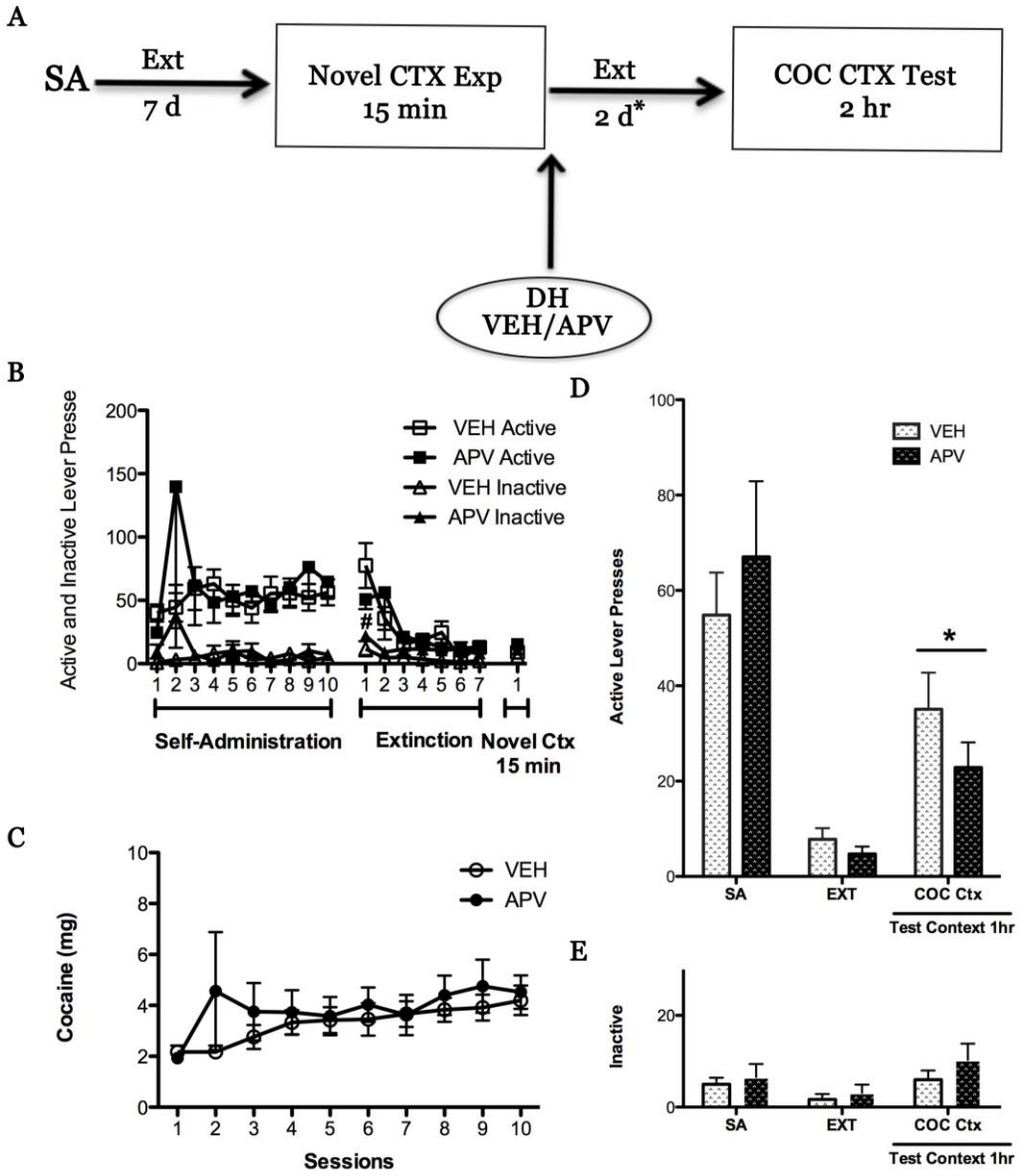

Figure 3. NMDA receptor antagonism in the DH is dependent on the memory retrieval of the cocaine-context associated memory. A. Schematic depicting timeline for experiment 1a. Abbreviations: self-administration training (SA), extinction training (Ext), preciously cocaine-paired context (COC CTX). B. Mean (± SEM) lever responses (active and inactive) over SA, Ext and reactivation sessions C. Mean (± SEM) cocaine (mg) intake across session. D. Mean (± SEM) active lever responses during SA (mean of last 3 sessions of training) and during tests of cocaine seeking behavior in the extinction context (last day before test in the cocaine-paired context) and test of cocaine seeking in the cocaine context (COC-paired). E.

Mean (± SEM) inactive lever presses. *Asterisks* represent significant difference relative to responding in the extinction context (ANOVA context simple main effect, $p < 0.05$).

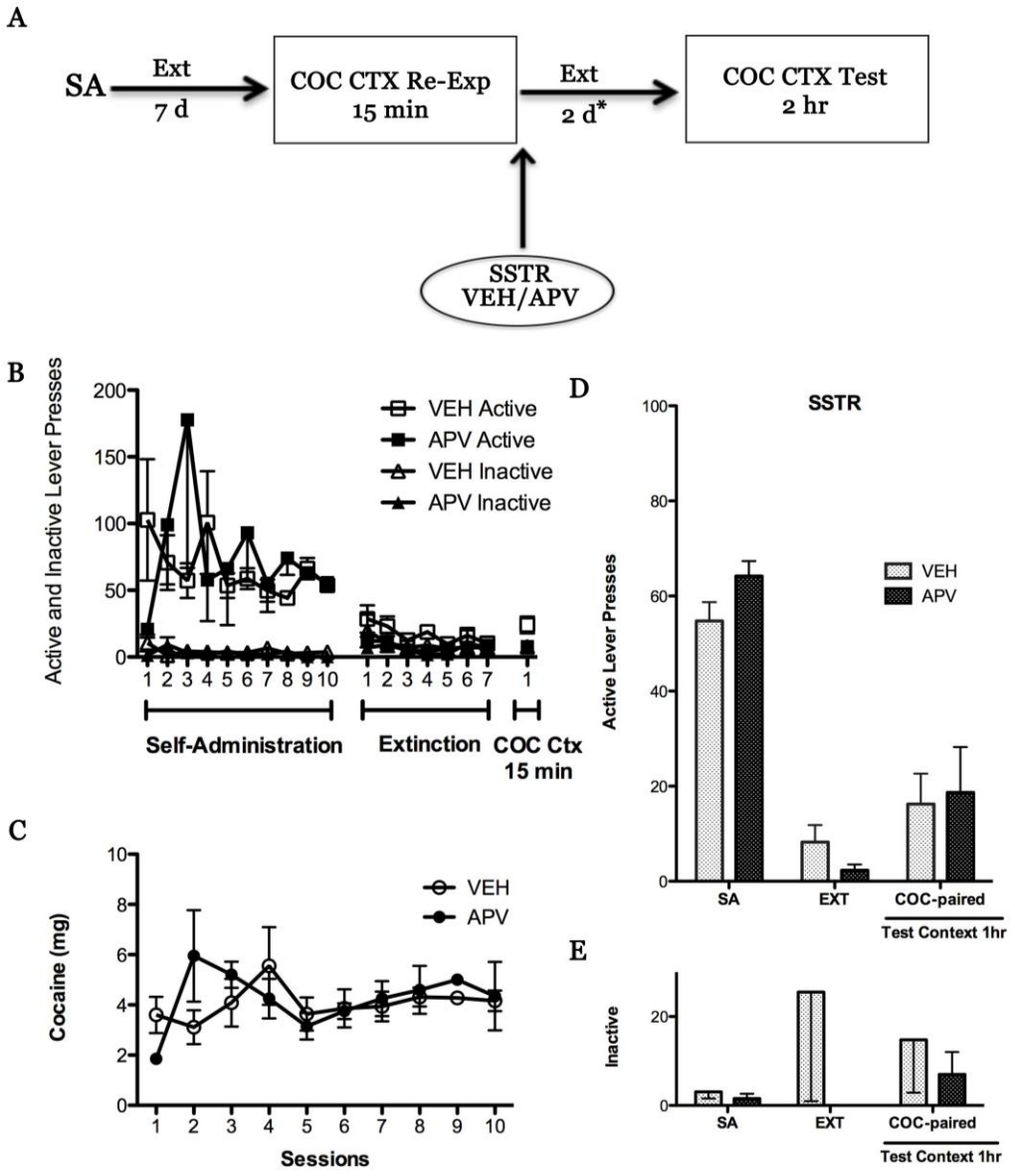

Figure 4. NMDA receptor antagonism in the SSTR directly after cocaine-context associated memory retrieval did not alter subsequent cocaine seeking behaviors. A. Schematic depicting timeline for experiment 1a. Abbreviations: self-administration training (SA), extinction training (Ext), preciously cocaine-paired context (COC CTX). B. Mean (± SEM) lever responses (active and inactive) over SA, Ext and reactivation sessions C. Mean (± SEM) cocaine (mg) intake across session. D. Mean (± SEM) active lever responses during SA (mean of last 3 sessions of training) and during tests of cocaine seeking behavior in the extinction context (last day before test in the cocaine-paired context) and test of cocaine seeking in the cocaine context (COC-paired). E. Mean (± SEM) inactive lever presses. *Asterisks* represent significant difference relative to responding in the extinction context (ANOVA context simple main effect, $p < 0.05$).

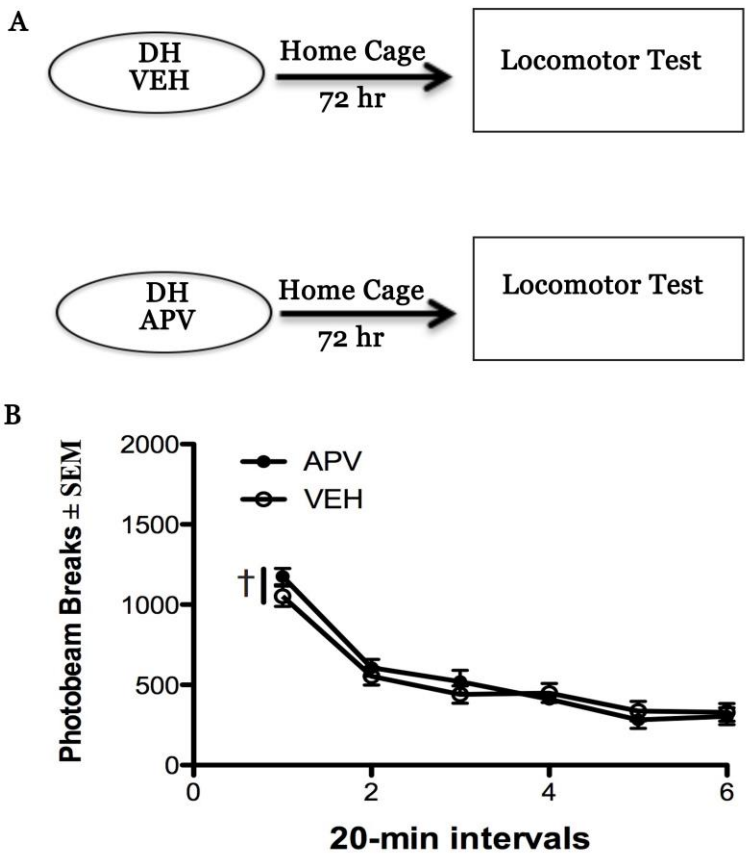

Figure 5. NMDA receptor blockade in the dorsal hippocampus does not alter general motor activity relative to control animals. A. Schematic depicting Experiment 2. 5 Animals received intra-DH infusions of VEH and APV in a counterbalanced order. An additional 9 animals received either VEH or APV, and only one test of locomotion. Animals were injected with either VEH or APV and returned to their home cage for 72 hours and then given a test of locomotion. There was no significant difference in the groups on locomotor activity determined by photo beam breaks (N_{VEH}=9, N_{APV}=10). There was a significant main effect by time ($F(1,17)=471.94$, $p<0.0001$, Tukey's test Interval 1>Interval 2-6), the first 20-minute interval was greater than intervals 2-6. *Dagger* represents significant difference relative to all other time intervals (ANOVA time simple main effects, $p < 0.05$).

REFERENCES

Alaghband Y, O'Dell SJ, Azamia S, Khalaj AJ, Guzowski JF, & Marshall JF (2014). Retrieval-induced NMDA receptor-dependent Arc expression in two models of cocaine-cue memory. *Neurobiol Learn Mem* 16:79-89 [epub ahead of print]

Alaghband Y & Marshall JF (2013). Common influences of non-competitive NMDA receptor antagonists on the consolidation and reconsolidation of cocaine-cue memory. *Psychopharmacology(Berl)* 226(4):707-719

Boccia M, Freudenthal R, Blake M, de la Fuente V, Acosta G, Baratti C, Romano A (2007). Activation of hippocampal nuclear factor-kappa B by retrieval is required for memory reconsolidation. *J Neurosci* 27(49):13436-13445

Chen Q, He S, Hu XL, Yu J, Zhou Y, Zhen J, Zhang S, Zhang C, Duan WH, Xiong ZQ (2007). Differential roles of NR2A- and NR2B-containing NMDA receptors in activity-dependent brain0derived neurotrophic factor gene regulation and limbic epileptogenesis. *J Neurosci* 27:542-552

Chia C & Otto T (2013). Hippocampal Arc (Arg3.1) expression is induced by memory recall and required for memory reconsolidation in trace fear conditioning. *Neurobiol Learn Mem.* 106:48-55

Collingridge G (1987) Synaptic plasticity: the role of NMDA receptors in learning and memory. *Nature* 330:604-605

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). The neuropschological basis of addiction. *Brain Res Brain Res Rev, 36*(2-3), 129-138.

Foltin, R. W., & Haney, M. (2000). Conditioned effects of environmental stimuli paired with smoked cocaine in humans. [Clinical Trial Controlled Clinical Trial Research Support, U.S. Gov't, P.H.S.]. *Psychopharmacology (Berl), 149*(1), 24-33.

Fuchs, R. A., Eaddy, J. L., Su, Z. I., & Bell, G. H. (2007). Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats. [Research Support, N.I.H., Extramural]. *Eur J Neurosci, 26*(2), 487-498. doi: 10.1111/j.1460-9568.2007.05674.x

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., & See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. [Research Support, U.S. Gov't, P.H.S.]. *Neuropsychopharmacology, 30*(2), 296-309. doi: 10.1038/sj.npp.1300579

Furkukawa H, Singh SK, Mancusso R & Gouaux E (2005). Subunity arrangement and function in NMDA receptors. *Nature* 438:185-192

Garcia-Delatorre P, Perez-Sanchez C, Guzman-Ramos K & Bermudez-Rattoni F (2014). Role of glutamate receptors of central and basolateral amygdala nuclei on retrieval and reconsolidation of taste aversion memory. *Neurobiol Learn Mem.* 111:35-40

Hellemans KG, Everitt BJ & Lee JL (2006). Disrupting reconsolidation of conditioned withdrawal memories in the basolateral amygdala reduces supression of heroin seeking in rats. *J Neurosci* 26(49): 12694-12699

Johnson JW & Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature*, 325:529-531

Kantak, K. M., Black, Y., E., V., Green-Jordan, K., & Eichenbaum, H. B. (2002). Dissociable effects of lidocaine inactivation on the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. *Journal of Neuroscience, 22*, 1126-1136.

Kelley, J. B., Anderson, K. L., & Itzhak, Y. (2007). Long-term memory of cocaine-associated context: disruption and reinstatement. *Neuroreport, 18*(8), 777-780.

Kelly A, Laroche S, & Davis S (2003). Activation of mitogen-activated protein kinsase/extracellular signal-regulated kindase in hippocampal circuitry is requred for consolidation and reconsolidation of recognition memory. *J of Neuro* 23(12):5354-5360

Lasseter, H. C., Wells, A. M., Xie, X., & Fuchs, R. A. (2011). Interaction of the basolateral amygdala and orbitofrontal cortex is critical for drug context-induced reinstatement of cocaine-seeking behavior in rats.. *Neuropsychopharmacology, 36*(3), 711-720.

Lee, JL, Everitt, BJ, & Thomas, KL (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science, 304*(5672), 839-843.

Lee, JL, & Hynds, RE(2013). Divergent Cellular Pathways of Hippocampal Memory Consolidation and Reconsolidation. *Hippocampus*,23(3):233-244

Lee JL, Milton AL, & Everitt BJ (2006). Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. *J Neurosci*

Lee, JL, Milton, AL, & Everitt, BJ(2006a). Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. [Research Support, Non-U.S. Gov't]. *J Neurosci, 26*(22), 5881-5887. doi: 10.1523/JNEUROSCI.0323-06.2006

Lee, JL, Milton, AL, & Everitt, BJ(2006b). Reconsolidation and extinction of conditioned fear: inhibition and potentiation. [Research Support, Non-U.S. Gov't]. *J Neurosci, 26*(39), 10051-10056. doi: 10.1523/JNEUROSCI.2466-06.2006

Liddie S & Itzhak Y (2014). Variations in the stimulus salience of cocaine reward influences drug-associated contextual memory. *Addiction Biology* [epub ahead of print]

Liu YN, Yang X, Suo ZW, Xu YM & Hu XD (2014). Fyn kinase-regulated NMDA receptor- and AMPA receptor-dependent pain sensitization in spinal dorsal horn of mice. *Eur J Pain, 18*(8):1120-1128

Mayer M, Westbrook GL & Guthrie PB (1984). Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309: 261-263

Meil WM, & See RE (1997). Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. [Research Support, Non-U.S. Gov't]. *Behav Brain Res, 87*(2), 139-148.

Milton AL, Lee JL, Butler VJ, Gardner R & Everitt BJ (2008). Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired drug-seeking behaviors. *J Neurosci* 28(33):8230-8237

Milton AL, & Everitt BJ(2010). The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *Eur J Neurosci, 31*, 2308-2319.

Monyer H, Nail B, Laurie DJ, Sakmann B, and Seeburg PH (1994). Developmental and Regional Expression in the Rat Brain and Functional Properties of Four NMDA Receptors. *Neuron* 12:529-540

Nader K, Schafe GE, & LeDoux JE(2000a). The labile nature of consolidation theory. *Nat Rev Neurosci, 1*(3): 216-219

Nader K, Schafe GE, Le Doux JE (2000b). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797):722-726

Nicolle MM, Bizon JL, & Gallagher M (1996). *In vitro* autoradiography of ionotropic glutamate receptors in hippocampus and striatum of aged Long-Evans rats: relationship to spatial learning. *Neuroscience 74*(3): 741-756

Pandis C, Sotiriou E, Kouvaras E, Asprodini E, Papatheodoropoulos C, & Angelatou F. Differential expression of NMDA and AMPA receptor subunits in rat dorsal and ventral hippocampus. *Neuroscience, 140(*1): 163-175

Praxinos G & Watson C (1997). The rat brain in stereotaxic coordinates. Academic Press New York, NY

Prybylowski K, Chang K, Sans N, Kan L, Vicini S, Wenthold RJ (2005). The synaptic localization of NR2B-containing NMDA receptors is controlled by interactions with PDZ proteins and AP-2. *Neuron* 47:845-857

Ramirez, DR, Bell, GH, Lasseter, HC, Ziaohu, X, Traina, SA, & Fuchs, R. A. (2009). Dorsal hippocampal regulation of memory reconsolidation processes that facilitate drug context-induced cocaine-seeking behavior in rats. *Eur J Neurosci, 30*: 901-912.

Roche KW, Standley S, McCallum J, Dune Ly C, Ehlers MD, Wenthold RJ (2001). Molecular determinants of NMDA receptor internalization. *Nat Neurosci* 4:794-802

Sanchez, H, Quinn, JJ, Torregrossa, MM, & Taylor, JR (2010). Reconsolidation of a cocaine-associated stimulus requires amygdalar protein kinase A. *J Neurosci, 30*(12), 4401-4407

Suzuki, A.,Josselyn, SA, Frankland, PW, Masushige, S, Silva, SJ, & Kida, S (2004). Memory Reconsolidation and Extinction Have Distinct Temporal and Biochemical Signatures. *The Journal of Neuroscience, 24*(20), 4787-4795.

Tedesco V, Mutti A, Auber A, & Chiamulera C. Nicotine-seeking reinstatement is reduced by inhibition of instrumental memory reconsolidation. *Behav Pharmacol, 25*(8):725-31

Tronson, N. C., & Taylor, J. R. (2007). Molecular mechanisms of memory reconsolidation. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S. Review]. *Nat Rev Neurosci, 8*(4), 262-275.

Valjent E, Corbille AG, BertranGonzalez J, Herve D, & Girault JA (2014). Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *PNAS* 103(8):2932-2937

Wells, A. M., Arguello, A. A., Xie, X., Blanton, M. A., Lasseter, H. C., Reittinger, A. M., & Fuchs, R. A. (2012). 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*.

Wells, A. M., Lasseter, H. C., Ziaohu, X., Cowhey, K. E., Reittinger, A. M., & Fuchs, R. A. (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. *Learning and Memory, 18*, 693-702.

Wu, Y., Yonghui L., Gao J., & Sui N. (2012). Differential effect of NMDA receptor antagonist in the nucleus accumbens on reconsolidation of morphine-related positive and aversive memory in rats. *European Journal of Pharmacology, 674*, 321-326.