Dorsal Hippocampus Inhibition Disrupts Acquisition and Expression, but Not Consolidation, of Cocaine Conditioned Place Preference

Ryan A. Meyers, Arturo R. Zavala, Colenso M. Speer, and Janet L. Neisewander
Arizona State University

Cocaine abusers may experience drug craving upon exposure to environmental contexts where cocaine was experienced. The dorsal hippocampus (DHC) is important for contextual conditioning, therefore the authors examined the specific role of the DHC in cocaine conditioned place preference (CPP). Muscimol was used to temporarily inhibit the DHC and was infused before conditioning sessions or tests for CPP to investigate acquisition and expression of cocaine CPP, respectively. To investigate consolidation, rats received intra-DHC muscimol either immediately or 6 hr after conditioning sessions. Inhibition of DHC, but not the overlying cortex, disrupted acquisition and expression of cocaine CPP. It is interesting to note that there was no effect of postconditioning DHC inhibition. The findings suggest that the DHC is important for both acquisition and recall, but not consolidation, of context–cocaine associations.

Keywords: hippocampus, drug abuse, cocaine-seeking behavior, memory, incentive motivation

Stimuli present during drug administration, such as drug paraphernalia or the environment in which the drug was taken, can acquire incentive motivational effects that may manifest as drug craving and may contribute to relapse (Childress, Ehrman, McLellan, & O'Brien, 1988). Drug-paired environmental stimuli are thought to acquire such incentive motivational effects through associative processes (Stewart, 1983), and this has been studied in animals by using the conditioned place preference (CPP) model (Tzschentke, 1998). To establish CPP, a rewarding unconditioned stimulus (US), such as food or cocaine, is paired with exposure to a distinct environment (place), whereas another environment is paired with saline. During conditioning, the drug-paired environment acquires incentive motivational effects through association with drug reward. Subsequently, these effects are reflected as a shift in preference for the drug-paired environment on the test day relative to a preconditioning baseline (Bardo & Bevins, 2000).

The dorsal hippocampus (DHC) plays a role in the mechanisms underlying CPP. For example, we have previously shown that excitotoxic lesions of the DHC performed prior to conditioning disrupt cocaine CPP (Meyers, Zavala, & Neisewander, 2003), consistent with the disruptive effects of preconditioning DHC lesions on food CPP (Ferbinteanu & McDonald, 2001). Disruption of cocaine CPP by DHC lesions is unlikely to result from nonspecific behavioral disruption, because we have shown that locomotion during testing for CPP is not altered in lesion animals, suggesting intact exploratory behavior that is needed to express CPP

(Meyers et al., 2003). Nevertheless, the specific role of the DHC in cocaine CPP remains unclear but could involve disruption of cocaine reward, the association of reward with the environment, and/or recall of the association.

Several studies support the notion that the DHC is involved in reward. For instance, the DHC supports intracranial self-stimulation (Collier & Routtenberg, 1984; Ursin, Ursin, & Olds, 1966), which is thought to measure central reward pathways. In addition, microinjection of morphine into the DHC establishes CPP (Corrigall & Linseman, 1988; Olmstead & Franklin, 1997; Wise, 1989), suggesting a role for the DHC in morphine reward. Moreover, inactivation of the dorsal subiculum, a major output structure of the DHC (Amaral & Witter, 1995), regulates cocaine self-administration (Black, Green-Jordan, Eichenbaum, & Kantak, 2004), suggesting a role in cocaine reinforcement. Collectively, these findings suggest the DHC contributes to reward processes.

DHC manipulations are known to alter acquisition of context-conditioned fear, which, together with findings on acquisition of CPP, suggests the DHC plays a more general role in learning associations between a US and the context in which it occurs. For instance, the hippocampal formation is necessary for aversive conditioning to contextual, but not discrete, conditioned stimuli (CSs; Maren, 2001; Phillips & LeDoux, 1992; Selden, Everitt, Jarrard, & Robbins, 1991). Furthermore, preconditioning manipulations of the DHC disrupt acquisition of contextual fear conditioning (Bast, Zhang, & Feldon, 2003; Kim, Rison, & Fanselow, 1993; Maren, Aharonov, & Fanselow, 1997). For example, either preconditioning electrolytic lesions of the DHC (Kim et al., 1993) or infusions of the glutamate N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, into the DHC (Bast et al., 2003) prior to conditioning trials disrupts acquisition of contextual fear conditioning. Preconditioning DHC infusions of dopamine D1 or D2 receptor agonists enhance acquisition of morphine CPP (Rezayof, Zarrindast, Sahraei, & Haeri-Rohani, 2003), which may reflect facilitation of learning and/or morphine reward.

The DHC also serves an important role in consolidation of context–US associations. In animals, consolidation is typically studied via postconditioning manipulations given during a period in which memories progress from short-term to long-term memory (McGaugh, 2000). Postconditioning DHC manipulations alter consolidation of inhibitory avoidance and contextual fear conditioning (Anagnostaras, Gale, & Fanselow, 2001; Fanselow, 2000; Izquierdo & Medina, 1997; Morris et al., 2003). For example, immediate (< 30 min), but not delayed (6 hr), posttraining infusion of the gamma-aminobutyric acid (GABA) receptor agonist, muscimol, disrupts memory consolidation for inhibitory avoidance training (Izquierdo et al., 1992; Rossato et al., 2004; Zanatta et al., 1997). In contrast, manipulations of monoaminergic transmission in the DHC are ineffective when administered immediately after training, but delayed postconditioning administration (3 to 6 hr) produces retrograde memory deficits (Bernabeu et al., 1997; Bevilaqua et al., 1997), suggesting that different neural systems may be engaged in the DHC during different phases of consolidation (Morris et al., 2003). The role of the DHC in consolidation of drug-induced CPP has not yet been explored.

Finally, the DHC has been implicated in processing recall of context-based memories. In particular, lesions of the DHC made after conditioning and prior to testing disrupt expression of context-elicited cocaine-seeking behavior (Fuchs et al., 2005) and context-conditioned fear (Kim & Fanselow, 1992; Maren et al., 1997; Maren & Fanselow, 1997). Furthermore, pretest intra-DHC microinjections of dopamine D1 or D2 receptor antagonists prior to testing impair expression of morphine CPP (Rezayof et al., 2003). Thus, an intact DHC is likely necessary for recall of associations between a US and the contextual CS in which it occurs.

The purpose of this study was to examine the specific role of the DHC in cocaine CPP. To this end, the DHC was temporarily inhibited with an amnestic dose (Zarrindast, Bakhsha, Rostami, & Shafaghi, 2002) of the GABA-A agonist, muscimol, either prior to conditioning sessions to examine acquisition, before testing for cocaine CPP to examine expression of cocaine CPP, or after conditioning sessions to examine consolidation of cocaine CPP. Postconditioning DHC inhibition occurred either immediately or 6 hr after each conditioning session.

Method

Subjects

Male Sprague–Dawley rats (Charles River, Raleigh, NC) weighing 275–300 g at the time of surgery were housed individually under a 12:12-hr light–dark cycle (lights off at 6 a.m.). Behavioral testing was conducted during the dark cycle. Prior to surgery, rats were acclimated to handling for at least 5 days. Housing facilities and animal care were in accordance with the conditions set forth in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH, 1996).

Surgery

Rats were pretreated with atropine sulfate (10 mg/kg, ip; Sigma, St. Louis, MO) and then anesthetized 5 min later with Nembutal (50 mg/kg, ip; Abbot Laboratories, Chicago). Following placement into a stereotaxic instrument, a midline incision was made through the clean shaven scalp, the tissue was reflected back, and four small jeweler's screws were placed into the skull. With the skull set flat (e.g., bregma level with lambda),

bilateral 23-gauge guide cannula aimed at the DHC (−3.8 AP, ±2.5 ML, −2.5 DV) or overlying cortex (−3.8 AP, ±2.5 ML, −0.5 DV) were implanted and secured to the skull and the screws with dental acrylic. Coordinates are given relative to bregma and were adapted from previous work by Holt and Maren (1999) and using the atlas of Paxinos and Watson (1998). Following surgery, wire stylets were inserted into the guide cannula to maintain patency.

Apparatus

Conditioning took place in Plexiglas chambers divided into two equal compartments ($36 \times 24 \times 30$ cm each) by a removable partition. One compartment had three white walls and a wire mesh floor above pine shavings. The other compartment had three black walls and a bar-grid floor above cedar shavings. Fluorescent lights were situated 32 cm above the black compartment to balance light intensity across the two sides of the chamber. Previous work from our laboratory has shown that rats do not express a strong bias for a particular side, with roughly half preferring the white compartment and half preferring the black compartment (O'Dell, Khroyan, & Neisewander, 1996). The front wall of both sides of the apparatus was clear Plexiglas to allow for observation during testing. On test days, the removable partition was replaced with a partition containing an opening (8×12 cm) to allow free access to both compartments. Each side of the chambers was equipped with two sets of photobeam emitters and detectors located 27 cm apart and 3 cm above the floor. During the acquisition experiment, a computer relay system recorded consecutive beam breaks, which occurred when rats crossed 27 cm from one side of a compartment to the other. This measure of locomotion is referred to as *crosses*. For expression experiments, the number of times a rat shuttled from one compartment to the other on the test day (i.e., *crossovers*) was used as a measure of locomotion.

Baseline Preference

Baseline preferences were assessed in a series of three daily tests where rats were placed into one of the compartments (half in white and half in black) and given free access to both sides of the apparatus for 15 min. Entry into a compartment was operationally defined as the rats' two front paws touching the floor of the respective compartment. The amount of time rats spent in each compartment was averaged across tests, and the side in which rats spent the least amount of time on average was designated as the rats' initially nonpreferred side.

Microinjection Procedure

Rats were habituated to the microinjection procedure 24 hr after their last baseline preference test (i.e., Day 4). A 30-gauge injection cannula was placed into each of the bilateral guide cannulas to a depth 1–2 mm beyond the tip of the guide. Injection cannulas were connected via polyethylene-10 tubing to 10 μl Hamilton syringes mounted on a syringe pump (World Precision Instruments, Sarasota, FL). Once the injection cannulas were inserted, 0.6 μl phosphate-buffered saline (PBS) was infused over 1 min. Movement of a small air bubble in the tubing to the appropriate distance was used to verify that the accurate volume had been infused. The injection cannulas were left in place for an additional minute after infusion to allow for diffusion away from the tip. Following successful infusion, injection cannulas were removed and replaced with wire obturators, and the rat was returned to its home cage. The same microinjection procedure was used during other phases of the experiments to deliver infusions of either vehicle or muscimol.

Conditioning

Conditioning began 48 hr after habituation to the microinjection procedure. Conditioning took place in four sessions on Days 6, 8, 10, and 12

with a rest day intervening on Days 7, 9, 11, and 13. On Day 6, half of the rats received either an injection of cocaine-HCl (15 mg/kg ip; RTI International, Research Triangle Park, NC) immediately prior to placement into their initially nonpreferred (i.e., drug-paired) side, and half received an equal volume of saline (1 ml/kg) prior to placement into their initially preferred side. Rats remained confined to the compartments for 30 min. Day 7 was a rest day during which rats were left undisturbed in their home cage, except for brief handling. On Day 8, rats received the opposite treatment from Session 1 (i.e., those that received cocaine paired with their nonpreferred environment received saline paired with their preferred environment, etc.). Day 9 was another rest day. This exact same sequence was repeated across Days 10–13. Rest days occurred between the four conditioning days in order to reduce possible residual effects of repeated intracranial injections of muscimol. Cocaine was paired with the rats' initially nonpreferred side in order to increase sensitivity for detecting a shift in preference. It is important to note that CPP in controls was evident as > 50% (> 450 s) of the total test time spent in the cocaine-paired side on average, suggesting a true preference for the drug-paired side. Thus disruption of acquisition and expression of CPP reported herein reflects a reduction in preference.

CPP Test

On Day 14, CPP was assessed by allowing rats free access to both sides of the apparatus for 15 min. Entry into a compartment was operationally defined as the rats' two front paws touching the floor of the compartment, and time spent in each side of the chamber was recorded by an observer blind to the rats' treatment conditions. Most of the rats were retested for CPP on Days 17 and 20 with rest days between these tests.

Specific Experiments

Table 1 presents an outline of the specific manipulations performed to investigate acquisition, consolidation, and expression of cocaine CPP in this study. A high, amnestic dose of muscimol (Zarrindast et al., 2002) was used to temporarily inhibit neurotransmission in the DHC in each experiment. Although this technique may not affect all neurons within the infusion area, it offers the advantage that inhibition is temporary, thereby allowing us to target specific processes that occur at different times (Martin, 1991; Martin & Ghez, 1999).

Effects of Presession DHC Inhibition on Acquisition of Cocaine CPP

Rats were randomly assigned to groups that received either vehicle ($n = 11$) or muscimol (1 mg/0.6 ml; $n = 10$) infusions into the DHC prior to each of the four conditioning sessions. Rats were first tested for baseline preference and habituated to microinjection as described previously. The conditioning procedure began the following day. Rats received their assigned infusion pretreatment 15 to 25 min prior to their cocaine and saline treatments, the latter of which preceded placement into the appropriate compartment of the apparatus. Forty-eight hr after completing the conditioning procedure, rats were tested for CPP.

Effects of Pretesting Inhibition of the DHC on Expression of Cocaine CPP

Rats from the previous experiment examining the effects of preconditioning DHC inhibition that had been assigned to the vehicle pretreatment group and that exhibited CPP on the test day (> 450 s in the drug-paired side) were used in this experiment ($n = 8$). On Day 17, all of these rats were pretreated with muscimol (1 mg/0.6 ml), and then retested for CPP 15–25 min later. On Day 20, the rats were pretreated with vehicle (0.6 ml)

microinjected into the DHC, and were then tested a third time for CPP 15 to 25 min later.

Effects of Postsession Inhibition of the DHC on Consolidation of Cocaine CPP With 30-min Conditioning Sessions

Rats were randomly assigned to groups that received bilateral vehicle ($n = 7$–8) or muscimol (1 μg/0.6 μl; $n = 8$–9) infusions into the DHC within 3 min (referred to as *immediate*) or 6 hr after completing the 30-min session on each conditioning day. Microinjection procedures were performed in a different room located close to the colony room. Following microinjection, rats were returned to their home cages in the colony. Except for the timing of the microinfusion, the procedures in this experiment were identical to our previous experiment investigating the effects of preconditioning DHC inhibition on acquisition of cocaine CPP. Immediate or 6-hr delayed infusions were selected because we had predicted that immediate, but not 6-hr delayed, infusion of muscimol would disrupt consolidation, which is consistent with previous research that has used DHC muscimol infusions to examine inhibitory avoidance (Izquierdo et al., 1992).

Effects of Postsession Inhibition of the DHC on Consolidation of CPP With 15-min Conditioning Sessions

Because postsession inhibition of the DHC in the previous experiment failed to affect acquisition of cocaine CPP, this experiment tested whether involvement of the DHC in consolidation of cocaine CPP is more *time limited* (i.e., occurs early during the conditioning session). If this is the case, then our manipulation must occur earlier in order to disrupt consolidation. Thus, in this experiment, conditioning sessions lasted only 15 min. This time frame, and not shorter time frames, was elected due to previous work demonstrating that strength of conditioning for cocaine CPP is reduced by conditioning sessions lasting less than 30 min (Bardo, Rowlett, & Harris, 1995). Within 3 min after these 15-min sessions, rats were microinjected bilaterally with either vehicle (PBS, 0.6 ml; $n = 9$) or muscimol (1 mg/0.6 ml; $n = 7$) as described previously. Rats were placed into their home cages and returned to the colony following microinjection. All other procedures were identical to the previous experiment.

Replication of the Effects of Pretesting Inhibition of the DHC on Expression of Cocaine CPP as a Manipulation Check

Because muscimol treatment after a 15-min conditioning session failed to affect acquisition of cocaine CPP, as a manipulation check we examined whether these same rats pretreated with muscimol prior to testing would fail to express CPP as demonstrated previously. Rats from our previous experiment that exhibited CPP for the drug-paired side on their test day (> 450 s) were used as subjects in this experiment ($n = 8$) and received microinjections of vehicle or muscimol counterbalanced for order prior to retesting for CPP. Seventy-two hours after their initial CPP test, half of the rats were microinjected with vehicle (PBS; 0.6 μl) and half were microinjected with muscimol (1 μg/0.6 μl) 15–25 min prior to retesting for expression of CPP. Seventy-two hr later, rats were retested a second time for CPP following the pretreatment opposite to that given on the previous test.

Effects of Presession Inhibition of the Cortex Overlying the DHC on Acquisition of Cocaine CPP

To determine the anatomical specificity of results observed in the acquisition experiment, we examined the effects of preconditioning inhi-

Table 1
Timeline of Experimental Manipulations by Cohort

Day	Experimental phase	Manipulations		
		Cohort 1	Cohorts 2 and 3	Cohort 4
1–3	Baseline tests			
4	Microinfusion of vehicle			
5, 7, 9, 11, 13	Intervening rest days			
6, 10	Conditioning sessions and specific pairing conditions: (a) saline/initially preferred side (b) cocaine/initially nonpreferred side	Acquisition DHC manipulations given to the following groups 15–25 min prior to all 30-min conditioning sessions: (1) vehicle ($n = 11$) (2) muscimol ($n = 10$)	Consolidation DHC manipulations given to the groups ($n = 7$–9) below after all 30-min conditioning sessions (Cohort 2): (1) vehicle immediately after all sessions (2) muscimol immediately after all sessions (3) vehicle 6 hr delayed (4) muscimol 6 hr delayed or after all 15-min conditioning sessions (Cohort 3): (5) vehicle immediately after sessions (6) muscimol immediately after sessions	Acquisition Cortex overlying DHC manipulations given to the following groups 15–25 min prior to all 30-min conditioning sessions: (1) vehicle ($n = 10$) (2) muscimol ($n = 12$)
8, 12	Conditioning sessions with the opposite pairings from those above (i.e., animals that received [a] now receive [b], and vice versa)			
14	Initial test for CPP			
15, 16, 18, 19	Intervening rest days			
17	Second CPP test	Expression DHC manipulation given to animals from group 2 only with initial CPP > 450 s ($n = 8$): muscimol 15–25 min prior to testing	Expression DHC manipulation given to animals with initial CPP > 450 s from Cohort 3 only ($n = 8$): (a) muscimol 15–25 min prior to testing (b) vehicle 15–25 min prior to testing	Expression DHC or cortex overlying the DHC manipulation given to animals with initial CPP > 450 s ($n = 10$): (a) DHC muscimol 15–25 min prior to testing (b) Cortex overlying DHC muscimol 15–25 min prior to testing
20	Third CPP test	Expression DHC manipulation given to same animals as Day 17: vehicle 15–25 min prior to testing	Expression manipulation opposite that given on Day 17 (i.e., animals that received [a] now received [b]).	Expression manipulation opposite that given on Day 17 (i.e., animals that received [a] now receive [b]).

Note. The dose of cocaine HCl was 15 mg/kg ip, and the dose of muscimol was 1 μg/side. DHC = dorsal hippocampus; CPP = conditioned place preference.

bition of the cortex overlying the DHC on acquisition of cocaine CPP. Rats were randomly assigned to groups that received either vehicle ($n = 10$) or muscimol (1 μg/0.6 μl; $n = 12$) infused bilaterally into the cortex overlying the DHC 15–25 min prior to placement into the apparatus for each conditioning session. They were then conditioned and tested by using the same procedures as previous experiments.

Effects of Pretesting Inhibition of Cortex Overlying the DHC on Expression of Cocaine CPP

To determine the anatomical specificity of results observed in the expression experiment, we compared the effects of pretesting inhibition of the cortex overlying the DHC or the DHC itself on expression of cocaine CPP. Rats underwent the conditioning procedure described previously and received infusions prior to each session. Those exhibiting CPP for the cocaine-paired environment on their test day (> 450 s) were used as subjects in this experiment ($n = 10$). Seventy-two hr after their initial CPP test, half of the rats were pretreated with muscimol (1 μg/0.6 μl) microinjected into the DHC, and half were pretreated with muscimol (1 mg/0.6 μl) infused into the overlying cortex 15–25 min prior to retesting for expression of CPP. Seventy-two hr after this second test, rats were retested a third time for expression of CPP following the pretreatment opposite to that given on the previous test. The site of infusion was achieved by lowering the injection cannulas to the appropriate depth.

Data Analysis

CPP was defined as a significant increase in time spent in the drug-paired side postconditioning relative to preconditioning baseline. For acquisition experiments, time spent in the drug-paired side was analyzed by using a 2×2 mixed factor analysis of variance (ANOVA) with treatment condition (vehicle/muscimol) as a between-subjects factor and baseline versus test day as a within-subjects factor. In our experiment investigating the effects of immediate and 6-hr delayed postsession DHC inhibition on consolidation, we analyzed data by using a $2 \times 2 \times 2$ mixed factor ANOVA with treatment condition (vehicle/muscimol) and inhibition time (immediate/6-hr delay) as between-subjects factors and baseline versus test day as a within-subjects factor. Locomotor activity crosses during conditioning sessions in our first experiment, defined as *consecutive photobeam breaks*, were analyzed by using a 2×2 ANOVA with pretreatment condition (vehicle/muscimol) as a between-subjects variable and crosses following saline versus cocaine administration as a within-subjects variable. For expression experiments, we analyzed time spent in the drug-paired side and number of crossovers between compartments during the test for CPP by using a one-way repeated measures ANOVA of day (baseline, initial expression test, muscimol test, vehicle test). Post hoc Tukey's tests were conducted to further analyze significant main effects and interactions.

Histology

Cannula placement was determined from coronal, cresyl violet-stained 40 μm sections by an experimenter blind to the rats' behavioral performance.

Results

Histology

Figure 1A illustrates representative cannula placements in the DHC and panel B illustrates the boundaries of correct cannula placements in the targeted structure, represented by the shaded areas. Only rats with both cannula tips located in the targeted structure were included in the data analyses, with the exception of
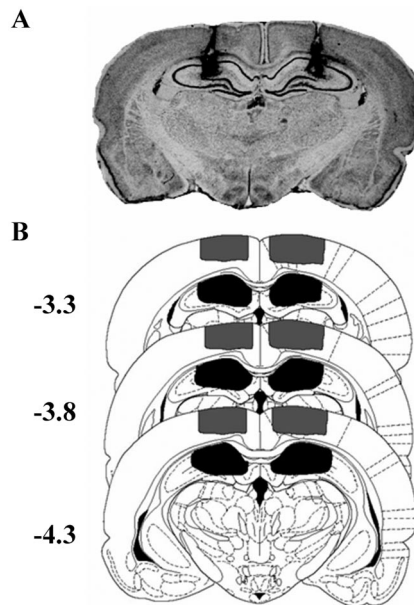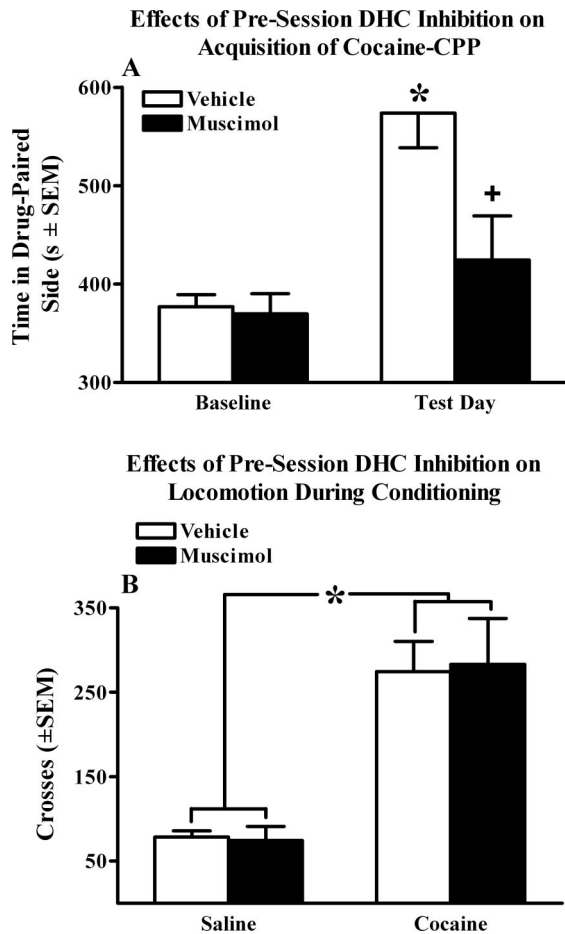

Figure 1. (A) Photomicrograph of a coronal, cresyl violet-stained section illustrating representative cannula placement into the dorsal hippocampus (DHC) and (B) schematic representation of acceptable areas of cannula placements in the DHC (solid black) and cortex (gray scale). Numbers indicate mm of each section from bregma according to the atlas of G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates* (4th ed.). Copyright 1986 by Elsevier. Adapted with permission.

one control rat assigned to the vehicle conditions in both the preconditioning and postconditioning (30-min sessions) DHC inhibition experiments, for which correct placement was unilateral with the other placement just above the dorsal border of the DHC. All ns reported previously are final ns based on the results of histology.

Effects of Presession DHC Inhibition on Acquisition of Cocaine CPP and Locomotion

A repeated measures ANOVA of time spent in the drug-paired side for rats receiving intra-DHC infusions of muscimol or vehicle prior to each conditioning session revealed a significant main effect of day, $F(1, 19) = 21.44$, $p < .05$, and a Day $\times$ Treatment interaction, $F(1, 19) = 6.81$, $p < .05$. Post hoc Tukey's tests showed that there were no group differences at baseline in the time spent in the drug-paired side, but during testing for CPP the vehicle-pretreated group spent more time in the drug-paired side relative to the muscimol-pretreated group ($p < .05$). Furthermore, only vehicle-pretreated rats exhibited an increase in time spent in the drug-paired side on the test day relative to baseline ($p < .05$). These results indicate that presession muscimol administration disrupts acquisition of cocaine CPP (see Figure 2A).

A repeated measures ANOVA of locomotor activity during conditioning sessions in this experiment indicated an effect of cocaine, $F(1, 19) = 42.61$, $p < .05$, but no Cocaine $\times$ Pretreatment interaction. These results indicate that cocaine increased locomotion and muscimol pretreatment prior to conditioning sessions failed to alter locomotion (see Figure 2B).

Effects of Pre-Session DHC Inhibition on Acquisition of Cocaine-CPP

Effects of Pre-Session DHC Inhibition on Locomotion During Conditioning

Figure 2. Effects of presession dorsal hippocampus (DHC) inhibition on (A) acquisition of cocaine conditioned place preference (CPP) and (B) cocaine-induced locomotion during conditioning. Values represent $M \pm SEM$. Respective muscimol (1 μg/0.6 μl; $n = 10$) and vehicle ($n = 11$) infusions occurred 15–25 min prior to all four conditioning sessions. Asterisks represent a significant difference from (A) respective baseline value or (B) saline condition ($p < .05$, Tukey's test). The plus sign represents a significant difference from respective vehicle control ($p < .05$, Tukey's test).
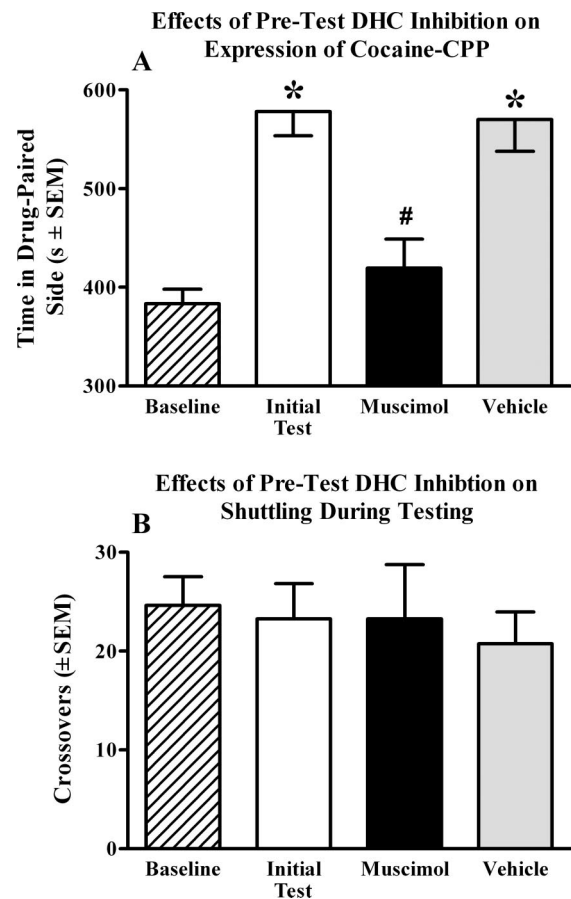
Effects of Pretesting Inhibition of the DHC on Expression of Cocaine CPP and Locomotion

A repeated measures ANOVA of time spent in the drug-paired side in rats receiving intra-DHC infusions prior to the CPP test revealed a significant main effect of test day, $F(3, 21) = 22.09$, $p < .05$, on expression of CPP. Post hoc Tukey's tests indicated that relative to the initial, drug-free test for CPP, muscimol pretreatment prior to testing for CPP decreased the amount of time spent in the drug-paired side ($p < .05$), whereas vehicle pretreatment prior to testing failed to alter the amount of time spent in the drug-paired side (see Figure 3A). Further post hoc tests revealed that time spent in the drug-paired side during both the first test for CPP and the vehicle-pretreatment test for CPP differed significantly from baseline, whereas there was no difference from baseline on the muscimol pretreatment test day ($p < .05$). A repeated

measures ANOVA of crossovers between compartments during testing for CPP revealed no effect of test day on locomotion (see Figure 3B).

Effects of Postsession Inhibition of the DHC on Consolidation of Cocaine CPP Using 30-min or 15-min Conditioning Sessions

A repeated measures ANOVA of time spent in the drug-paired side for rats undergoing 30-min conditioning sessions followed immediately or 6 hr later by intra-DHC infusions revealed a significant main effect of test day, $F(1, 28) = 36.57$, $p < .05$, but no Day × Treatment, Day × Time, or Day × Treatment × Time interactions. Similarly, a repeated measures ANOVA of time spent in the drug-paired side for rats undergoing 15-min conditioning sessions followed immediately by intra-DHC infusions revealed a

Effects of Pre-Test DHC Inhibition on Expression of Cocaine-CPP

Effects of Pre-Test DHC Inhibtion on Shuttling During Testing

Figure 3. Effects of pretesting inhibition of the dorsal hippocampus DHC on (A) expression of cocaine conditioned place preference (CPP) and (B) crossovers during testing. Values represent $M \pm SEM$. Rats ($n = 8$) were tested three times for cocaine CPP with at least 3 rest days between tests. There was no pretreatment given prior to the initial test for CPP. On the second and third tests for CPP, rats received muscimol (1 μg/0.6 μl) infusions or vehicle infusions, respectively, 15–25 min prior to testing. Asterisks represent a significant difference from baseline value ($p < .05$, Tukey's test). The pound sign represents a significant difference from both the initial and vehicle-pretreatment tests for expression of cocaine CPP ($p < .05$, Tukey's test).

significant main effect of test day, $F(1, 14) = 17.43$, $p < .05$, but no Day $\times$ Treatment interaction. In both experiments, rats exhibited an increase in time spent in the drug-paired side on the test day relative to preconditioning baseline, regardless of DHC treatment or time of treatment (see Figure 4).

Replication of the Effects of Pretesting Inhibition of the DHC on Expression of Cocaine CPP

A repeated measures ANOVA of time spent in the drug-paired side in rats receiving intra-DHC infusions prior to testing revealed a significant main effect of test day, $F(3, 21) = 10.44$, $p < .05$, on expression of CPP. Post hoc Tukey's tests indicated that muscimol pretreatment on the test day decreased the amount of time spent in the drug-paired side relative to the initial, drug-free test for CPP, whereas the vehicle pretreatment did not alter CPP ($p < .05$). Further post hoc tests revealed that both the initial test for CPP and vehicle-pretreatment test for CPP differed significantly from baseline, whereas the muscimol-pretreatment test day did not differ from baseline ($p < .05$). These results replicate our previous findings that muscimol pretreatment disrupts expression of cocaine CPP (see Figure 5).

Effects of Presession Inhibition of the Cortex Overlying the DHC on Acquisition of Cocaine CPP

A repeated measures ANOVA of time spent in the drug-paired side in rats receiving intracortical infusions prior to conditioning sessions revealed a significant main effect of test day, $F(1, 20) = 63.89$, $p < .05$, but no Day $\times$ Treatment interaction. Rats exhibited a significant increase in time spent in the drug-paired side on the test day relative to preconditioning baseline, regardless of cortex pretreatment. These results indicate that muscimol microinjected into the cortex overlying the DHC prior to conditioning sessions fails to disrupt acquisition of cocaine CPP (see Figure 6).

Effects of Pretesting Inhibition of the Overlying Cortex on Expression of Cocaine CPP

A repeated measures ANOVA of time spent in the drug-paired side in rats receiving intracranial infusions prior to testing revealed a significant main effect of test day, $F(3, 27) = 8.67$, $p < .05$, on expression of CPP. Post hoc Tukey's tests indicated that, relative to the initial test for CPP, muscimol inhibition of the DHC decreased the amount of time spent in the drug-paired side ($p < .05$), whereas similar pretreatment to the overlying cortex failed to alter expression of CPP. These results demonstrate that inhibition of the DHC, but not overlying cortex, disrupts expression of cocaine CPP (see Figure 7).

Discussion

The present study is the first to examine the specific role of the DHC in processes related to learning and memory for cocaine CPP. The findings suggest a role for the DHC in acquisition and expression, but not consolidation, of cocaine CPP. Preconditioning DHC inhibition disrupted acquisition of cocaine CPP (Figure 2A), an effect anatomically specific to the DHC because inhibition of the overlying cortex failed to produce an effect (see Figure 6).

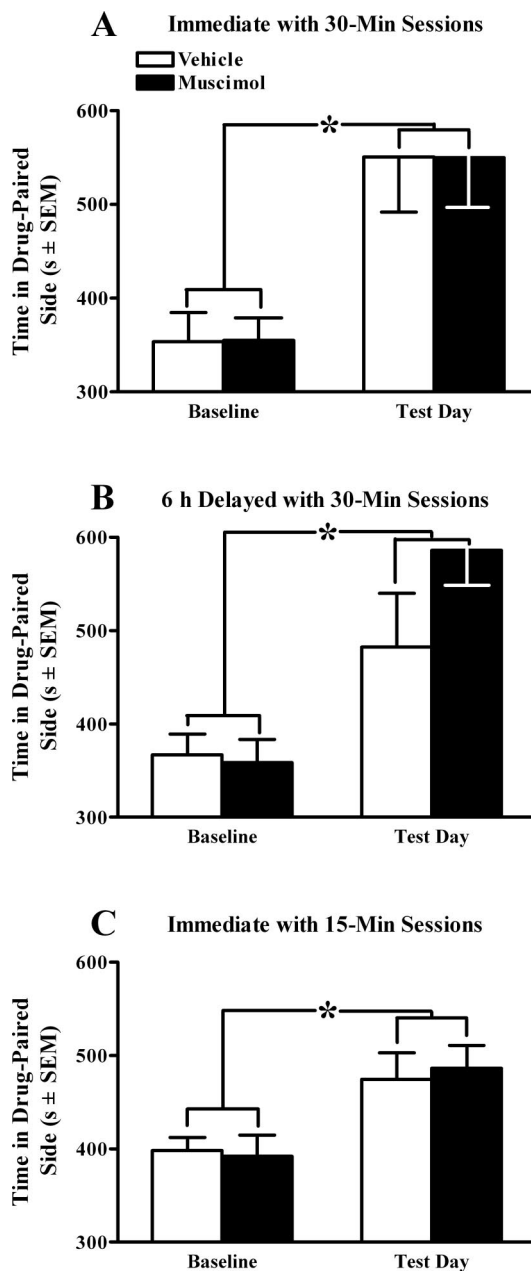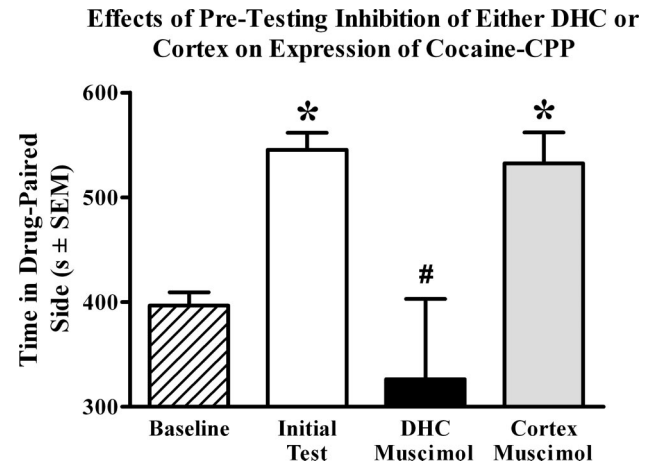**Effects of Post-Session DHC Inhibition on Consolidation of Cocaine-CPP**

Figure 4. Effects of (A) immediate or (B) 6-hr postsession dorsal hippocampus (DHC) muscimol (1 μg/0.6 μl; $n = 8$–9) or vehicle ($n = 7$–8) infusions with 30-min conditioning sessions or immediate postconditioning DHC muscimol (1 μg/0.6 μl; $n = 7$) or vehicle ($n = 9$) infusions following 15-min conditioning sessions (C) on consolidation of cocaine conditioned place preference (CPP). Asterisks represent a significant difference between baseline and postconditioning tests ($p < .05$; ANOVA main effect).

DHC inhibition did not affect cocaine-induced locomotion during conditioning (Figure 2B), suggesting the disruption of cocaine CPP was not due to a nonspecific motor effect. Pretesting DHC

Replication of Effects of Pre-Test DHC Inhibition on Expression of Cocaine-CPP
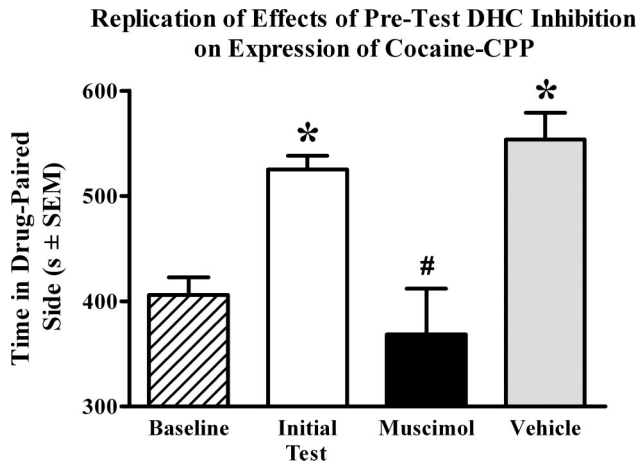
Figure 5. Replication of the effects of pretesting inhibition of the dorsal hippocampus (DHC; 1 μg/0.6 μl muscimol) on expression of cocaine conditioned place preference (CPP) in rats that had exhibited cocaine CPP in the previous experiment. Rats ($n = 8$) were tested three times for cocaine CPP with 3 rest days between tests. No pretreatment was given prior to the initial test for CPP. For the second and third tests for CPP, rats received muscimol (1 μg/0.6 μl) infusions or vehicle infusions, 15–25 min prior to testing in a counterbalanced order. Asterisks represent a significant difference from baseline ($p < .05$, Tukey's test). The pound sign represents a significant difference from both the initial and vehicle-pretreatment tests for expression of cocaine CPP ($ps < .05$, Tukey's tests).

Effects of Pre-Testing Inhibition of Either DHC or Cortex on Expression of Cocaine-CPP

Figure 7. Effects of pretesting inhibition of either the overlying cortex or dorsal hippocampus (DHC) on expression of cocaine conditioned place preference CPP. Rats ($n = 10$) were tested three times for CPP with at least 3 rest days between tests. No pretreatment was given prior to the initial test for CPP. For the second and third tests for CPP, rats received muscimol (1 μg/0.6 μl) infusions into either the DHC or the cortex 15–25 min prior to testing in a counterbalanced order. Asterisks represent a significant difference from baseline value ($p < .05$, Tukey's test). The pound sign represents a significant difference from both the initial and the cortex inhibition tests for expression of cocaine CPP ($ps < .05$, Tukey's tests).
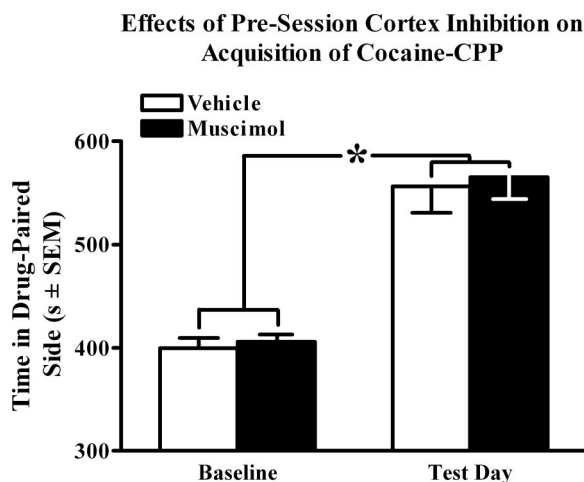
inhibition disrupted expression of cocaine CPP (Figures 3A and 5), an effect not likely due to changes in exploratory activity because rats exhibited similar numbers of crossovers between compartments on test days regardless of drug pretreatment (Figure 3B). The disruptive effect of DHC inhibition on expression of cocaine CPP was anatomically specific to the DHC because, in the same

Effects of Pre-Session Cortex Inhibition on Acquisition of Cocaine-CPP

Figure 6. Effects of presession muscimol (1 μg/0.6 μl; $n = 12$) or vehicle ($n = 10$) infusions into the cortex overlying dorsal hippocampus DHC on acquisition of cocaine conditioned place preference (CPP). Infusions occurred 15–25 min prior to each conditioning session. The asterisk represents a significant difference from respective baseline ($p < .05$, Tukey's test).

rats, inhibition of the overlying cortex prior to testing failed to disrupt expression of CPP (see Figure 7). Given our selection of pharmacological inhibition rather than a histologically verifiable lesion, it is difficult to estimate the extent of DHC inhibition produced by our infusion parameters. However, recent evidence has shown that a similar volume of muscimol (0.5 μl) infused into the DHC spreads roughly 2 mm rostro-caudally through the structure, with some spread into the overlying cortex but not into the ventral hippocampus or thalamus (Corcoran, Desmond, Frey, & Maren, 2005). Thus, this evidence and our finding that inhibition of the cortex fails to produce effects similar to DHC inhibition supports the idea that disruption of acquisition and expression is due to impaired function of the DHC. Postsession DHC inhibition immediately following either 30-min or 15-min conditioning sessions or 6 hr after 30-min conditioning sessions did not affect acquisition (see Figure 4); thus, the present study failed to find evidence that the DHC is necessary for consolidation of cocaine CPP.

The effects of preconditioning DHC inhibition on acquisition in the present study extend our previous findings demonstrating disruption of cocaine CPP through the use of preconditioning excitotoxic DHC lesions (Meyers et al., 2003). The present findings are complementary to those observed with manipulation of dopaminergic neurotransmission prior to conditioning sessions for morphine CPP, which indicate that stimulation of either D1 or D2 dopamine receptors in the DHC facilitates acquisition of morphine CPP (Rezayof et al., 2003). Collectively, these results suggest an important role for the DHC in the acquisition of drug-induced CPP. This role may involve disruption of reward because the DHC regulates cocaine self-administration (Black et al., 2004), supports intracranial self-stimulation (Olds, 1969), and supports CPP estab-

lished with localized administration of morphine (Corrigall & Linseman, 1988; Olmstead & Franklin, 1997; Stevens, Shiotsu, & Stein, 1991; Wise, 1989). Alternatively, the DHC may be involved in forming the association between cocaine reward and the drug-paired environment, which is necessary for the environment to acquire incentive motivational effects, and thereby, elicit preference in the absence of the drug. This explanation is in accordance with previous literature demonstrating DHC involvement in contextual CS–US associations established with aversive USs (Bast et al., 2003; Kim et al., 1993; Maren, Aharonov, & Fanselow, 1997; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999).

Deficits in expressing cocaine CPP following pretesting DHC inhibition are consistent with previous findings demonstrating that pretesting microinjections of dopamine antagonists into the DHC disrupt expression of morphine CPP. Thus, dopamine in the DHC may mediate expression of morphine CPP (Rezayof et al., 2003) and similar dopaminergic mechanisms may be involved in expression of cocaine CPP. Collectively, these findings are consistent with earlier studies suggesting a role for the hippocampus in cocaine-seeking behavior motivated by cocaine-associated explicit and contextual stimuli (Neisewander et al., 2000) or stimulation of hippocampus itself (Vorel, Liu, Hayes, Spector, & Gardner, 2001). In addition, recent evidence has shown that DHC inactivation disrupts contextual, but not explicit cue or cocaine-primed reinstatement of cocaine-seeking behavior (Fuchs et al., 2005). In contrast, explicit cue reinstatement of cocaine-seeking behavior is disrupted by inactivation of the ventral subiculum of the hippocampal formation (Sun & Rebec, 2003). Collectively, these findings suggest that the DHC is involved in expression of drug-conditioned effects established with contextual stimuli, whereas the ventral hippocampus may be involved in conditioned effects established with explicit drug-paired stimuli.

A likely explanation for the effects of DHC inhibition prior to testing is impaired recall of previous contextual CS–US associations. Consistent with this explanation, pretesting DHC lesions impair conditioned freezing to a contextual, but not an explicit, CS (Kim & Fanselow, 1992; Maren et al., 1997; Matus-Amat, Higgins, Barrientos, & Rudy, 2004). These findings implicate a role for the DHC in the recall of contextual conditioning and suggest that during expression of cocaine CPP, the DHC mediates retrieval of the context-cocaine reward association.

It is unlikely that the effects of DHC inhibition on expression of cocaine CPP were caused by extinction due to repeated testing (Bardo, Neisewander, & Miller, 1986) because repeated testing following cortex inhibition or vehicle pretreatment failed to affect expression of CPP (Figures 3A, 5, and 7) and cocaine CPP in our laboratory is highly resistant to extinction (Fuchs, Weber, Rice, & Neisewander, 2002; Zavala, Weber, Rice, Alleweireldt, & Neisewander, 2003). The effects of pretesting DHC inhibition are also not likely the result of behavioral disruption because the number of crossovers on the test day was similar across groups, suggesting intact exploratory behaviors during CPP testing (Figure 3B). Pretesting DHC inhibition may have interfered with the incentive motivational effects of the cocaine-paired environment that underlie approach behaviors during testing (Bardo & Bevins, 2000). However, this possibility is mitigated by previous findings that suggest the amygdala, and not the hippocampus, processes stimulus incentive value. For example, rats run slower down a runway toward a goal after the reward at the end of the alley is reduced in

size relative to previous trials, thus reflecting reduced incentive value of the reward (Amsel, 1958; Amsel & Roussel, 1952). Manipulations disrupting amygdala function, but not hippocampal function, result in preservative running speeds following reward reduction (Kemble & Beckman, 1970; Kesner & Williams, 1995; Salinas, Packard, & McGaugh, 1993). We have also found that preconditioning basolateral amygdala (BLA) lesions disrupt acquisition of cocaine CPP, whereas postconditioning BLA lesions fail to disrupt expression and increase resistance to extinction of cocaine CPP, suggesting a role for the amygdala in the assignment and modification of incentive value of cocaine-associated stimuli (Fuchs et al., 2002). In any case, if DHC inhibition interfered with incentive motivation, given the differences in effects of postconditioning manipulations of BLA and DHC on expression of cocaine CPP, it is likely that the role of the DHC in this process is different from that of the amygdala.

We failed to find evidence for DHC involvement in consolidation of cocaine CPP. Upon failing to observe an effect of immediate or 6-hr postsession DHC inhibition following 30-min conditioning sessions, we examined the effects of immediate DHC inhibition following 15-min conditioning sessions to address the possibility that DHC involvement in consolidation of cocaine CPP is more time limited (i.e., occurs early during the conditioning session). We reasoned that shortening conditioning trial duration would reveal such an effect, but our data failed to support this idea. The lack of effect of postconditioning DHC muscimol is not likely the result of insufficient inhibition because similar pretesting inhibition in the same rats was effective in disrupting expression of cocaine CPP. Moreover, shortening conditioning trials to less than 15 min is unlikely to support place conditioning (Bardo et al., 1995), and effects on consolidation of psychomotor stimulant-CPP have been observed in the amygdala following 30-min conditioning sessions (Hsu, Schroeder, & Packard, 2002; Schroeder & Packard, 2002).

It is surprising that no effect of postconditioning DHC inhibition on consolidation of cocaine CPP was observed, as the DHC has been shown to be a crucial site for memory consolidation in rats across a variety of context-conditioned tasks (Anagnostaras et al., 2001; Berman & Kesner, 1976; Cammarota et al., 2004; Holland & Bouton, 1999; Izquierdo & Medina, 1997; Nadel & Moscovitch, 1997), including studies that have used immediate postconditioning muscimol infusions (Holahan, 2005; Izquierdo et al., 1992) as well as 6-hr delayed catecholamine manipulations (Bernabeu et al., 1997; Bevilaqua et al., 1997; Izquierdo & Medina, 1997). It is interesting to note that a recent parallel study that used DHC inhibition to examine its role in acquisition, consolidation, and expression of a food-conditioned cue preference task based on spatial cues found that the DHC was necessary for consolidation, but not for acquisition or expression, of the task (Holahan, 2005). Because similar manipulations of the DHC were used in the present study, but with a different behavioral paradigm, it is likely that the opposite patterns of results may be attributed to differences in processing needed across tasks. Alternatively, the role of the DHC in consolidation of CS–US associations may be influenced by the type of CS (context vs. explicit cue) or type of US (natural vs. drug rewards) used to establish CPP. For instance, cocaine present at the time of treatment may interfere with DHC inhibition. Indeed, Rezayof et al. (2003) have shown that enhancing dopamine neurotransmission in the DHC facilitates acquisition of mor-

phine CPP. Enhanced dopamine activity in the DHC from cocaine administration may counteract effects of postconditioning DHC inhibition. However, our finding that acquisition of cocaine CPP was disrupted even though cocaine was present during inhibition mitigates this idea.

The lack of a consolidation effect in the present study does not preclude DHC involvement in this process for CPP because the DHC may mediate postconditioning consolidation at other time points or via mechanisms (such as second messenger systems) that are not altered by muscimol. For example, following infusion into the DHC, detectable amounts of muscimol remain for at least 1 hr (Corcoran et al., 2005), leaving open the possibility that other time points between 1 and 6 hr or beyond may be important for DHC-dependent consolidation of cocaine CPP. Nevertheless, the present findings raise the interesting possibility that consolidation of cocaine CPP may not require the DHC and instead might be mediated by the amygdala, which has been shown to be necessary for consolidation of food and amphetamine CPP (Holahan, 2005; Hsu et al., 2002; Schroeder & Packard, 2002).

There are some methodological issues that need to be addressed with the use of the CPP model. First, we used a biased design that involves pairing the drug to the individual rats' nonpreferred side. With this design it can be difficult to interpret whether CPP reflects reduction of initial aversion or induction of a preference for the drug-paired side. This was not a problem in the present study because CPP was evident in control rats as > 50% of time was spent in the drug-paired side during testing, indicating true preference rather than reduction of initial aversion. Furthermore, because we used a nonbiased apparatus in which naive rats do not exhibit a strong bias for a particular side, it is unlikely that rats found their initially nonpreferred side to be aversive. A second methodological issue is that drug-induced deficits in CPP expression are a result of state-dependent learning because experimental rats are in a different state on test day than that experienced during conditioning. Studies that have examined state-dependent learning effects by using the CPP model have primarily found similar CPP expression regardless of whether rats are tested in a drugged versus nondrugged state (Mucha & Iversen, 1984; Nomikos & Spyraki, 1988; Shoblock, Wichmann, & Maidment, 2005; Tzschentke & Schmidt, 1997; but see Olmstead & Franklin, 1997). Furthermore, on the basis of the present findings with muscimol infusion into the cortex, it seems unlikely that our dose of muscimol disrupted CPP expression simply by changing the state of the rats. For instance, rats trained under the influence of cortical muscimol infusions and tested in a nondrug state exhibited CPP of the same magnitude as rats trained and tested in a nondrug state. Furthermore, rats trained in a nondrug state and tested under the influence of cortical muscimol also exhibited CPP of the same magnitude as rats trained and tested in a nondrug state (data not shown). Although it is possible that DHC muscimol may produce a site-specific state-dependent learning effect, it must be noted that our dose of muscimol is thought to pervasively inhibit neuronal activity in the treated region (Martin, 1991; Martin & Ghez, 1999), thus making disruption of the neural processes necessary for CPP a more plausible explanation for our behavioral effects.

In conclusion, these results provide novel information demonstrating a functional role for the DHC in acquisition and expression, but not consolidation, of cocaine CPP and suggest that the DHC is important for behaviors elicited by cocaine conditioned contextual stimuli. Furthermore, the findings provide the first attempt to investigate the role of the DHC in consolidation of drug-induced CPP. The lack of support for DHC involvement in this process is intriguing given the established role of this structure in consolidation of other context-conditioned behaviors. Further research is needed to explore this discrepancy. Moreover, the role of the DHC in drug reward and behaviors conditioned by stimuli associated with other drugs of abuse such as morphine or nicotine remains to be elucidated. Information regarding the role of brain regions in processes related to learning and memory of drug conditioning will contribute significantly to the understanding of craving and relapse elicited by drug-associated stimuli, and understanding these processes could lead to the development of treatments for cocaine dependence.

References

Amaral, D., & Witter, M. (1995). Hippocampal formation. In G. Paxinos (Ed.), *The rat nervous system* (2nd ed., pp. 443–493). Los Angeles: Academic Press.

Amsel, A. (1958). The role of frustrative nonreward in noncontinuous reward situations. *Psychological Bulletin, 55,* 102–119.

Amsel, A., & Roussel, J. (1952). Motivational properties of frustration. I. Effect on a running response of the addition of frustration to the motivational complex. *Journal of Experimental Psychology, 43,* 363–366.

Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). Hippocampus and contextual fear conditioning: Recent controversies and advances. *Hippocampus, 11,* 8–17.

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology, 153,* 31–43.

Bardo, M. T., Neisewander, J. L., & Miller, J. S. (1986). Repeated testing attenuates conditioned place preference with cocaine. *Psychopharmacology, 89,* 239–243.

Bardo, M. T., Rowlett, J. K., & Harris, M. J. (1995). Conditioned place preference using opiate and stimulant drugs: A meta-analysis. *Neuroscience and Biobehavioral Reviews, 19,* 39–51.

Bast, T., Zhang, W. N., & Feldon, J. (2003). Dorsal hippocampus and classical fear conditioning to tone and context in rats: Effects of local NMDA-receptor blockade and stimulation. *Hippocampus, 13,* 657–675.

Berman, R. F., & Kesner, R. P. (1976). Posttrial hippocampal, amygdaloid, and lateral hypothalamic electrical stimulation: Effects on short- and long-term memory of an appetitive experience. *Journal of Comparative and Physiological Psychology, 90,* 260–267.

Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., et al. (1997). Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proceedings of the National Academy of Science, 94,* 7041–7046.

Bevilaqua, L., Ardenghi, P., Schroder, N., Bromberg, E., Schmitz, P. K., Schaeffer, E., et al. (1997). Drugs acting upon the cyclic adenosine monophosphate/protein kinase A signalling pathway modulate memory consolidation when given late after training into rat hippocampus but not amygdala. *Behavioural Pharmacology, 8,* 331–338.

Black, Y. D., Green-Jordan, K., Eichenbaum, H. B., & Kantak, K. M. (2004). Hippocampal memory system function and the regulation of cocaine self-administration behavior in rats. *Behavioural Brain Research, 151,* 225–238.

Cammarota, M., Bevilaqua, L. R., Bonini, J. S., Rossatto, J. I., Medina, J. H., & Izquierdo, N. (2004). Hippocampal glutamate receptors in fear memory consolidation. *Neurotoxicity Research, 6,* 205–212.

Childress, A., Ehrman, R., McLellan, A. T., & O'Brien, C. (1988). Con-

ditioned craving and arousal in cocaine addiction: A preliminary report. *NIDA Research Monograph, 81,* 74–80.

Collier, T. J., & Routtenberg, A. (1984). Electrical self-stimulation of dentate gyrus granule cells. *Behavioral and Neural Biology, 42,* 85–90.

Corcoran, K. A., Desmond, T. J., Frey, K. A., & Maren, S. (2005). Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. *Journal of Neuroscience, 25,* 8978–8987.

Corrigall, W. A., & Linseman, M. A. (1988). Conditioned place preference produced by intra-hippocampal morphine. *Pharmacology, Biochemistry, and Behavior, 30,* 787–789.

Fanselow, M. S. (2000). Contextual fear, gestalt memories, and the hippocampus. *Behavioural Brain Research, 110,* 73–81.

Ferbinteanu, J., & McDonald, R. J. (2001). Dorsal/ventral hippocampus, fornix, and conditioned place preference. *Hippocampus, 11,* 187–200.

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., 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,* 296–309.

Fuchs, R. A., Weber, S. M., Rice, H. J., & Neisewander, J. L. (2002). Effects of excitotoxic lesions of the basolateral amygdala on cocaine-seeking behavior and cocaine conditioned place preference in rats. *Brain Research, 929,* 15–25.

Holahan, M. R. (2005). Complementary roles for the amygdala and hippocampus during different phases of appetitive information processing. *Neurobiology of Learning and Memory, 84,* 124–131.

Holland, P. C., & Bouton, M. E. (1999). Hippocampus and context in classical conditioning. *Current Opinion in Neurobiology, 9,* 195–202.

Holt, W., & Maren, S. (1999). Muscimol inactivation of the dorsal hippocampus impairs contextual retrieval of fear memory. *Journal of Neuroscience, 19,* 9054–9062.

Hsu, E. H., Schroeder, J. P., & Packard, M. G. (2002). The amygdala mediates memory consolidation for an amphetamine conditioned place preference. *Behavioural Brain Research, 129,* 93–100.

Izquierdo, I., da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M. B., & Medina, J. H. (1992). Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behavioral and Neural Biology, 58,* 16–26.

Izquierdo, I., & Medina, J. H. (1997). Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory, 68,* 285–316.

Kemble, E. D., & Beckman, G. J. (1970). Runway performance of rats following amygdaloid lesions. *Physiology and Behavior, 5,* 45–47.

Kesner, R. P., & Williams, J. M. (1995). Memory for magnitude of reinforcement: Dissociation between the amygdala and hippocampus. *Neurobiology of Learning and Memory, 64,* 237–244.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256,* 675–677.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behavioral Neuroscience, 107,* 1093–1098.

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience, 24,* 897–931.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behavioural Brain Research, 88,* 261–274.

Maren, S., & Fanselow, M. S. (1997). Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiology of Learning and Memory, 67,* 142–149.

Martin, J. H. (1991). Autoradiographic estimation of the extent of reversible inactivation produced by microinjection of lidocaine and muscimol in the rat. *Neuroscience Letters, 127,* 160–164.

Martin, J. H., & Ghez, C. (1999). Pharmacological inactivation in the analysis of the central control of movement. *Journal of Neuroscience Methods, 86,* 145–159.

Matus-Amat, P., Higgins, E. A., Barrientos, R. M., & Rudy, J. W. (2004). The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *Journal of Neuroscience, 24,* 2431–2439.

McGaugh, J. L. (2000). Memory—A century of consolidation. *Science, 287,* 248–251.

Meyers, R. A., Zavala, A. R., & Neisewander, J. L. (2003). Dorsal, but not ventral, hippocampal lesions disrupt cocaine place conditioning. *NeuroReport, 14,* 2127–2131.

Morris, R. G., Moser, E. I., Riedel, G., Martin, S. J., Sandin, J., Day, M., et al. (2003). Elements of a neurobiological theory of the hippocampus: The role of activity-dependent synaptic plasticity in memory. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences, 358,* 773–786.

Mucha, R. F., & Iversen, S. D. (1984). Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: A procedural examination. *Psychopharmacology, 82,* 241–247.

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia, and the hippocampal complex. *Current Opinion in Neurobiology, 7,* 217–227.

National Institutes of Health. (1986). *Guide for the care and use of laboratory animals* (DHEW Publication No. 86–23). Washington, DC: U.S. Government Printing Office.

Neisewander, J. L., Baker, D. A., Fuchs, R. A., Tran-Nguyen, L. T., Palmer, A., & Marshall, J. F. (2000). Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *Journal of Neuroscience, 20,* 798–805.

Nomikos, G. G., & Spyraki, C. (1988). Cocaine-induced place conditioning: Importance of route of administration and other procedural variables. *Psychopharmacology, 94,* 119–125.

O'Dell, L. E., Khroyan, T. V., & Neisewander, J. L. (1996). Dose-dependent characterization of the rewarding and stimulant properties of cocaine following intraperitoneal and intravenous administration in rats. *Psychopharmacology, 123,* 144–153.

Olds, J. (1969). The central nervous system and the reinforcement of behavior. *American Psychologists, 24,* 114–132.

Olmstead, M. C., & Franklin, K. B. (1997). The development of a conditioned place preference to morphine: Effects of microinjections into various CNS sites. *Behavioral Neuroscience, 111,* 1324–1334.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego, CA: Academic Press.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106,* 274–285.

Rezayof, A., Zarrindast, M. R., Sahraei, H., & Haeri-Rohani, A. (2003). Involvement of dopamine receptors of the dorsal hippocampus on the acquisition and expression of morphine-induced place preference in rats. *Journal of Psychopharmacology, 17,* 415–423.

Rossato, J. I., Bonini, J. S., Coitinho, A. S., Vianna, M. R., Medina, J. H., Cammarota, M., et al. (2004). Retrograde amnesia induced by drugs acting on different molecular systems. *Behavioral Neuroscience, 118,* 563–568.

Sacchetti, B., Lorenzini, C. A., Baldi, E., Tassoni, G., & Bucherelli, C. (1999). Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. *Journal of Neuroscience, 19,* 9570–9578.

Salinas, J. A., Packard, M. G., & McGaugh, J. L. (1993). Amygdala modulates memory for changes in reward magnitude: Reversible post-training inactivation with lidocaine attenuates the response to a reduction in reward. *Behavioural Brain Research, 59,* 153–159.

Schroeder, J. P., & Packard, M. G. (2002). Posttraining intra-basolateral amygdala scopolamine impairs food- and amphetamine-induced conditioned place preferences. *Behavioral Neuroscience, 116,* 922–927.

Selden, N. R., Everitt, B. J., Jarrard, L. E., & Robbins, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience, 42,* 335–350.

Shoblock, J. R., Wichmann, J., & Maidment, N. T. (2005). The effect of a systemically active ORL-1 agonist, Ro 64–6198, on the acquisition, expression, extinction, and reinstatement of morphine conditioned place preference. *Neuropharmacology, 49,* 439–446.

Stevens, K. E., Shiotsu, G., & Stein, L. (1991). Hippocampal μ-receptors mediate opioid reinforcement in the CA3 region. *Brain Research, 545,* 8–16.

Stewart, J. (1983). Conditioned and unconditioned drug effects in relapse to opiate and stimulant drug self-adminstration. *Progress in Neuropsychopharmacology and Biological Psychiatry, 7,* 591–597.

Sun, W., & Rebec, G. V. (2003). Lidocaine inactivation of ventral subiculum attenuates cocaine-seeking behavior in rats. *Journal of Neuroscience, 23,* 10258–10264.

Tzschentke, T. M. (1998). Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology, 56,* 613–672.

Tzschentke, T. M., & Schmidt, W. J. (1997). Interactions of MK-801 and GYKI 52466 with morphine and amphetamine in place preference conditioning and behavioural sensitization. *Behavioural Brain Research, 84,* 99–107.

Ursin, R., Ursin, H., & Olds, J. (1966). Self-stimulation of hippocampus in rats. *Journal of Comparative and Physiological Psychology, 61,* 353–359.

Vorel, S. R., Liu, X., Hayes, R. J., Spector, J. A., & Gardner, E. L. (2001). Relapse to cocaine-seeking after hippocampal theta burst stimulation. *Science, 292,* 1175–1178.

Wise, R. A. (1989). Opiate reward: Sites and substrates. *Neuroscience and Biobehavioral Reviews, 13,* 129–133.

Zanatta, M. S., Quillfeldt, J. H., Schaeffer, E., Schmitz, P. K., Quevedo, J., Medina, J. H., et al. (1997). Involvement of the hippocampus, amygdala, entorhinal cortex and posterior parietal cortex in memory consolidation. *Brazilian Journal of Medical and Biological Research, 30,* 235–240.

Zarrindast, M. R., Bakhsha, A., Rostami, P., & Shafaghi, B. (2002). Effects of intrahippocampal injection of GABAergic drugs on memory retention of passive avoidance learning in rats. *Journal of Psychopharmacology, 16,* 313–319.

Zavala, A. R., Weber, S. M., Rice, H. J., Alleweireldt, A. T., & Neisewander, J. L. (2003). Role of the prelimbic subregion of the medial prefrontal cortex in acquisition, extinction, and reinstatement of cocaine-conditioned place preference. *Brain Research, 990,* 157–164.