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Hippocampal Regulation of Contextual Cue-Induced Reinstatement of Cocaine-Seeking Behavior

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Abstract

Associations between cocaine and cues facilitate development and maintenance of addiction. We hypothesized that the ventral hippocampus is important for acquisition of these associations. Rats were trained to self-administer cocaine, with or without pre-exposure to distinct sets of cocaine- and saline-paired contextual cues. Next, rats were conditioned for 3 days with the distinct sets of contextual cues paired with cocaine and saline along with distinct discrete cues. Vehicle or lidocaine was infused into the ventral hippocampus prior to conditioning sessions. Following extinction, reinstatement of cocaine-seeking behavior was examined following exposure to contextual cues, discrete cues, or their combination. Inactivation of the ventral hippocampus during conditioning blocked acquisition of the association between cocaine and cocaine-paired contextual cues in that only lidocaine-treated rats with short-term cue exposure failed to reinstate responding in the presence of cocaine-paired contextual cues. Lidocaine also prevented rats in both cue exposure groups from discriminating between cocaine-and saline-paired contextual cues during reinstatement tests. Reinstatement induced by cocaine-paired discrete cues or by contextual and discrete cues together was not impaired for either cue exposure condition. The hippocampus is important for acquisition of the association between cocaine and context and in maintaining discrimination between cocaine-relevant and -irrelevant contextual cues.

Keywords

Cocaine; Discrete stimulus cues; Contextual cues; Hippocampus; Reinstatement; Self-administration

Introduction

Environmental cues associated with cocaine use play a role in craving and relapse experienced by addicts, thus contributing to development and maintenance of cocaine addiction (Shaham et al., 2003). Several brain areas involved in learning and memory processes are activated in imaging studies during craving induced by cocaine-associated cues in human subjects (Risinger et al., 2005; Volkow et al., 2006). This suggests a role for these brain areas in mediating cocaine craving and relapse and indicates that associative learning processes may play a role in regulating cocaine addiction. The focus of the experiments described herein is on the hippocampal memory system and its role in mediating acquisition of associations between cocaine and environmental stimuli.

The hippocampus forms episodic memories by integrating the contextual details of the environment into a temporally and spatially unified memory representation and is activated during performance of tasks that require formation of associations between stimuli within an event (Ongur et al., 2005). Though many studies examining the role of the hippocampus on behavior do not differentiate between subregions of the hippocampus, there are important functional differences between the dorsal hippocampus and the ventral hippocampus (Hock and Busney, 1998; McDonald et al., 2006). Fear conditioning studies support a role for the

ventral hippocampus in processing contextual cues associated with aversive stimuli (Hobin and Maren, 2006; Rudy and Matus-Amat, 2005). There is also evidence that the ventral hippocampus is involved in drug-seeking behavior (Vorel et al., 2001). Dopaminergic innervation to the hippocampus is almost entirely limited to the ventral hippocampus, which provides a significant source of innervation to the nucleus accumbens (Verney et al., 1985; Yang and Mogenson, 1986). Dopamine in the nucleus accumbens is a major substrate of drug reward (Wise and Bozarth, 1985). Thus, the hippocampal memory system may be involved in mediating neurocognitive aspects of addiction-related behaviors via pathways between the ventral hippocampus, the nucleus accumbens, and the prefrontal cortex, another component of reward circuitry (Cooper et al., 2006). Specifically, the ventral hippocampus may be involved in the processing of environmental stimuli associated with cocaine self-administration (Rogers and See, 2007; Sun and Rebec, 2003).

Hippocampal learning theory indicates that the hippocampus is required for memory storage of associative information involving contextual cues during acquisition of this information, but not after consolidation has taken place (Alvarez and Squire, 1994; Zola-Morgan and Squire, 1990). Previous self-administration work from this laboratory has demonstrated that lidocaine inactivation of the ventral subiculum did not impact cue-induced reinstatement of cocaine-seeking behavior following long-term experience with cocaine-paired discriminable contextual and discrete conditioned stimulus cues (Black et al., 2004). Lidocaine may have been ineffective in modifying cocaine-seeking behavior in this study due to the lengthy exposure rats had to drug and drug-paired cues prior to inactivation, thus measuring the effects of lidocaine on learned behavior that was well beyond the acquisition stage.

Given that the hippocampus is important during acquisition of contextual information, we hypothesized that inactivation of the ventral hippocampus during cocaine self-administration in rats with short-term contextual cue exposure would prevent these cues from acquiring motivational salience. In contrast, in rats with long-term contextual cue exposure during cocaine self-administration, we did not expect inactivation of the ventral hippocampus to alter the motivational salience of these cues. To test if the salience of these cues were altered, we measured responding during a reinstatement test in the presence of cocaine-paired contextual cues and absence of cocaine after responding had been extinguished. We expected lidocaine treatment during conditioning sessions in animals with short-term exposure to the contextual cues to interfere with acquisition of the association between cocaine and the contextual cues as evidenced by a reduction in drug-seeking behavior during the drug-free reinstatement test. We also tested discrete cue-induced reinstatement, with only short-term exposure to the discrete cues in all groups, to examine whether long-term exposure to contextual cues had any impact on acquisition of new associations between cocaine and cocaine-paired discrete cues, and whether the ventral hippocampus plays a role in this. We hypothesized that lidocaine inactivation of the ventral hippocampus would have no effect on discrete cue-induced reinstatement of cocaine-seeking behavior, since others have shown that processing of discrete cues is hippocampal-independent (Huff and Rudy, 2004; Walker et al., 2005).

Methods

Animals

Male Wistar strain rats [Crl(WI)BR, Charles River Laboratories, Portage, MI], weighing approximately 275–300 g upon arrival, were maintained at 85–90% of their free-feeding body weight throughout the study by restricting food to approximately 16 g per day. They had continuous access to water in their home cages, which consisted of individual clear plastic boxes (24 × 22 × 20 cm). The animal facility was temperature- (21–23° C) and light-(lights on at 8 am and off at 8 pm) controlled, and the policies and procedures in *Guide for the Care and Use of Laboratory Animals* published by the National Academies Press (1996) were followed.

Apparatus

The experimental chambers (model ENV-008CT, Med Associates, East Fairfield, VT) contained 2 levers located 7 cm from the floor, with a white stimulus light located 2 cm above the right (active) lever, as described in Kantak et al. (2002). Motor-driven syringe pumps (model PHM-100, Med Associates) located outside of each cubicle were used for drug delivery. A computer programmed in MedState Notation and connected to an interface (Med Associates) controlled experimental events and recorded data.

Drugs

Cocaine hydrochloride (gift from NIDA, Bethesda, MD) was dissolved in sterile 0.9% saline solution containing 3 IU heparin/mL. During self-administration, animals received a 1.0 mg/kg unit infusion dose of cocaine, made in a concentration of 2.68 mg/mL and delivered intravenously at a rate of 1.8 mL/minute. Infusion volume was adjusted individually for body weight, resulting in drug delivery times of 1.2 sec/100 g body weight. This dose has been shown to produce clinically relevant peak plasma cocaine levels following repeated intravenous administration (Booze et al., 1997; Lau and Sun, 2002). During saline self-administration, heparinized saline solution was substituted for cocaine.

Lidocaine hydrochloride (Sigma, St. Louis, MO) was prepared daily as a 20% solution in 0.9% saline and administered bilaterally into the ventral hippocampus (100 μg) just prior to each conditioning session. The effects of lidocaine have been reported to disappear within 30–90 minutes, with higher concentrations such as that used in the present study resulting in a time course of inactivation in the high end of this range (Lomber, 1999). Vehicle infusions consisted of 0.9% saline. A total volume of 0.5 μL was infused per side at a rate of 0.59 μL/min. by a motor-driven syringe pump (Model PHM-100; Med Associates, Georgia, VT). Lidocaine is a sodium channel blocker that inhibits nerve conductance. Its inactivation is reversible, restricted to the infusion site, and has measurable behavioral effects without affecting other measures such as motor coordination (Pereira de Vasconcelos et al., 2006).

Food Pellet Shaping Sessions

Rats were initially trained to self-administer chocolate-flavored sucrose pellets under a fixed-ratio 1 (FR1) schedule of reinforcement in Context A (Table 1). Rats were considered shaped if they self-administered a minimum of 100 pellets per session, and in most cases, this only required one overnight autoshaping session.

Surgery

Rats were then surgically implanted with intravenous jugular catheters and bilateral cannulae into the ventral hippocampus (AP −5.7, L±4.5, DV-7.8). Rats were anesthetized with an intraperitoneal injection of 90 mg/kg ketamine plus 10 mg/kg xylazine, and surgery was performed as described in Black et al. (2004). Cannulae were placed 1 mm above the intended site. Cannula placement was based on the bregma coordinate system provided by Swanson (1992). All rats had a minimum of 1 week to recover from surgery before starting the experiment.

Throughout the experiment, catheters were maintained by flushing them daily (Monday through Friday) with 0.1 mL of a 0.9% saline solution containing 0.3 IU heparin (LymphoMed, Rosemont, IL), an anticoagulant, and 67 mg Timentin (SmithKline Beecham Pharmaceuticals, Philadelphia, PA), an antibiotic. On weekends, a locking solution consisting of glycerol and undiluted (1000 IU/ml) heparin (3:1) was used to fill the catheter dead space. In addition, catheters were checked for function at least once per week by infusing 0.1 mL solution

containing 1 mg methohexital sodium (King Pharmaceuticals, Bristol, TN) and checking for the presence of sedation.

Self-Administration Training Sessions

All rats with short-term exposure to the contextual cues (n=16) underwent cocaine self-administration training in Context A (same context as used for food pellet shaping sessions), and half the rats with long-term exposure to contextual cues (n=16) underwent cocaine self-administration training in Context B and the other half in Context C (Table 1, Figure 1A). Rats were trained to self-administer cocaine starting with an FR1, and incrementing up to a terminal FR5, schedule of drug delivery for a minimum of 21 sessions until responding was stable. During each cocaine infusion, the stimulus light above the active lever was lit. Immediately after the infusion, there was a 20-second time-out period during which the stimulus light remained on and the house light was off. Responses on the active lever during the time-out were recorded but had no consequences. After cocaine self-administration training, rats underwent 5 days of saline self-administration, in Context A for the short-term group, and in Context C or B (opposite context as used for cocaine self-administration sessions) for the long-term group (Figure 1A). After this, rats were given 2 additional days of cocaine self-administration training, in the Context A for the short-term group and in the Context B or C for the long-term group (Figure 1A). Training sessions took place once per day, 5 days per week, and lasted 2 hours with a maximum of 30 infusions per session. It is important to note that during this phase of the experiment, Context A was not equivalent to Contexts B or C; Context A did not contain distinct visual, auditory, and olfactory cues, and Contexts B and C did (Table 1). Contexts B and C were counterbalanced between groups in the long-term contextual cue exposure group during the self-administration training and conditioning (described below) phases of the experiment, and Contexts B and C were counterbalanced between groups later in the experiment (during the conditioning phase) for the short-term contextual cue exposure groups. Context A was not included in counterbalancing of contexts among groups because it was intended to be a neutral training context lacking the distinct visual, auditory, and olfactory cues that would be introduced to the short-term contextual cue exposure groups later in the experiment.

The above experimental design for self-administration training was used for two reasons. First, using more than 21 cocaine self-administration training sessions in Context B or C in the long-term rats helped ensure that retrieval of the associative contextual memory was no longer hippocampal-dependent, based on a previous study in this laboratory (Black et al., 2004). Because rats in the long-term group required at least 21 sessions of training to ensure this task was no longer hippocampal-dependent, rats in the short-term group were given an equivalent number of cocaine self-administration sessions in Context A so that all groups received the same amount of cocaine exposure, and only differed in the amount of Context B or C exposure. Secondly, by exposing rats to at least 21 sessions with cocaine, followed by 5 sessions with saline and then an additional 2 sessions with cocaine, rats were predisposed to maintain high rates of responding when cocaine was available and low rates of responding when saline was available prior to the 3-day conditioning phase, facilitating discrimination between cocaine and saline during conditioning sessions. If the first experience with saline were during the three conditioning sessions, then rats would likely show high rates of accelerated extinction responding (Harris et al., 2007), making it difficult to detect whether rats were discriminating cocaine from saline. The additional 2 sessions with cocaine were provided following the 5 sessions with saline in order to ensure that responding maintained by cocaine was at baseline levels prior to initiating the conditioning sessions. We based this design on pilot data from rats that did not have exposure to saline prior to the three-day conditioning procedure and were not able to successfully learn the discrimination between cocaine and saline in three days. We subsequently found that introducing saline prior to the conditioning procedure without

reintroducing cocaine resulted in a much lower rate of responding at the start of the conditioning sessions. So, providing several saline sessions followed by two cocaine sessions facilitated learning of the discrimination between saline and cocaine while maintaining high response levels for cocaine during the conditioning sessions in all groups of rats.

Self-Administration Conditioning Baseline Sessions

Following self-administration training, rats from each cue exposure condition were randomly subdivided into 2 groups (n=8) and underwent 3 days of conditioning (Figure 1B). Rats from each cue exposure group had 1-hr access to cocaine in Context B or C and 1-hr access to saline in Context C or B under an FR5 schedule of reinforcement, with 1-hr between sessions. Providing 1-hr between sessions was based on previous studies using a similar training procedure with discrimination between cocaine- and saline-paired cues that used a period of 40–60 minutes between cocaine and subsequent saline sessions (Cervo et al., 2003; Weiss et al., 2001). There was a novel discrete cue (stimulus light flashing on for 2 sec. then off for 2 sec. for a total of 20 sec.) delivered concurrently with each cocaine infusion and a different one (stimulus light flashing on for 1 sec. then off for 1 sec. for a total of 20 sec.) for saline. We added novel discrete cues for the conditioning part of the experiment to examine whether length of exposure to cocaine-associated contextual cues would impact the role of the ventral hippocampus in mediating acquisition of associations between cocaine and cocaine-paired discrete cues. Rats in both short-term and long-term contextual cue exposure groups had an equal amount of exposure to the discrete cues, so the only difference between the groups was length of exposure to the distinct sets of contextual cues. The order of presentation of cocaine and saline sessions was counterbalanced between rats in each group. One group from each exposure condition was given bilateral lidocaine infusions into the ventral hippocampus just prior to each of the two daily conditioning sessions, and the other group was given bilateral vehicle infusions just prior to each of the two daily conditioning sessions, such that each rat received a total of six bilateral lidocaine or vehicle infusions, two per day for three days. Each individual rat always received the same infusion, (e.g., a lidocaine rat received lidocaine all six times).

Following conditioning sessions, extinction training (2-hr sessions) was provided to all rats for 15 sessions, or until active lever responding was 25% or less of baseline responding that was measured during cocaine availability for each cue exposure condition. We used a percentage of baseline responding rather than a specific number of responses in order to accommodate individual differences in responding during self-administration training. In order to extinguish lever pressing without altering the salience of the cocaine-paired or saline-paired cues, extinction sessions took place in Context D (Table 1).

Reinstatement Test Sessions

Following extinction, rats were given a series of three reinstatement tests with three days between tests (Figure 1C). Neither cocaine nor saline were available during any of the tests. We designed the experiment with three separate reinstatement tests so that we could test effects of contextual and discrete cues separately before testing the combination of their effects. To evaluate contextual cue-induced reinstatement of cocaine seeking, rats were placed into the cocaine-paired context (Context B or C) and the saline-paired context (Context C or B) and no discrete cues were delivered. To evaluate discrete cue-induced reinstatement of cocaine seeking, rats were presented with the discrete cues previously paired with cocaine and saline after each completion of an FR5 on the active lever in Context D. To evaluate contextual/discrete cue-induced reinstatement of cocaine seeking, rats were presented with the discrete cues after each completion of an FR5 on the active lever in the cocaine-paired context (B or C) and the saline-paired context (C or B). Each reinstatement test consisted of 1 hr with one set of cues and 1 hr with the other set of cues with 1 hr between the two sessions. The order of

presentation of cocaine-paired and saline-paired cues was the same as during conditioning for individual rats.

The three reinstatement tests were always given in the same order, with the contextual cue-induced reinstatement test first, the discrete cue-induced reinstatement test second, and the contextual/discrete cue-induced reinstatement test third. We expected, based on previous research showing that processing of discrete cues is hippocampal-independent (Huff and Rudy, 2004; Walker et al., 2005), that we would only find a significant reduction in reinstatement in the short-term cue exposure group as compared to the long-term cue exposure group in the contextual cue-induced reinstatement test. Also, there is evidence from previous studies that discrete cue-induced reinstatement of drug-seeking behavior tends to be stronger than contextual cue-induced reinstatement, and contextual/discrete cue-induced reinstatement tends to be the strongest in terms of number of responses (Fuchs et al., 2005; Tsiang and Janak, 2006). We always carried out the contextual cue-induced reinstatement test first, followed by the discrete cue-induced reinstatement test and then the contextual/discrete cue-induced reinstatement test, because we wanted the contextual cue-induced reinstatement test to be sensitive to differences between groups despite expected lower responding across groups, and we expected responding in the presence of discrete and contextual/discrete cues to be more robust, and thus less susceptible to reductions in responding due to repeated testing.

Histology

At the end of the experiment, rats were given an overdose of sodium pentobarbital (364 mg) and then intracardially perfused with saline followed by a 10% formalin solution. Brains were extracted, post-fixed in 10% formalin for 2 days, and then transferred to a 30% sucrose solution for 2–4 days. Brains were then sliced, mounted on slides, and stained with thionin to verify cannula placement.

Data Analyses

The dependent measures were number of active lever responses, number of inactive lever responses, and number of infusions earned. To establish the self-administration training baseline, data were averaged from the last three 2-hr cocaine or saline baseline sessions in individual animals prior to analysis. Response data were analyzed by four-way ANOVA, with drug, lever, context during training, and future assignment to treatment during conditioning as factors. The number of infusions earned was analyzed by three-way ANOVA, since lever was not a factor for this measure.

To calculate the dependent variables during conditioning, data were averaged from the last two days of the procedure for 1-hr cocaine and saline sessions in individual animals prior to analysis. Data from the 1st hr of the last extinction session were used to equate amount of time between conditions. Conditioning and extinction response data were analyzed together by four-way ANOVA, with session type, lever, cue exposure, and treatment during conditioning as factors. Number of infusions was analyzed by three-way ANOVA, since lever was not a factor for this measure.

Active lever responses during reinstatement were analyzed as difference scores, with responding during the 1st hour of the last extinction session subtracted from responding during the 1-hr reinstatement session for each individual animal. We used difference scores to normalize the data because there was a significant difference between short-term and long-term contextual cue exposure groups in responding for cocaine during the conditioning phase of the experiment, and thus the value represented by 25% of baseline for the extinction criterion varied in individual subjects. We wanted to normalize the differences in the absolute number of responses made during extinction that resulted from using a percent of baseline criterion

rather than a criterion of a fixed number of responses to define extinction. Using difference scores thus provided a more accurate assessment of responding above extinction levels. To determine whether cocaine-paired cues reinstated active lever responding significantly above extinction levels, difference scores were compared via one-sample t-tests to a value of zero. Difference scores statistically greater than a value of zero would indicate significant reinstatement of drug-seeking behavior above extinction levels of responding, whereas a difference score statistically equal to zero would indicate no change from extinction responding, and a difference score statistically less that zero would indicate reduced responding during the reinstatement test as compared to extinction. To compare amount of reinstatement of drug-seeking behavior between cue exposure groups for each treatment condition, data from cocaine-paired cue sessions were analyzed by one-way ANOVA by group. Lastly, to determine whether rats discriminated between cocaine-paired and saline-paired cues, difference scores were analyzed by three-way ANOVA, with session type, cue exposure, and treatment during conditioning as factors. This series of analyses was run separately for each of the three types of reinstatement tests.

Results

Histology

Histological verification of placements and functional spread of lidocaine or vehicle are depicted separately by cue exposure and treatment groups (Figure 2). All placements depicted were within 1.1 mm of the anterior-posterior position of the intended site. In addition, all fell within the ventral hippocampal region, with the spread of lidocaine, which is estimated to spread spherically with a radius of 0.50 mm from the infusion site (Tehovnik and Sommer, 1997), overlapping with the ventral subiculum for all but 1 rat whose had a unilateral placement overlapping with the CA region within the ventral hippocampus. The spherical volume equation is an approximate rather than an exact estimate of the spread of lidocaine, establishing that it is unlikely that lidocaine spread outside of the ventral hippocampus. The functional spread of lidocaine is dependent on the infusion volume and rate of infusion rather than the concentration of lidocaine (Martin and Ghez, 1999;Nakamura et al., 2003;Tehovnik and Sommer, 1997). Several previous studies using microinjection procedures, infusion volumes, and rates of infusion similar to those in the present study have shown the spread of lidocaine to be consistent with calculations using the spherical volume equation (Martin, 1991;Martin and Ghez, 1999;Sandkuhler and Gebhart, 1984;Martin, 1991;Tehovnik and Sommer, 1997;Martin and Ghez, 1999). Data of two animals from the long-term cue exposure lidocaine treatment group were discarded; one dislodged his head mount, and the other failed to respond during the three-day conditioning period, resulting in a group size of n=6 for this condition.

Self-Administration Training Sessions

By the end of self-administration training, all rats were able to successfully discriminate between cocaine and saline and between the active and inactive levers, and this did not differ between exposure groups or between treatment groups prior to any treatment. For lever responses, a four-way ANOVA revealed a significant main effect of drug [$F(1, 26)=13.9$, $p\leq0.001$], a significant main effect of lever [$F(1, 26)=28.3$, $p\leq0.001$], and a significant drug × lever interaction [$F(1, 26)=16.8$, $p\leq0.001$]. Tukey's post-hoc tests revealed that responding was significantly higher on the active lever during cocaine baseline sessions compared to the inactive lever during cocaine baseline sessions ($p\leq0.001$) and compared to the active ($p\leq0.001$) or inactive ($p\leq0.001$) levers during saline baseline sessions. Similarly, there was only a significant main effect of drug in a three-way ANOVA comparing number of infusions earned [$F(1, 26)=347.7$, $p\leq0.001$], with the number of cocaine infusions significantly higher than the number of saline infusions in all groups. These analyses indicate that there were no baseline differences between groups prior to treatment with vehicle or lidocaine, and no baseline

differences between groups based on complexity of training context (Context A vs. Context B or C). In addition, rats in all groups received an equivalent amount of cocaine during the self-administration training phase of the experiment.

Self-Administration Conditioning Sessions

The numbers of active and inactive lever responses during self-administration conditioning sessions and extinction as well as the number of infusions earned during self-administration conditioning sessions are shown in Figure 3. A four-way ANOVA of lever responses revealed significant main effects of session type [$F(2, 52)=72.5$, $p\leq0.001$], lever [$F(1, 52)=112.7$, $p\leq0.001$], and length of cue exposure [$F(1, 26)=5.7$, $p\leq0.05$]. Tukey's post-hoc tests revealed that overall, responding was higher during cocaine-self administration sessions than during saline self-administration sessions ($p\leq0.01$) or extinction ($p\leq0.01$). Responding was also higher overall on the active than on the inactive lever and higher overall in the short-term cue exposure group than in the long-term cue exposure group. Additionally, there were significant interactions of session type × cue exposure [$F(2, 52)=4.2$, $p\leq0.05$], session type × lever [$F(2, 52)=76.3$, $p\leq0.001$], and session type × lever × cue exposure [$F(2, 52)=3.5$, $p\leq0.05$], such that active lever responding was higher during cocaine self-administration sessions for rats in the short-term cue exposure group than for rats in the long-term cue exposure group ($p\leq0.01$). However, this difference in the number of active lever responses in the short-term and long-term groups is only 32 responses on average. Importantly, lidocaine treatment during conditioning sessions did not significantly impact responding, either overall or through an interaction with cue exposure and/or session type.

Similarly, a three-way ANOVA of average number of infusions earned during conditioning revealed a significant main effect of session type [$F(1, 26)=178.5$, $p\leq0.001$] and a significant session type × cue exposure interaction [$F(1, 26)=6.5$, $p\leq0.05$]. Tukey's post-hoc tests revealed that, overall, rats with short-term cue exposure earned significantly more infusions during cocaine sessions than rats with long-term cue exposure ($p\leq0.05$), with no effect of lidocaine vs. vehicle treatment during conditioning.

Contextual Cue-Induced Reinstatement Test

Lidocaine treatment blocked contextual cue-induced reinstatement of cocaine-seeking behavior in rats with short-term exposure to the contextual cues, but not in rats with long-term exposure to the contextual cues (Figure 4, inset). One-sample t-tests comparing difference scores revealed reinstatement values that were significantly greater than zero for rats with short-term cue exposure treated with vehicle during conditioning ($p\leq0.005$) and for rats with long-term cue exposure treated with vehicle ($p\leq0.001$) or lidocaine ($p\leq0.05$) during conditioning, but not for rats with short-term cue exposure treated with lidocaine during conditioning. A one-way ANOVA comparing the four groups was significant [$F(3, 26)=5.5$, $p\leq0.005$]. Tukey's post-hoc tests revealed that difference scores were lower in rats with short-term cue exposure treated with lidocaine than all other groups ($p\leq0.005$).

Lidocaine also impaired rats' ability to discriminate between cocaine-paired and saline-paired contextual cues regardless of length of cue exposure (Figure 4, main graph). For the three-way ANOVA, the interaction of session type × conditioning treatment was significant [$F(1, 26)=12.0$, $p\leq0.05$]. Tukey's post-hoc tests revealed that regardless of length of cue exposure, rats treated with vehicle during conditioning had significantly higher difference scores for active lever responding during cocaine-paired contextual cue sessions than during saline-paired contextual cue sessions ($p\leq0.01$), indicating discrimination between the two types of contextual cues. In contrast, rats treated with lidocaine during conditioning did not discriminate the two types of contextual cues, as shown by similar difference scores for active lever responses in the cocaine context vs. the saline context. Although difference scores for inactive lever

responses were significantly higher during saline-paired cue sessions than during cocaine-paired cue sessions [F (1, 26)=4.8, p≤0.05], the actual difference between these two conditions was relatively small (mean=1.3±0.8 for cocaine-paired cue sessions, mean=4.1±1.2 for saline-paired cue sessions).

Discrete Cue-Induced Reinstatement Test

Lidocaine treatment had no effect on discrete cue-induced reinstatement of cocaine-seeking behavior (Figure 5, inset). One-sample t-tests comparing difference scores revealed reinstatement values that were significantly greater than zero for rats in all four groups (p≤0.05). A one-way ANOVA of these difference scores indicated that there were no significant group differences in the degree of responding above extinction levels.

Only rats in the long-term contextual cue exposure group were able to discriminate the cocaine-paired and saline-paired discrete cues (Figure 5, main graph). Based on the three-way ANOVA, there was a significant main effect of cue exposure [F (1, 26)=16.4, p≤0.001] and a session type × cue exposure interaction [F (1, 26)=4.7, p≤0.05]. Tukey's post-hoc tests of the interaction effect revealed that rats with long-term contextual cue exposure had significantly higher difference scores for active lever responding during cocaine discrete cue reinstatement test sessions than during saline discrete cue reinstatement test sessions (p≤0.05). Difference scores for responding maintained by discrete cocaine and saline cues did not significantly differ in the short-term contextual cue exposure groups, indicating that discrimination of these two discrete cue types was impaired. Importantly, lidocaine pretreatment did not impact the ability of rats to discriminate the cocaine-paired vs. saline-paired discrete cues as the main effect of treatment and its interaction with session type and/or cue exposure were not significant. For inactive lever responses, difference scores were significantly higher for rats in the short-term cue exposure group than for rats in the long-term cue exposure group [F (1, 26)=7.7, p≤0.01]. However, the actual difference between these two groups was relatively small (mean=8.4±1.9 for rats in the short-term cue exposure group, mean=0.4±2.0 for rats in the long-term cue exposure group).

Contextual/Discrete Cue-Induced Reinstatement Test

Lidocaine treatment had no effect on contextual/discrete cue-induced reinstatement of cocaine-seeking behavior in rats with long-term and short-term exposure to the contextual cues (Figure 6, inset). One-sample t-tests comparing difference scores revealed reinstatement values that were significantly greater than zero in all groups (p≤0.05). A one-way ANOVA comparing the degree of reinstatement above extinction levels revealed a no significant differences among the four groups.

A three-way ANOVA assessing discrimination between cues during the contextual/discrete cues reinstatement test (Figure 6, main graph) revealed only a significant main effect of session type [F (1, 26)=7.2, p≤0.05]. Though this analysis indicated that there was significant discrimination between cocaine-paired and saline-paired cue contextual/discrete cues overall, it appeared discrimination was driven mainly by the long-term cue exposure group treated with vehicle during conditioning. However, the session type × cue exposure × treatment interaction did not reach statistical significance. An analysis of difference scores for inactive lever responding showed no significant differences due to treatment, cue exposure, or cue condition.

Discussion

Role of the Hippocampus in Regulating Contextual Cue-Induced Reinstatement of Cocaine-Seeking Behavior

Lidocaine inactivation of the ventral hippocampus during conditioning blocked contextual cue-induced reinstatement of cocaine-seeking behavior in rats with 3 days of exposure to the contextual cues but not in rats with at least 21 days of exposure to the contextual cues. A previous study showed that tetrodotoxin inactivation of the dorsal hippocampus just prior to reinstatement testing blocked contextual cue-induced but not discrete cue-induced reinstatement of cocaine-seeking behavior (Fuchs et al., 2005). In that study, reinstatement tests were given after rats had only 10 sessions with cocaine and cocaine-paired cues. Collectively, these findings suggest an important role for both the dorsal and ventral hippocampus in regulating contextual cue-induced reinstatement of cocaine-seeking behavior and that this role is consistent with hippocampal learning theory.

Based on present and previous findings, we suggest that hippocampal processing of drug-related information is still in progress after 10 contextual stimulus-cocaine pairings, but not after 21 contextual stimulus-cocaine pairings. Other types of learning and memory studies support a similar timeline of ventral hippocampal involvement during acquisition of associative information, with the ventral hippocampus required 1–5 days after training, but not 25–30 days after training (Bontempi et al., 1999; Gusev et al., 2005). Because we only looked at two time points in the present study, further investigation is needed to determine a more specific time course for hippocampal involvement in the associations between drugs and drug-paired contextual cues. Moreover, future studies should inactivate brain sites outside the ventral hippocampus during conditioning to assess the anatomical selectivity of the effects of lidocaine for reducing reinstatement of cocaine-seeking behavior after short-term contextual cue exposure.

An alternative explanation for these results in the cocaine-paired context could be that the effect of lidocaine was simply due to a depression of locomotor activity caused by inactivation of the ventral hippocampus. Ventral hippocampal inactivation has been reported to significantly reduce locomotor activity in some studies (Bast et al., 2001) but not in others (Bardgett and Henry, 1999; Rogers and See, 2007). Because we found differences between lidocaine-treated rats in the short-term and long-term cue exposure groups, the effect of lidocaine into the ventral hippocampus was not likely to be due to a general reduction in locomotor activity in the present study. Perhaps most pertinent, the reduction in reinstatement of cocaine-seeking behavior in the short-term group was observed more than two weeks after lidocaine was administered.

The self-administration results during conditioning indicate that the ventral hippocampus is not involved in mediating responding for or intake of cocaine. This is supported by an earlier finding from this laboratory showing that the dorsal, but not ventral, hippocampus was involved in mediating cocaine-seeking and cocaine-taking behavior studied under a second-order schedule of reinforcement (Black et al., 2004). Also, the fact that there were no differences between lidocaine- and vehicle-treated rats in lever responding associated with saline and saline-paired cues indicates that lidocaine did not have any non-specific effects on responding, since this condition also serves as a control for the effects of lidocaine on responding measured in the absence of cocaine and the cocaine-paired cues. In support of this, Rogers and See (2007) reported that GABA agonist inactivation of the ventral hippocampus did not significantly impact lever responding during extinction or lever responding following a saline injection. Regardless of length of exposure to the contextual cues, lidocaine impaired ability of rats to discriminate between contextual cocaine-paired (CS^+) and saline-paired (CS^-) cues. There is evidence from fear conditioning studies that the hippocampus is involved in discrimination between contextual cues, such that pre-training hippocampal lesions did not

block contextual cue-induced reinstatement of freezing behavior, but did impair discrimination between contextual shock-paired (CS^+) and non-shock-paired (CS^-) cues (Desmedt et al., 2003; Frankland et al., 1998). These findings suggest that the ventral hippocampus is involved in the processing of contextual CS^- cues, and is required for discrimination between contextual CS^+ and CS^- cues. Though the rats in the long-term contextual cue exposure group had previously learned the discrimination between the contextual CS^+ and CS^- cues, lidocaine inactivation of the ventral hippocampus during the last three days of experience with these cues was enough to disrupt cue discrimination without disrupting reinstatement of cocaine-seeking behavior in the presence of the cocaine-paired cues. A dissociation between the effects of lidocaine inactivation of the ventral hippocampus on reinstatement of cocaine-seeking behavior and cue discrimination in rats with long-term contextual cue exposure is in keeping with pharmacological evidence indicating that although the processes involved in regulating reinstatement of cocaine-seeking behavior and discrimination overlap, these behaviors are not invariably linked and do not reflect different behavioral expressions of a unitary neurobiological process (Spealman et al., 1999).

Role of the Hippocampus in Regulating Discrete Cue-Induced Reinstatement of Cocaine-Seeking Behavior

In contrast to results for contextual cue-induced reinstatement, lidocaine inactivation of the ventral hippocampus during conditioning did not affect performance in tests for discrete cue-induced reinstatement of cocaine-seeking behavior, even though rats had only three sessions of exposure to the discriminable discrete stimulus cues prior to extinction sessions. In contrast, Rogers and See (2007) recently showed that GABA agonist inactivation of the ventral hippocampus reduced discrete cue-induced reinstatement of cocaine-seeking behavior. Similarly, Sun and Rebec (2003) found that lidocaine inactivation of the ventral subiculum just prior to the reinstatement test reduced discrete cue-induced reinstatement of cocaine-seeking behavior. The discrepancy may be due to the fact that rats in the present study learned the association between cocaine and the cocaine-paired discrete cues in the presence of a distinct context, whereas rats in the other studies did not. This suggests that during processing of associations involving discrete cues, the ventral hippocampus may be more recruited in situations that lack distinct contextual cues, but less recruited when distinct contextual cues are also present. In support of this idea, fear conditioning with discrete cues presented within a distinct context was shown to be hippocampal-independent (Huff and Rudy, 2004; Walker et al., 2005).

Rats with short-term exposure to both contextual and discrete cues showed an inability to discriminate between the discrete cocaine-paired (CS^+) and saline-paired (CS^-) cues. This suggests that although three days of exposure to these cues is sufficient for the cocaine-paired cues to reinstate responding, this number of sessions is not sufficient for rats to successfully discriminate discrete CS^+ and CS^- cues. However, rats with long-term contextual cue exposure but equivalent short-term discrete cue exposure were able to successfully discriminate between the discrete cues. This may have been because the task was more difficult for rats in the short-term contextual cue exposure group, since consolidation of information acquired in novel environments is not as good as in familiar environments (Kentros et al., 2004). Another contributing factor may be the compound nature of the stimuli presented during the 3-day conditioning procedure (3 types of contextual cues plus a discrete stimulus cue in each cue condition). Since all of these components were novel to the rats in the short-term contextual cue exposure group, they may not have been able to attend to the contextual cues and the discrete cues separately.

Role of the Hippocampus in Regulating Contextual/Discrete Cue-Induced Reinstatement of Cocaine-Seeking Behavior

Previous research from this laboratory has shown that lidocaine inactivation of either the dorsal or ventral subiculum of the hippocampus had no effect on contextual/discrete cue-induced reinstatement of cocaine-seeking behavior (Black et al., 2004). In that study, lidocaine may have been ineffective in blocking reinstatement of cocaine-seeking behavior due to the rats' long-term exposure to cocaine and cocaine-paired cues prior to inactivation of the hippocampal brain sites. In the present study, however, even lidocaine-treated rats with short-term exposure to the contextual/discrete cues showed reinstatement of cocaine-seeking behavior in the compound stimulus test. Given impairment in the contextual, but not discrete, cue-induced reinstatement test in lidocaine-treated rats with short-term cue exposure, it is likely that the discrete cue in the compound stimulus test was sufficiently salient to induce reinstatement of cocaine-seeking behavior in the short-term cue exposure group.

Though all rats reinstated responding in the presence of the cocaine-paired contextual/discrete cues, they were impaired in ability to discriminate between the contextual/discrete CS^+ and CS^- cues. Rats in the short-term cue exposure group may have failed to discriminate between the cues regardless of treatment because they were more highly motivated to respond than the long-term cue exposure group. This is suggested by the fact that rats in the short-term cue exposure group had more active lever responses during cocaine sessions and earned more cocaine infusions than rats in the long-term cue exposure group during conditioning. The novelty of the cues to the short-term cue group may have enhanced their motivation to self-administer cocaine, which is supported by evidence that cocaine self-administration in the presence of novel contextual cues enhances motivation to work for cocaine on a progressive ratio schedule (Caprioli et al., 2007).

In contrast, vehicle-treated rats with long-term exposure to contextual cues and short-term exposure to discrete cues showed an improved ability to discriminate CS^+ and CS^- cues regardless of whether they were contextual or discrete. The long-term exposure to contextual cues in this group easily explains their improved ability to discriminate the cocaine-paired and saline-paired contextual cues. The ability of this group to discriminate between the discrete cues after only 3 days of exposure may be due to the fact that they learned the significance of these cues in a familiar environment. Previous studies have shown animals preferentially attend to novel cues in the presence of both novel and familiar cues (Besheer et al., 1999; Kentros et al., 2004). Also, presentation of a novel cue in a familiar context increases the salience of the novel cue as compared to presentation of a novel cue in a novel context in humans (McDermott et al., 2006). Attended cues are preferentially consolidated (Kentros et al., 2004). Because rats in the long-term cue exposure group had a great deal of prior experience with the contextual cues and had habituated to the presence of these cues during self-administration training sessions, they may have been better able to attend to and subsequently remember the only novel element of the conditioning sessions, the discrete cues, and use this information appropriately during the contextual/discrete cue-induced reinstatement test.

In summary, the present study provides evidence that the ventral hippocampus plays a role both in regulating acquisition of the association between cocaine and cocaine-paired contextual cues and in maintaining the discrimination of cocaine-paired and saline-paired contextual cues. These findings suggest that through an associative learning process, the hippocampal memory system is involved early on in the development of cocaine addiction. Once the cocaine-paired contextual cues are no longer novel, it plays less of a role. Other brain areas, such as the prefrontal cortex, may be more relevant for processing contextual cues that are no longer novel. Previous studies have shown that both lidocaine inactivation of the prelimbic prefrontal cortex or agranular insular prefrontal cortex (Di Pietro et al., 2006) and tetrodotoxin inactivation of dorsomedial prefrontal cortex (Fuchs et al., 2005) prior to reinstatement testing blocked

contextual cue- or contextual/discrete cue-induced reinstatement of cocaine-seeking behavior. These findings suggest that information about contextual cues may be stored in the prefrontal cortex. Further study of the interaction between hippocampal and cortical brain areas in an animal model of relapse would be useful for understanding the cognitive contributions to the addiction process in humans, leading to development of effective cognitive and pharmacological treatments.

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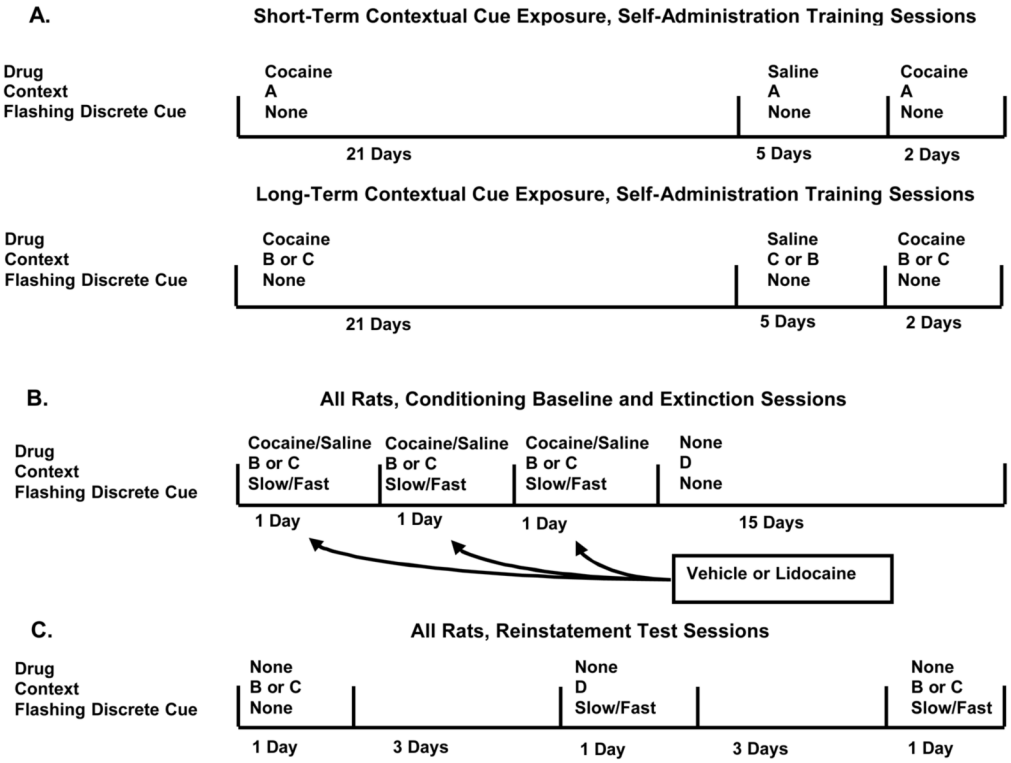

Figure 1.
Timeline of experimental procedures during self-administration training sessions (A), self-administration conditioning baseline sessions and extinction sessions (B), and reinstatement test sessions (C).

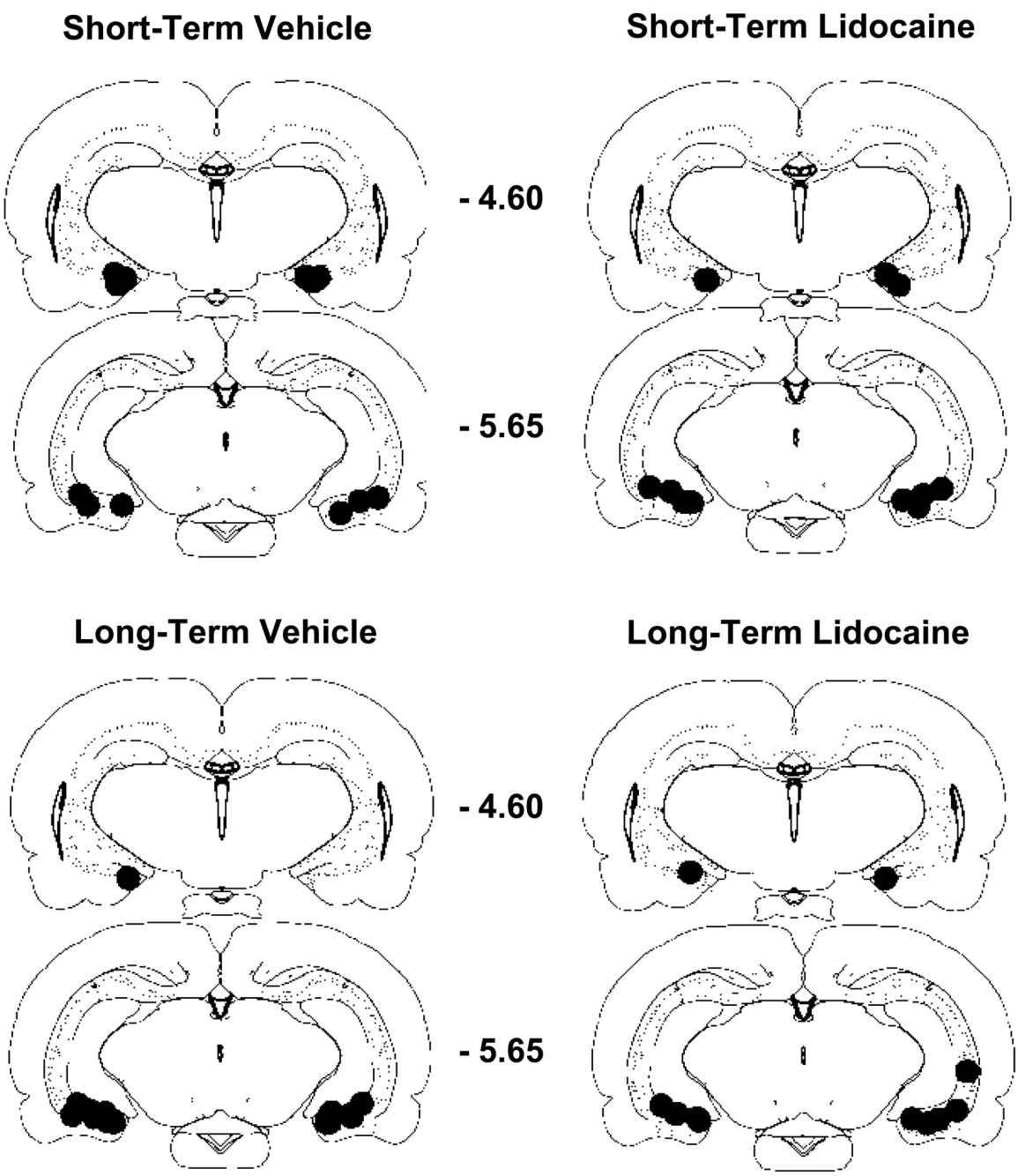

Figure 2.

Cannulae placements and functional spread of lidocaine and vehicle (circles with a 0.50 mm radius) in short-term and long-term cue exposure groups at AP levels −4.60 and −5.65 mm from bregma. The volume of lidocaine required to inactivate >90% of neurons within a particular radius from the infusion site is specified by the spherical volume equation, V=4/3Πr3 (Tehovnik and Sommer, 1997). Based on the spherical volume equation, the radius of the functional spread of 0.5 μL lidocaine, the volume used in the present study, is estimated to be 0.50 mm from the infusion site.

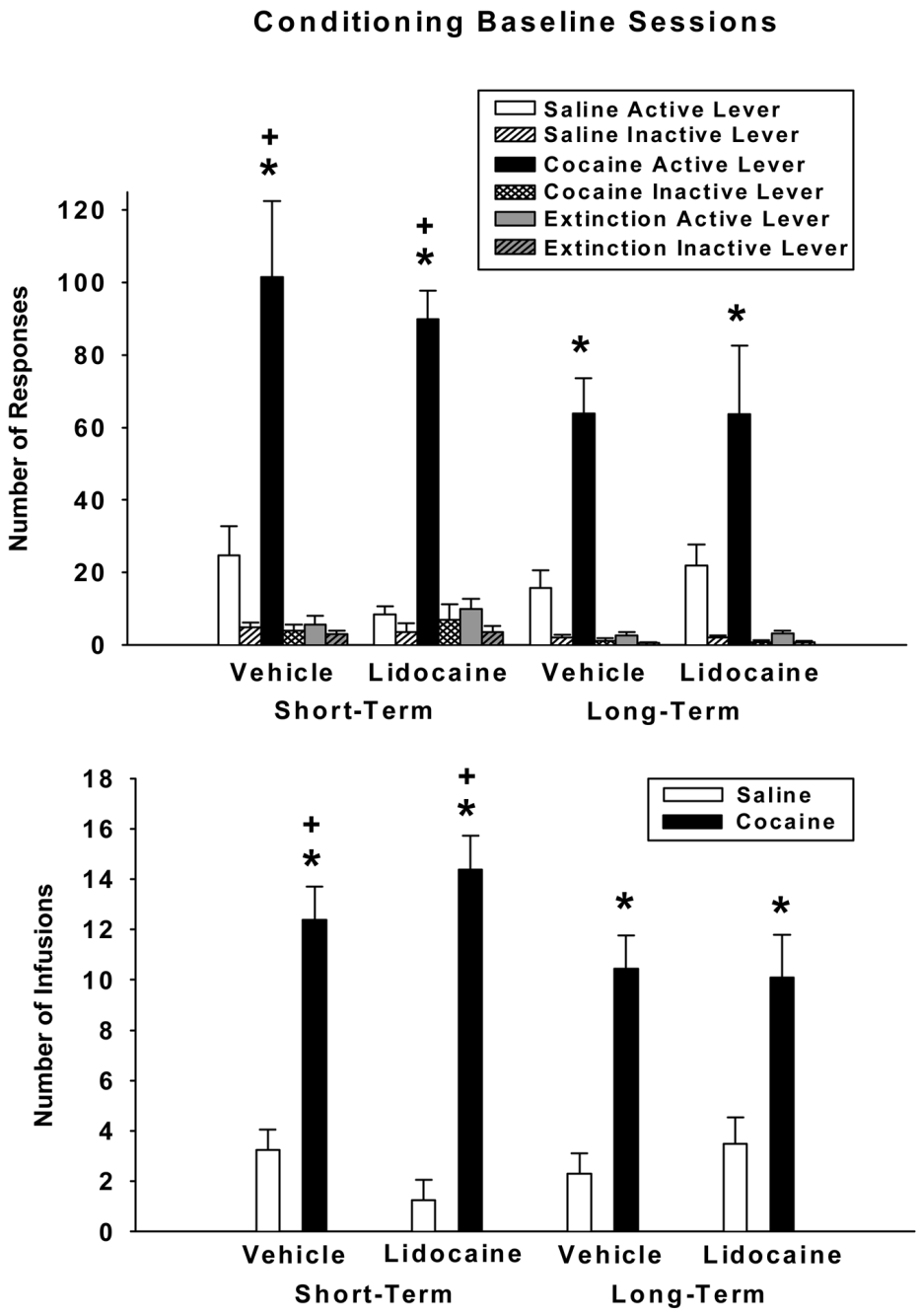

Figure 3.

Mean ± S.E.M. active and inactive lever responses during cocaine, saline and extinction sessions (top panel) and infusions during cocaine and saline sessions (bottom panel) for the last 2 days of the 3-day conditioning procedure and the last day of extinction in rats with short-term or long-term exposure to contextual cues. Prior to each session, animals received bilateral infusions of either lidocaine or vehicle into the ventral hippocampus. * $p \leq 0.05$ compared to all other conditions within the same treatment and cue exposure group; and + $p \leq 0.05$ compared to the corresponding long-term cue exposure condition within the same treatment group.

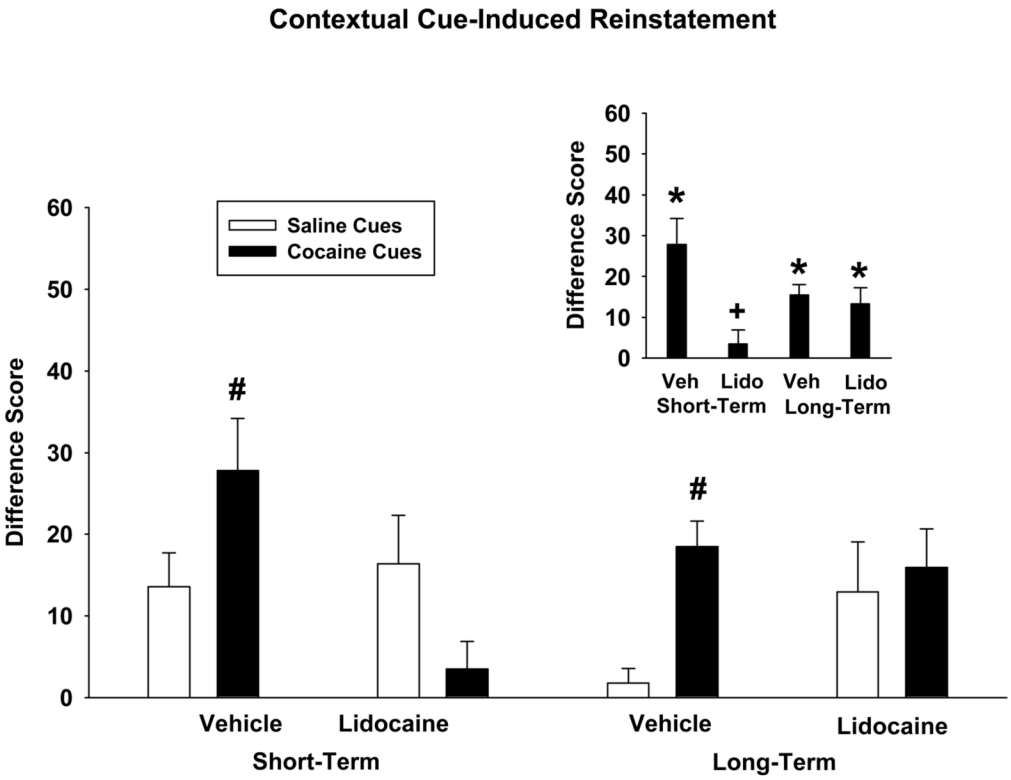

Figure 4.

Mean ± S.E.M. active lever response difference scores during cue-induced reinstatement tests with saline-paired and cocaine-paired contextual cues in rats with short-term and long-term exposure to the contextual cues. Inset shows active lever responses during cocaine-paired contextual cue sessions only. Raw score means for cocaine sessions were as follows: short-term vehicle=33.4±5.5, short-term lidocaine=13.4±3.6, long-term vehicle=18.0±2.6, long-term lidocaine=16.5±4.1. Inset: * $p \leq 0.05$ compared to zero, which indicates no increase in responding above extinction levels; + $p \leq 0.05$ compared to the other three groups. Main graph: # $p \leq 0.05$ compared to saline responses within treatment group.

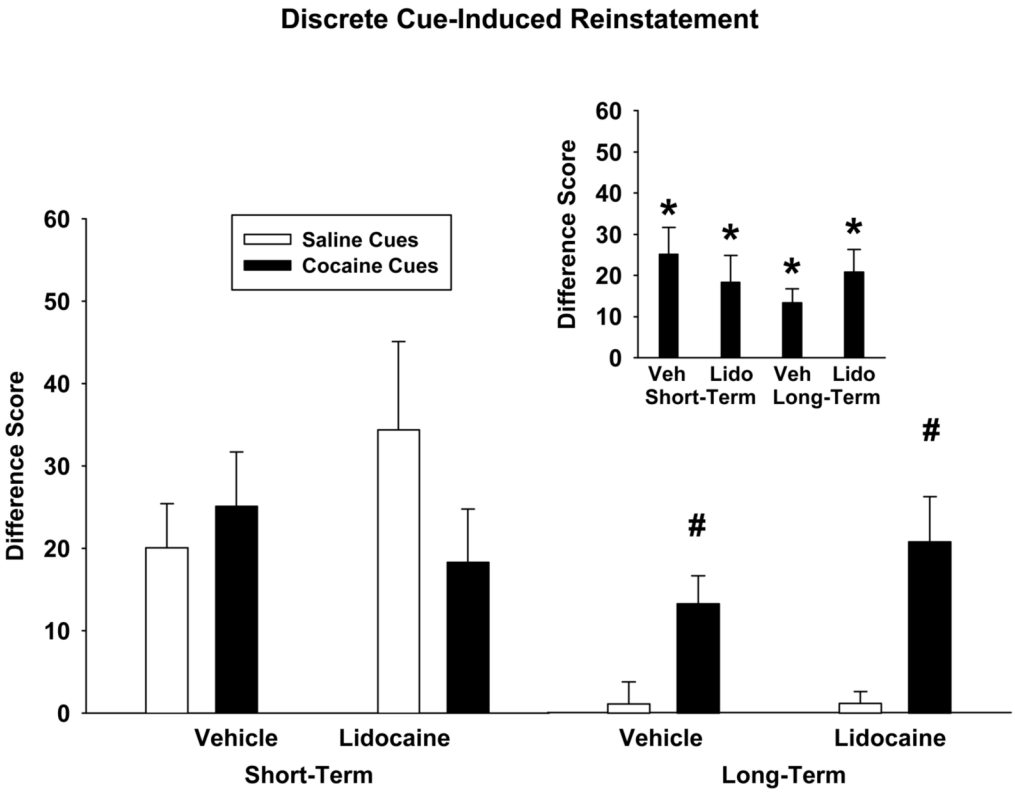

Figure 5.

Mean ± S.E.M. active lever response difference scores during cue-induced reinstatement tests with saline-paired and cocaine-paired discrete cues in rats with short-term and long-term exposure to the contextual cues. Inset shows active lever responses during cocaine-paired discrete cue sessions only. Raw score means for cocaine sessions were as follows: short-term vehicle=30.8±7.0, short-term lidocaine=28.1±8.1, long-term vehicle=15.9±3.7, long-term lidocaine=24.0±5.8. Inset: * $p \leq 0.05$ compared to zero, which indicates no increase in responding above extinction levels. Main graph: # $p \leq 0.05$ compared to saline responses within treatment group.

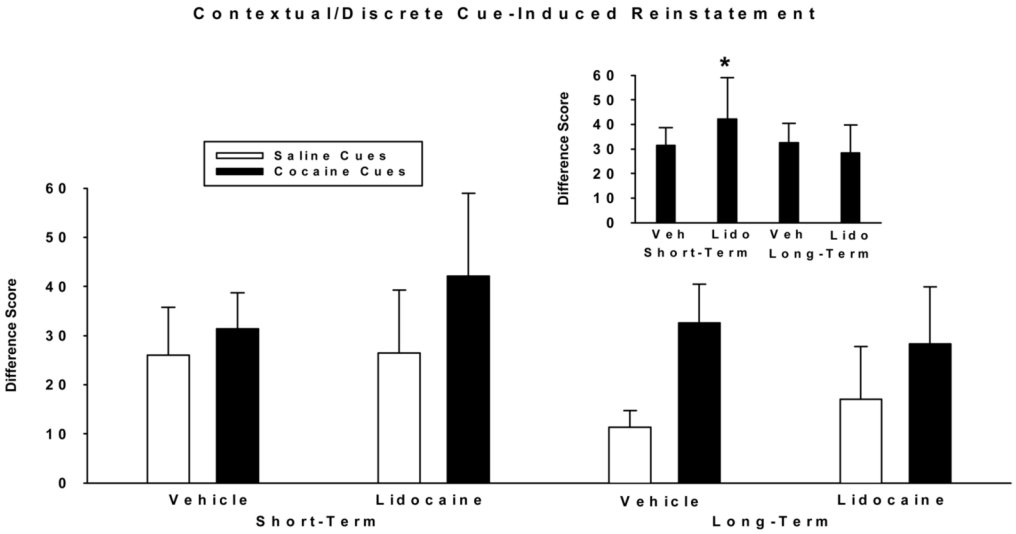

Figure 6.

Mean ± S.E.M. active lever response difference scores during cue-induced reinstatement tests with saline-paired and cocaine-paired contextual/discrete cues in rats with short-term and long-term exposure to the contextual cues. Inset shows active lever responses during cocaine-paired contextual/discrete cue sessions only. Raw score means for cocaine sessions were as follows: short-term vehicle=37.0±7.6, short-term lidocaine=52.0±14.9, long-term vehicle=35.3±8.4, long-term lidocaine=31.5±11.8. Inset: * $p \leq 0.05$ compared to zero, which indicates no increase in responding above extinction levels.

Table 1

Characteristics of the four environmental contexts used in this study.

Environment	Added Olfactory Contextual Cues	Added Auditory Contextual Cues	Added Visual Contextual Cues
Context A	Sani-Chip hardwood bedding (same as used in the home cages)	Ventilation fan noise	None (house light remained on)
Context B	Cedar bedding	Ventilation fan noise and intermittent tone (70 db; 7 kHz; 0.5 sec. duration every sec.)	Black panel on rear wall of chamber (house light remained on)
Context C	Pine bedding	Ventilation fan noise and continuous white noise (70 db)	Black and white striped panel on rear wall of chamber (house light remained on)
Context D	None (odorless Care Fresh bedding was used)	None (ventilation fan remained off)	None (house light remained on)