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NMDA receptor blockade in the basolateral amygdala disrupts consolidation of stimulus-reward memory and extinction learning during reinstatement of cocaine-seeking in an animal model of relapse

Matthew W. Feltenstein and Ronald E. See

Department of Neurosciences, Medical University of South Carolina, Charleston, SC, 29425, USA

Abstract

Previous research from our laboratory has implicated the basolateral amygdala (BLA) complex in the acquisition and consolidation of cue-cocaine associations, as well as extinction learning, which may regulate the long-lasting control of conditioned stimuli (CS) over drug-seeking behavior. Given the well established role of NMDA glutamate receptor activation in other forms of amygdalar-based learning, we predicted that BLA-mediated drug-cue associative learning would be NMDA receptor dependent. To test this hypothesis, male Sprague-Dawley rats self-administered i.v. cocaine (0.6 mg/kg/infusion) in the absence of explicit CS pairings (2-h sessions, 5 days), followed by a single 1-h classical conditioning (CC) session, during which they received passive infusions of cocaine discretely paired with a light+tone stimulus complex. Following additional cocaine self-administration sessions in the absence of the CS (2-h sessions, 5 days) and extinction training sessions (no cocaine or CS presentation, 2-h sessions, 7 days), the ability of the CS to reinstate cocaine-seeking on three test days was assessed. Rats received bilateral intra-BLA infusions (0.5 μ l/hemisphere) of vehicle or the selective NMDA receptor antagonist, 2-amino-5-phosphonovalerate (AP-5), immediately prior to the CC session (acquisition), immediately following the CC session (consolidation), or immediately following reinstatement testing (consolidation of conditioned-cued extinction learning). AP-5 administered before or after CC attenuated subsequent CS-induced reinstatement, whereas AP-5 administered immediately following the first two reinstatement tests impaired the extinction of cocaine-seeking behavior. These results suggest that NMDA receptor-mediated mechanisms within the BLA play a crucial role in the consolidation of drug-CS associations into long-term memories that, in turn, drive cocaine-seeking during relapse.

Keywords

amygdala; NMDA; glutamate; cocaine; relapse; reinstatement

Address correspondence to: Dr. Ronald E. See, Department of Neurosciences, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425, TEL: 843-792-2487, FAX: 843-792-4423, E-mail: seere@musc.edu.

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Introduction

One of the most significant problems for the long-term treatment of drug dependence is the high propensity of users to relapse to drug-seeking and drug-taking behaviors following prolonged periods of abstinence from drug use (Dackis and O'Brien, 2001; Wagner and Anthony, 2002). Of particular note, abstinent cocaine users often report intense drug craving when exposed to stimuli that have been previously associated with the drug of abuse (Childress, Hole, Ehrman, Robbins, McLellan, and O'Brien, 1993; Ehrman, Robbins, Childress, and O'Brien, 1992; Volkow, Wang, Telang, Fowler, Logan, Childress, Jayne, Ma, and Wong, 2006). Through the process of associative learning, previously neutral stimuli acquire incentive motivational value when repeatedly paired with drug use, and encounters with these conditioned stimuli (CS) may serve as environmental triggers of relapse to cocaine use (Wallace, 1989). Similarly, exposing animals to CS following withdrawal from chronic cocaine self-administration will robustly reinstate drug-seeking as measured by responding on a previously cocaine-paired lever (de Wit and Stewart, 1981; Meil and See, 1996; Shaham, Shalev, Lu, De Wit, and Stewart, 2003). The reinstatement model of relapse has not only allowed for the testing of compounds that may prevent relapse in abstinent drug users (Feltenstein, Altar, and See, 2007; Heidbreder, 2005), but has provided a model for extensive exploration of the neural circuitry that underlies conditioned cues and their role in relapse (Kruzich and See, 2001; Meil and See, 1997; Neisewander, Baker, Fuchs, Tran-Nguyen, Palmer, and Marshall, 2000; Weiss, Maldonado-Vlaar, Parsons, Kerr, Smith, and Ben-Shahar, 2000).

One neural substrate of particular interest in the study of cue-induced relapse is the basolateral complex of the amygdala (BLA). Using brain imaging techniques, a number of studies have noted an increase in amygdala activity when abstinent cocaine users are presented with drug-associated cues or drug-related imagery (Bonson, Grant, Contoreggi, Links, Metcalfe, Weyl, Kurian, Ernst, and London, 2002; Breiter, Gollub, Weisskoff, Kennedy, Makris, Berke, Goodman, Kantor, Gastfriend, Riorden, Mathew, Rosen, and Hyman, 1997; Childress, Mozley, McElgin, Fitzgerald, Reivich, and O'Brien, 1999; Grant, London, Newlin, Villemagne, Liu, Contoreggi, Phillips, Kimes, and Margolin, 1996; Kilts, Gross, Ely, and Drexler, 2004; Kilts, Schweitzer, Quinn, Gross, Faber, Muhammad, Ely, Hoffman, and Drexler, 2001). Consistent with these findings, studies in rodent models using excitotoxic lesions (Meil and See, 1997) or reversible pharmacological inactivation (Grimm and See, 2000; Kantak, Black, Valencia, Green-Jordan, and Eichenbaum, 2002; McLaughlin and See, 2003) have shown the importance of the BLA in the expression of conditioned-cued reinstatement of cocaine-seeking. However, in contrast to the fairly extensive studies of amygdalar substrates of fear conditioning (Davis, Rainnie, and Cassell, 1994; LeDoux, 2000), the role of the BLA in the acquisition and consolidation of drug-cue memories has only recently begun to be explored.

In order to assess the dynamic process of stimulus-cocaine associative learning, we developed a paradigm whereby stimuli are discretely paired with i.v. cocaine infusions during a classical conditioning session in rats with prior cocaine self-administration experience (Kruzich, Congleton, and See, 2001; See, 2005). Based on a single session, these stimuli will later reinstate drug-seeking long after their initial presentation and after prolonged withdrawal from cocaine. Using this procedure, we have demonstrated that sodium channel blockade with tetrodotoxin (Kruzich and See, 2001), a muscarinic acetylcholine receptor antagonist (See, McLaughlin, and Fuchs, 2003), or dopamine receptor antagonists (Berglund, Case, Parker, Fuchs, and See, 2006) within the BLA disrupt the acquisition of cocaine-stimulus associations, as evidenced by attenuation of conditioned-cued reinstatement. More recently, we have also applied this appetitive learning paradigm to demonstrate that the BLA is important in the consolidation of cocaine-cue associative learning, since neuronal inactivation after the classical conditioning session attenuated subsequent conditioned-cued reinstatement (Fuchs,

Feltenstein, and See, 2006b). Thus, the BLA is not only important during the expression of reinstatement of drug-seeking, but also for the acquisition and consolidation of cocaine-cue associations that can maintain cocaine-seeking during relapse.

The BLA has a large concentration of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors (Monaghan and Cotman, 1985) and a substantial amount of evidence suggests these receptors are critical for the formation of learned associations. For example, microinfusions of NMDA receptor antagonists (e.g., AP-5 or MK-801) into the BLA have been found to inhibit both the initial acquisition (Goosens and Maren, 2003; Lee and Kim, 1998; Maren, Aharonov, Stote, and Fanselow, 1996; Walker, Paschall, and Davis, 2005) and extinction (Lee and Kim, 1998; Lee, Milton, and Everitt, 2006) of conditioned fear behaviors. Similar effects have been noted for the acquisition (Ferry and Di Scala, 2000; Hatfield and Gallagher, 1995; Liang, Hon, and Davis, 1994) and consolidation (Ferry and Di Scala, 2000; Liang et al., 1994) of associative learning in other tasks, including the inhibitory avoidance task and taste-potentiated odor aversion. AP-5 administration into the BLA has also been found to inhibit the acquisition of learning in appetitive tasks, such as appetitive instrumental learning (Baldwin, Holahan, Sadeghian, and Kelley, 2000) and discriminative approach behaviors to an appetitive CS (Burns, Everitt, and Robbins, 1994). While our laboratory has previously shown that NMDA receptors in the BLA are not necessary for the expression of conditioned-cued reinstatement (See, Kruzich, and Grimm, 2001), it is unknown whether NMDA receptors are involved in the formation of CS-cocaine associations. Thus, the current study examined whether NMDA receptor antagonism in the BLA would impair the acquisition and/or consolidation of stimulus-reward associations and conditioned-cued extinction learning in an animal model of relapse.

Methods and Materials

Subjects

Male, Sprague-Dawley rats ($n = 32$, initial weight 250-275 g; Charles River, Wilmington, MA, USA) were individually housed in a temperature- and humidity-controlled vivarium on a 12-h reverse light-dark cycle (lights off 6 AM-6 PM). Animals were given water *ad libitum* and were maintained on 25 g of standard rat chow (Harlan, Indianapolis, IN, USA) per day for the duration of each experiment. Rats were acclimated to handling and allowed to adapt for a minimum of three days prior to the start of the experiment. Housing and care of the rats were carried out in accordance with the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

Lever response training

Rats were trained to lever press in standard self-administration chambers (30 × 20 × 20 cm) linked to a computerized data collection program (MED-PC, Med Associates, Inc., St. Albans, VT, USA). The chambers were equipped with two retractable levers, a white stimulus light above each lever, a food pellet dispenser between the levers, a tone generator (ENV-223HAM, Med Associates), and a house light on the wall opposite the levers. Each chamber was contained within a sound-attenuating cubicle equipped with a ventilation fan. Forty-eight hours prior to surgery, rats were food deprived overnight and trained to lever press along a fixed ratio (FR) 1 schedule of reinforcement (45 mg pellets; Noyes, Lancaster, NH, USA) during a 15-h overnight training session in the absence of explicit conditioned stimulus (CS) presentation (i.e., active lever presses resulted in the delivery of a food pellet only). Lever presses on an inactive lever were recorded, but had no programmed consequence. Following lever response training, food dispensers were permanently removed from the test chambers.

Surgery

Rats were anesthetized using a mixture of ketamine hydrochloride and xylazine (66 and 1.33 mg/kg, respectively, IP) followed by equithesin (0.5 ml/kg with a solution of 9.72 mg/ml pentobarbital sodium, 42.5 mg/ml chloral hydrate, and 21.3 mg/ml magnesium sulfate heptahydrate dissolved in a 44% propylene glycol, 10% ethanol solution, IP). Surgical procedures were conducted using aseptic techniques. Catheters were constructed using previously described methods (Fuchs, Evans, Parker, and See, 2004) and consisted of external guide cannulae with screw-type connectors (Plastics One, Inc., Roanoke, VA, USA), Silastic tubing (10 cm; ID = 0.64 mm; OD = 1.19 mm; Dow Corning, Midland, MI, USA), prolite polypropylene monofilament mesh (2 cm diameter, Atrium Medical Corporation, Hudson, NH, USA), and cranioplastic cement. A small incision was made on the back and chest of the rat 5 mm above the area where the jugular vein enters the rib cage. The external guide cannula exited from the incision on the rat's back and the open end of the Silastic tubing was passed subcutaneously to the area of the jugular vein. The free end of the tubing was inserted 33 mm into the right jugular vein and secured with 4.0 silk sutures. Both incisions were sutured with 4.0 sterile surgical thread.

Immediately after the catheter surgery, the rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL, USA). Bilateral stainless-steel guide cannulae (26 gauge, Plastics One, Inc.) were aimed 2 mm dorsal to the BLA (-2.7 mm AP, $\pm$ 5.0 mm ML, -6.6 mm DV) using standard stereotaxic procedures. Three small screws and cranioplastic cement secured the guide cannulae to the skull. Stylets (Plastics One, Inc.) were placed into the guide cannulae and catheter to prevent occlusions. To maintain catheter patency, catheters were flushed once daily for 4 days after surgery with 0.1 ml each of an antibiotic solution of cefazolin (100 mg/ml; Schein Pharmaceuticals, Florham Park, NJ, USA) dissolved in heparinized saline (70 U/ml; Elkins-Sinn, Cherry Hill, NJ, USA) and heparinized saline. For the duration of the experiment, each subject then received 0.1 ml of heparinized saline (10 U/ml) immediately prior to self-administration sessions and the cefazolin and 70 U/ml heparinized saline regimen following each session. To verify catheter patency, rats occasionally received a 0.12 ml infusion of methohexital sodium (10.0 mg/ml IV; Eli Lilly and Co., Indianapolis, IN, USA), a short-acting barbiturate that produces a rapid loss of muscle tone when administered intravenously.

Cocaine self-administration and classical conditioning

Fig.1 illustrates the phases of training and testing, and when the microinfusions occurred. Rats self-administered cocaine (cocaine hydrochloride dissolved in 0.9% sterile saline; cocaine provided by the National Institute on Drug Abuse, Research Triangle Park, NC, USA) during daily 2-h sessions according to an FR 1 schedule of reinforcement. At the start of each session, the catheter was connected to a liquid swivel (Instech, Plymouth Meeting, PA, USA) via polyethylene 20 tubing that was encased in steel spring leashes (Plastics One, Inc.). The swivels were suspended above the self-administration chamber and were connected to infusion pumps (model PHM-100, Med-Associates). The house light signaled the initiation of the session and remained illuminated throughout the entire session. Lever presses on the active lever resulted in a 2-s activation of the infusion pump (0.6 mg/kg cocaine per 50 μ l infusion) in the absence of explicit CS presentation (i.e., active lever presses resulted in the delivery of cocaine only). After each infusion, responses on the active lever had no consequences during a 20-s time-out period. During the sessions, responses on the inactive lever were recorded, but had no programmed consequences. Daily self-administration sessions continued until each rat had obtained the initial self-administration criterion of five sessions with at least ten infusions per session.

Twenty-four hours after the last day of initial cocaine self-administration training, rats underwent a single 1-h classical conditioning (CC) session in the self-administration chamber.

During this session, rats received passive infusions (i.e., levers were retracted) of cocaine and simultaneous 5-s presentations of a stimulus complex, consisting of activation of the stimulus light above the active lever and the tone generator (78 dB, 4.5 kHz). The number of pairings equaled the mean number of cocaine infusions earned during the first hour of the preceding two self-administration days as calculated for each individual rat.

CS-induced reinstatement of extinguished cocaine-seeking

To investigate the role of NMDA receptors in the BLA in the acquisition and consolidation of CS-cocaine associations, rats received bilateral intra-BLA microinfusions (0.5 μ l/hemisphere) of the selective NMDA receptor antagonist d-AP-5 (Tocris Bioscience, Ellisville, MO, USA) immediately prior to (0, 3, or 10 μ g) or immediately following (0 or 3 μ g) the CC session, respectively. The vehicle consisted of phosphate-buffered saline (PBS). Injection cannulae (33 gauge, Plastics One, Inc.) were inserted to a depth of 2 mm past the tip of the guide cannulae and remained in place for 2 min during the infusion and for 1 min both before and after the infusion.

After the CC session, rats underwent 5 additional days of cocaine self-administration sessions in the absence of explicit CS presentation. Subsequently, rats underwent daily 2-h extinction training, during which responses on both levers were recorded, but had no programmed consequences. Once the extinction criterion was reached (i.e., a minimum of seven extinction sessions with ≤ 25 active lever responses per session for 2 consecutive days), CS-induced reinstatement of extinguished cocaine-seeking behavior was assessed. During the 2-h reinstatement test session, responses on the active lever resulted in a 5-s presentation of the light + tone stimulus complex, followed by a 20-s time out, in the absence of cocaine reinforcement.

In our recent study, administration of TTX immediately following the first CS reinstatement test prevented the extinction of cocaine-seeking behavior on a subsequent reinstatement test (Fuchs et al., 2006b), suggesting that the BLA also plays an important role in conditioned-cued extinction memory consolidation. To investigate the role of NMDA receptors in the BLA in CS extinction memory consolidation, rats that had previously received vehicle treatment at the time of the CC session (either at acquisition or consolidation) were assigned to receive bilateral intra-BLA microinfusions (0.5 μ l/hemisphere) of AP-5 (3 μ g) or vehicle immediately after each of the first two reinstatement tests. Daily 2-h extinction sessions occurred between each reinstatement test to criterion.

Histology and data analysis

After all testing was completed, rats were transcardially perfused with PBS and 10% formaldehyde solution. The brains were dissected and stored in 10% formaldehyde solution prior to sectioning. Using a vibratome (Technical Products International, St. Louis, MO, USA), brains were sectioned in the coronal plane (75 μ m thickness), mounted on gelatin-coated slides, and stained for Nissl substance with cresyl violet (Kodak, Rochester, NY, USA). The sections were examined under a light microscope using 10x magnification. The most ventral point of the microinjector tips were mapped onto schematics of the appropriate plates using a rat brain atlas (Paxinos and Watson, 1997). Analyses of active and inactive lever responding and cocaine intake during self-administration, number of CC pairings, and lever responses during extinction and CS reinstatement testing were conducted using one- or two-way repeated measures ANOVA and one-way ANOVA or *t* tests, where appropriate.

Results

Cocaine self-administration, classical conditioning, and extinction

Schematic representations of the most ventral point of bilateral BLA injection cannulae for animals in the acquisition-vehicle ($n = 6$), acquisition-3 μg AP-5 ($n = 6$), acquisition-10 μg AP-5 ($n = 5$), consolidation-vehicle ($n = 9$) and consolidation-3 μg AP-5 ($n = 6$) groups are indicated in Fig. 2. Data for active and inactive lever responding are shown across the various experimental phases for the acquisition (Fig. 3) and consolidation (Fig. 4) groups. Both groups of rats readily acquired cocaine self-administration, responded preferentially on the active lever, and displayed stable lever responding and cocaine intake by the end of the first five days of self-administration. Data analyses failed to reveal any significant pre-existing treatment group differences in active or inactive lever responding or daily cocaine intake for animals in the acquisition group ($F_{2,14} = 0.70\text{--}1.32$, $P = 0.30\text{--}0.51$). Similar analyses for animals in the consolidation group also revealed no significant treatment group differences in active or inactive lever responding or cocaine intake ($t_{13} = 0.16\text{--}0.83$, $P = 0.42\text{--}0.87$). Following the first phase of cocaine self-administration, all animals underwent a 1-h CC session. No significant differences in the number of CS-cocaine pairings (mean $\pm$ SEM) were noted for animals in the acquisition (vehicle = 10.50 ± 2.25 , 3 μg AP-5 = 13.00 ± 1.92 , 10 μg AP-5 = 13.80 ± 0.7 , $F_{2,14} = 0.86$, $P = 0.44$) or the consolidation (vehicle = 14.67 ± 1.95 , 3 μg AP-5 = 10.83 ± 1.49 , $t_{13} = 1.43$, $P = 0.18$) groups. Moreover, data analyses failed to reveal any significant treatment group differences in active or inactive lever responding or cocaine intake during the second phase of cocaine self-administration training, nor were any treatment group differences found for active or inactive lever responding at the end of extinction training for animals in the acquisition ($F_{2,14} = 0.34\text{--}1.49$, $P = 0.26\text{--}0.72$) or the consolidation groups ($t_{13} = 0.59\text{--}1.53$, $P = 0.15\text{--}0.57$).

CS reinstatement testing

Following extinction training, exposure to the cocaine-paired CS led to a selective increase in responding on the previously drug-paired lever, an effect that was dose-dependently attenuated by pre-CC infusions of AP-5 in the BLA (Fig. 3). Active lever responding during the conditioned-cued reinstatement test was significantly increased over extinction in the vehicle-treated ($t_{10} = 3.96$, $P < 0.005$) and the 3 μg AP-5 ($t_{10} = 3.36$, $P < 0.01$) groups, but did not significantly differ from extinction level responding for animals in the 10 μg AP-5 group ($t_8 = 1.75$, $P = 0.12$). Compared to the vehicle group, animals that received 10 μg AP-5 ($t_9 = 2.56$, $P < 0.05$), but not 3 μg AP-5 ($t_{10} = 0.48$, $P = 0.64$), showed a significant attenuation in CS-induced reinstatement. Finally, there were no significant group differences in inactive lever responding during reinstatement testing for animals in the acquisition groups.

As seen in Fig. 4, vehicle-treated animals in the consolidation group showed a significant increase in active lever responding, relative to extinction responding, during the CS reinstatement test ($t_{13} = 6.90$, $P < 0.001$). Moreover, this effect was attenuated when AP-5 (3 μg) was selectively infused into the BLA ($t_{13} = 3.83$, $P < 0.005$). Although significantly reduced relative to the vehicle group, CS-reinstatement was not completely abolished, since AP-5 treated rats did show higher responding over extinction levels ($t_{10} = 3.70$, $P < 0.005$). There were also no significant group differences in inactive lever responding during CS reinstatement testing for animals in the consolidation groups.

CS extinction memory consolidation

Fig. 5 shows active and inactive lever responding for animals assigned to receive post-CS reinstatement testing infusions of vehicle ($n = 7$) or AP-5 ($n = 6$). Statistical analyses showed no significant pre-existing treatment group differences in active lever responding or cocaine intake during cocaine self-administration, extinction, or the first CS reinstatement test ($t_{11} =$

0.27-0.90, $P = 0.39-0.80$). Moreover, no significant differences in the number of CS-cocaine pairings (mean $\pm$ SEM) were noted (vehicle = 14.71 ± 2.52 , 3 μ g AP-5 = 11.83 ± 1.97 , $t_{11} = 0.88$, $P = 0.40$). Similar analyses for inactive lever responding only revealed a modest, but significant difference between these groups at the end of extinction training ($t_{11} = 2.78$, $P < 0.05$). A two-way repeated measures ANOVA on the CS reinstatement data revealed main effects for group ($F_{1,11} = 5.02$, $P < 0.05$) and test ($F_{2,22} = 6.78$, $P = 0.005$). The group $\times$ test interaction was not significant. Further one-way repeated measures ANOVA for each group showed a significant test effect for animals that received vehicle ($F_{2,12} = 11.03$, $P < 0.005$), but not AP-5 ($F_{2,10} = 0.81$, $P = 0.47$). For animals in the vehicle group, post-hoc comparisons of subsequent tests to CS test 1 revealed a significant decrease in active lever responding on both the second ($t_{12} = 2.29$, $P < 0.05$) and third ($t_{12} = 2.81$, $P < 0.05$) tests. Relative to extinction responding, rats that received intra-BLA vehicle infusions exhibited extinction learning across tests, as seen by a significantly higher level of active lever responding on the first CS-reinstatement test ($t_{12} = 4.43$, $P < 0.001$), but not on subsequent tests ($t_{12} = 0.91-1.66$, $P = 0.12-0.38$). In contrast, rats that received intra-BLA infusions of AP-5 immediately after the CS-reinstatement test maintained significantly higher responding over extinction levels ($t_{10} = 2.93-8.89$, $P < 0.001-0.05$). Relative to vehicle-treatment, intra-BLA infusions of AP-5 just after the previous test led to significantly greater active lever responding on the last CS-reinstatement test ($t_{11} = 2.23$, $P < 0.05$). Finally, no significant group differences in inactive lever responding were seen during CS-reinstatement testing or intervening extinction days.

Discussion

Previous research has implicated the BLA in the process of neutral stimuli acquiring incentive-motivational properties when paired with reward (Cador, Robbins, and Everitt, 1989; Everitt, Parkinson, Olmstead, Arroyo, Robledo, and Robbins, 1999; Hatfield, Han, Conley, Gallagher, and Holland, 1996; Whitelaw, Markou, Robbins, and Everitt, 1996). As we have previously demonstrated (Fuchs et al., 2006b), exposure to CS-cocaine pairings during a single 1-h CC session subsequently led to robust reinstatement of cocaine-seeking behavior (i.e., increased responding on the previously cocaine-paired lever) in vehicle-treated animals. Intra-BLA blockade of NMDA receptors by AP-5 at the time of either acquisition or consolidation significantly disrupted cocaine-CS associative learning as seen by the decrease in conditioned-reinstatement, although disruption of acquisition required a higher concentration of AP-5. When given immediately following a series of CS-reinstatement tests, AP-5 also attenuated the extinction of CS-induced cocaine-seeking behavior that normally occurs following exposure to non-reinforced CS presentations (i.e., the consolidation of CS-no cocaine associations). Thus, the current results show that NMDA receptor mediated activity in the BLA plays an important role in the associative learning of environmental stimuli relevant to cocaine-seeking behavior.

The current results are consistent with our previous work, which found that post-CC session inactivation of the BLA by the Na⁺-channel blocker tetrodotoxin (TTX) led to an attenuation of CS-induced reinstatement (Fuchs et al., 2006b). Although not an appetitive task, similar results have been noted for inhibition of memory formation of inhibitory avoidance via post-training intra-amygdala infusions of NMDA receptor antagonists (Jerusalinsky, Ferreira, Walz, Da Silva, Bianchin, Ruschel, Zanatta, Medina, and Izquierdo, 1992; Liang et al., 1994). These effects were dose- and time-dependent, in that AP-5 administered ≥ 90 min after training had no effect of memory retention. Further support for the role of the BLA in memory consolidation comes from studies examining the administration of protein synthesis inhibitors. In one such study, intra-BLA administration of protein synthesis (anisomycin) or protein kinase A (Rp-cAMPS) inhibitors immediately after training led to a significant dose-dependent reduction in Pavlovian fear conditioning (Schafe and LeDoux, 2000), and these effects were time-dependent, since administration 6 h after conditioning had no effects.

While we anticipated that NMDA receptors would be important in cocaine-cue associative learning, the differences in the dose of AP-5 required to disrupt acquisition vs. consolidation were unexpected (i.e., a higher dose of AP-5 was required when administered prior to CC to achieve a significant reduction in drug-seeking during cue-induced reinstatement). It is possible that this difference may have resulted from changes in neural activity that occurred in the presence of cocaine during the acquisition phase, specifically dopamine-mediated mechanisms in associative learning via cocaine-induced elevated DA levels. Previous research has indicated a significant increase in extracellular DA in the amygdala, including the lateral and basal nuclei, during exposure to psychostimulants (Harmer, Hitchcott, Morutto, and Phillips, 1997; Young and Rees, 1998). This enhancement in DA activity during the time of CC could have potentiated drug-CS associative learning, even in the presence of AP-5. In support of this possibility, we have previously found a potentiation of cue-induced reinstatement following intra-BLA infusions of d-amphetamine (Ledford, Fuchs, and See, 2003). Furthermore, we have shown that either a dopamine D₁ receptor antagonist (SCH23390) or a dopamine D_{2/3} receptor antagonist (raclopride) administered prior to the CC session attenuated the later expression of conditioned-cued reinstatement (Berglind et al., 2006). It is also possible that the higher dose of AP-5 was effective when given immediately prior to the CC session in that its effects carried over to the consolidation phase. That is, while the 3 µg dose of AP-5 may have been metabolized and/or removed over the course of the 1-h CC session, the 10 µg dose may have remained active after the conclusion of the session, thus inhibiting the consolidation of drug-CS associative learning. Alternatively, the higher dose of AP-5 could have had non-specific effects independent of learning. Indeed, higher doses of NMDA antagonists have been known to produce sensorial disturbances (Tan, Kirk, Abraham, and McNaughton, 1989; Tang and Ho, 1988). Similar to the current results, intra-accumbens infusions of AP-5 in a passive avoidance learning task produced only a modest dose-dependent effect when given prior to training, while the same doses produced greater effects when administered at the time of consolidation (De Leonibus, Costantini, Castellano, Ferretti, Oliverio, and Mele, 2003). In a morphine conditioned place preference paradigm, administration of a protein synthesis inhibitor (anisomycin) that was effective at preventing memory consolidation had no effect when given prior to training (Robinson and Franklin, 2007), suggesting that different processes may be involved in memory acquisition versus consolidation. Future studies should investigate the roles of different glutamate receptor subtypes during these various stages of drug-CS associative learning.

Based on previous amygdalar learning paradigms, it is likely that AP-5 disrupts cocaine-cue associative learning by attenuation of NMDA receptor-dependent long-term potentiation (LTP). This hypothesis is supported by studies in which intra-BLA infusions of AP-5 impaired the acquisition, but not the expression, of approach behaviors to an appetitive CS (Burns et al., 1994) and appetitive instrumental behaviors (Baldwin et al., 2000). LTP is a neuronal model of learning that has been demonstrated to occur *in vitro* (Chapman and Bellavance, 1992; Chapman, Kairiss, Keenan, and Brown, 1990) and *in vivo* (Clugnet and LeDoux, 1990; Maren and Fanselow, 1995) in the BLA, and appears to require the activation of NMDA receptors (Gean, Chang, Huang, Lin, and Way, 1993; Huang and Kandel, 1998; Maren and Fanselow, 1995), although there are some exceptions (Chapman and Bellavance, 1992).

Consistent with the effect we previously found with tetrodotoxin (Fuchs et al., 2006b), intra-BLA infusions of AP-5 administered immediately after the CS reinstatement tests inhibited subsequent conditioned-cued extinction learning, presumably by preventing the consolidation of new information about the changed value of the CS (i.e., CS-no drug associations). These effects are similar to anisomycin-induced prevention of incentive learning in the amygdala related to the reduction of a food reward when given immediately after a series of devaluation sessions (Wang, Ostlund, Nader, and Balleine, 2005), and the prevention of extinction of cocaine-seeking behavior in a context-induced reinstatement model of relapse (Fuchs, Eaddy,

Su, and Bell, 2006a). Contrary to short-term memories, the consolidation of long-term extinction memories appears to be an NMDA receptor-mediated event (Santini, Muller, and Quirk, 2001), as supported by a series of studies using fear-conditioning paradigms in which intra-BLA NMDA receptor blockade produced long-term deficits in extinction of conditioned-freezing (Lee and Kim, 1998; Lee et al., 2006) and fear-potentiated startle (Falls, Miserendino, and Davis, 1992) behaviors. Moreover, overexpression of an NMDA receptor subunit (NR2B) has been found to enhance extinction learning in mice (Tang, Shimizu, Dube, Rampon, Kerchner, Zhuo, Liu, and Tsien, 1999), an effect further validated by studies that have shown facilitation of extinction learning in animals that received systemic (Boutreau, Paolone, and Stewart, 2006; Lee et al., 2006) or intra-BLA infusions (Lee et al., 2006) of the partial NMDA receptor agonist, D-cycloserine.

According to the “trace dominance” hypothesis (Dudai, 2004; Nader, 2003), re-exposure to the CS alone can lead to 2 opposing processes: the “reconsolidation” of the original CS-US memory (i.e., reminder learning) and the consolidation of CS-no US memories (i.e., extinction learning). These processes are thought to occur simultaneously (Debiec, LeDoux, and Nader, 2002; Eisenberg, Kobilo, Berman, and Dudai, 2003); however, which of the two processes dominates depends on a number of factors. When exposure is limited, the CS produces internal reinforcement by its own conditioned reinforcing properties and “reconsolidation” of the original CS-US memory is thought to dominate (Eisenhardt and Menzel, 2007). However, continuous exposure to the CS in the absence of external reinforcement (i.e., the US) results in extinction learning becoming stronger, while the reminder memory becomes simultaneously weakened. Given this proposed interaction, it can be hypothesized that the administration of amnesic agents at different time points would lead to different outcomes, such that the inhibition of “reminder learning” would result in the CR decreasing, whereas the inhibition of “extinction learning” would result in the opposite (i.e., the CR is maintained). In support of this theory, intra-BLA infusions of antisense oligodeoxynucleotides that target the protein Zif268 following brief re-exposure (15 min) to a cocaine-paired CS prevented the ability of the CS to facilitate the acquisition of new cocaine-seeking behavior (Lee, Di Ciano, Thomas, and Everitt, 2005), consistent with an inhibition of reconsolidation. In a direct temporal comparison, intra-BLA infusions of anisomycin immediately after 15 min of re-exposure to a previously drug-paired context attenuated context-induced subsequent reinstatement of cocaine-seeking, whereas the same treatment after 120 min of re-exposure led to the opposite effect (i.e., continued reinstatement over subsequent tests), indicating time-dependent inhibition of reconsolidation or consolidation of extinction learning, respectively (Fuchs et al., 2006a). Thus, future studies should examine the temporal role of the BLA in the consolidation and reconsolidation of drug-stimulus associative learning, as well as any putative interactions among these competing processes.

Along with previous studies, the current results suggest a fundamental role of the amygdala in a number of stages of drug-cue associative learning. While it is impossible to intervene in the initial acquisition and consolidation of the drug-cue associations that play a critical role in both ongoing drug-seeking behavior and relapse (Gawin, 1991), various possibilities exist for the utilization of drugs that either inhibit the reconsolidation of drug-CS associations, or facilitate the consolidation of extinction learning (i.e., CS-no drug associations). While extinction therapy has been shown to attenuate conditioned responses to previously drug-paired stimuli (O'Brien, Childress, McLellan, and Ehrman, 1992), its effectiveness to date appears fairly limited. Thus, the administration of adjunctive pharmacotherapies that modulate glutamate receptors (e.g., D-cycloserine), in combination with behaviorally-based therapies, may prove especially beneficial for reducing the impact of drug-paired environmental stimuli to precipitate drug craving and relapse.

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Figure 1.

Schematic representing the phases of cocaine self-administration training (SA I and II), the classical conditioning session (CC), extinction (EXT), and CS reinstatement testing (CS I, II, and III). Arrows indicate when the bilateral intra-BLA microinfusions occurred. Animals in the acquisition and consolidation groups received their microinfusions immediately prior to and following the CC session, respectively, and underwent a single CS test. Animals in the consolidation of extinction learning group all received vehicle treatment at the time of the CC session (either at acquisition or consolidation) and then received subsequent microinfusions (either AP-5 or vehicle) immediately after each of the first two reinstatement tests.

Figure 2. Coronal sections (adapted from Paxinos and Watson, 1986) with graphical representations of cannula tip placements in the BLA. Numbers to the left of the sections indicate A/P distance from bregma in mm.

Figure 3.

Mean ($\pm$ SEM) active (top panel) and inactive (bottom panel) lever responses for the last 2 days of self-administration (SA) phases I and II, extinction (EXT), and during the CS reinstatement test. Animals received bilateral intra-BLA infusions of vehicle or AP-5 immediately prior to the CC session (acquisition). Significant differences are indicated as compared to extinction levels (** $P < 0.005$; ** $P < 0.01$) or vehicle ($\dagger P < 0.05$).

Figure 4.

Mean ($\pm$ SEM) active (top panel) and inactive (bottom panel) lever responses for the last 2 days of self-administration (SA) phases I and II, extinction (EXT), and during the CS reinstatement test. Animals received bilateral intra-BLA infusions of vehicle or AP-5 immediately after the CC session (consolidation). Significant differences are indicated as compared to extinction levels (** $P < 0.005$; ** $P < 0.01$) or vehicle ($\dagger P < 0.05$).

Figure 5. LEFT: Mean ($\pm$ SEM) active (top panel) and inactive (bottom panel) lever responses for the last 2 days of self-administration (SA) phases I and II and extinction (EXT). All animals received vehicle during the initial CC session. RIGHT: Mean ($\pm$ SEM) active (top panel) and inactive (bottom panel) lever responses during the three CS reinstatement tests. Animals received bilateral intra-BLA infusions of vehicle or AP-5 immediately after the first and second CS tests. Significant differences are indicated as compared to CS I (* $P < 0.05$) or vehicle († $P < 0.05$).