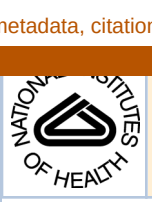

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Basolateral Amygdala Involvement in Memory Reconsolidation Processes that Facilitate Drug Context-induced Cocaine seeking

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Abstract

Understanding the neurobiological underpinnings of putative memory stabilization processes that maintain context-response-cocaine associations in long-term memory and underlie contextual control over addictive behavior is of great interest from an addiction treatment perspective. Using an instrumental animal model of contextual drug relapse, we show that the protein synthesis inhibitor, anisomycin, administered into the basolateral amygdala (BLA) immediately after limited (15- or 60-min) re-exposure to a previously cocaine-paired context subsequently disrupted the ability of the previously cocaine-paired context to reinstate extinguished cocaine-seeking behavior relative to vehicle. Consistent with a BLA-mediated memory reconsolidation deficit, similar impairment in cocaine-seeking behavior was not observed in "no-reactivation" control groups that received anisomycin into the BLA after (re)exposure to either a novel unpaired or an extinction-paired context nor in a neuroanatomical control group that received anisomycin into the posterior caudate-putamen, dorsally adjacent to the BLA, after re-exposure to the cocaine-paired context. Furthermore, anisomycin administered into the BLA after brief (5-min) or extensive (120-min) re-exposure to the cocaine-paired context (which was sufficient to extinguish cocaine-seeking behavior in a vehicle control group) also failed to alter responding. Together, these findings suggest that re-exposure to a cocaine-paired context in the absence of cocaine reinforcement is sufficient to trigger memory reconsolidation processes that support future drug-seeking behavior. The presence and duration of drug-related memory reactivation critically influences and anisomycin-sensitive mechanisms in the BLA selectively control this phenomenon. These findings support the feasibility of novel pharmacotherapeutic approaches that selectively inhibit the reconsolidation of cocaine-related memories in order to prevent drug relapse.

Keywords

cocaine; context; reinstatement; anisomycin; rat

Environmental contexts provide a setting where associations can form between drug-seeking behavior and the motivational effects of cocaine. The resulting long-term memories of context-response-drug associations underlie the ability of drug-associated environmental contexts to reinstate extinguished drug-seeking behavior in laboratory animals (Alleweireldt *et al*., 2001; Everitt *et al*., 2001; Crombag *et al*., 2002) and promote drug relapse in addicts (Ehrman *et al*., 1992; Foltin & Haney, 2000). It has been theorized that, upon retrieval, these memories can become destabilized and need to be reconsolidated into long-term memory in order to be maintained (Misanin *et al*., 1968; Lewis, 1979; Nader *et al*., 2000b). Thus, investigating the neurobiological mechanisms of memory stabilization may provide unique

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insight into suppressing abnormal learning and memory that contribute to addictive behaviors.

The basolateral amygdala (BLA) plays a critical role in the expression of context-induced reinstatement of cocaine-seeking behavior (Fuchs *et al*., 2005;2007) and in memory reconsolidation processes that regulate aversive and appetitive conditioned behaviors (Nader *et al*., 2000a;Bahar *et al*., 2004;Dudai & Eisenberg, 2004). Importantly, the BLA mediates memory reconsolidation processes that underlie the motivational effects of drug-associated Pavlovian conditioned stimuli and conditioned reinforcers. For instance, anisomycin (ANI)—induced inhibition of protein synthesis in the BLA after drug-context memory reactivation disrupts morphine-conditioned place preference in a manner consistent with a memory reconsolidation deficit (Milekic *et al*., 2006). Similarly, *zif*268 knockdown or NMDA receptor antagonism in the BLA in conjunction with drug-conditioned stimulus (CS) memory reactivation impairs subsequent CS-induced instrumental behaviors, including cocaine-seeking behavior (Lee *et al*., 2005;2006a;Milton *et al*., 2008). Unlike conditioned stimuli that signal imminent reward/reinforcement, drug-associated contexts in instrumental settings act as occasion setters that signal drug availability contingent upon responding (Fuchs *et al*., 2005). The associative structure that supports contextual control over drug-seeking behavior (i.e. context-response-drug associations) is distinctly different from that maintaining Pavlovian stimulus and CS control over behavior (i.e. CS/context-drug associations). Therefore, it is unclear whether the same or different neural mechanisms control the memory reconsolidation processes that underlie context-induced cocaine-seeking behavior as those that support Pavlovian contextual conditioned and CS-induced instrumental behaviors.

To start investigating this question, the present study evaluated the contribution of ANI-sensitive memory reconsolidation processes within the BLA to context-induced reinstatement of instrumental cocaine-seeking behavior, while the companion paper to this report focused on contributions of the dorsal hippocampus, dorsomedial prefrontal cortex, and dorsolateral caudate-putamen to this phenomenon (Ramirez *et al*., submitted). ANI or vehicle was administered into the BLA following a non-reinforced session during which drug seeking was permitted to occur in the previously cocaine-paired, extinction-paired, or an unpaired context. The effects of these manipulations on subsequent cocaine-seeking behavior were assessed in the cocaine-paired context. Based on previous studies (Pedreira & Maldonado, 2003; Suzuki *et al*., 2004; Power *et al*., 2006), we hypothesized that a brief memory reactivation session in the cocaine-paired context would trigger context-response-drug memory reconsolidation, and the involvement of ANI-sensitive processes in the BLA in this phenomenon would be demonstrated by ANI-induced, memory reactivation-dependent disruption of subsequent cocaine-seeking behavior.

MATERIALS AND METHODS

Animals

Experimentally naïve male Sprague-Dawley rats (Charles River, N=119), weighing 275-300 g at the start of the experiment, were individually housed in a temperature and humidity controlled vivarium on a reversed light-dark cycle. Rats were maintained on 20-25 g of rat chow/day, with water available *ad libitum*. Rat housing and treatment followed the guidelines of the “Guide for the Care and Use of Laboratory Rats” (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996) and protocols approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee.

Food training

In order to expedite the acquisition of cocaine self-administration, rats were first trained to lever press on a fixed ratio (FR) 1 schedule of food reinforcement (45 mg pellets; Purina, Richmond, IN, USA) in standard sound-attenuated operant conditioning chambers (26 × 27 × 27 cm high; Coulbourn Instruments, Allentown, PA, USA) during a 16-h overnight session. The food training chamber was distinctly different from Contexts 1, 2, and 3 used subsequently in the experiment. It contained a food pellet dispenser located between two retractable levers and an empty plastic tray with no bedding beneath the bar floor (26 cm × 27 cm). Visual, olfactory, and auditory stimuli were not programmed to occur during the training session aside from the sound of the food hopper and electric ventilation fan. During the session, each lever press on the one (active) lever resulted in food pellet delivery only. Lever presses on the other (inactive) lever had no programmed consequences. The contextual stimuli used for subsequent conditioning were not present in the operant conditioning chamber.

Surgery

Forty-eight h after food training, rats were fully anesthetized using ketamine hydrochloride and xylazine (66.6 mg/kg and 1.3 mg/kg, respectively; IP). Chronic indwelling catheters were constructed and implanted into the right jugular vein, as described previously (Fuchs *et al*., 2006b). The catheter ran subcutaneously and exited on the rat's back, posterior to the shoulder blades. After the catheter surgery, the rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL, USA). They received stainless steel guide cannulae (26 gauge, Plastics One), aimed bilaterally at the BLA or overlying posterior caudate-putamen (pCPu) using standard stereotaxic procedures (BLA: −2.7 mm AP, ± 5.2 mm ML, −6.8 mm DV; pCPu: −2.7 mm AP, ± 5.2 mm ML, −4.8 mm DV, relative to the skull surface at bregma). Rats were given minimum 5 days for post-operative recovery before the start of the experiment.

To extend catheter patency, catheters were flushed through once daily for 5 days following surgery with 0.1 ml of an antibiotic cefazolin solution (10.0 mg/ml, Schein Pharmaceutical, Florham Park, NJ, USA). Thereafter, catheters were flushed with 0.1 ml heparinized saline (10 U/ml; Baxter Healthcare Corp., Deerfield, IL, USA) before, and with 0.1 ml of the cefazolin solution and 0.1 ml of heparinized saline (70 U/ml) after, each self-administration session. Catheter patency was periodically verified by infusing 0.1 ml of propofol (10 mg/ml, IV; Abbott Labs., North Chicago, IL, USA), an ultra short-acting barbiturate which produces a rapid loss of muscle tone only when administered intravenously.

Self-administration

Self-administration training was conducted during 2-h sessions during the rats' dark cycle, as described before (Fuchs *et al*., 2007; see experimental timeline in Fig. 2A). Rats were trained to lever press on a FR 1 schedule of cocaine reinforcement (cocaine hydrochloride; 0.10 mg/0.05 ml/infusion; NIDA, Research Triangle Park, NC, USA) with a 20-s time out period. Active (right) lever presses resulted in a 2.5-s activation of the infusion pump only. During the subsequent time out period, responses on the active lever had no consequences. Responses on the inactive (left) lever had no programmed consequences. Daily training was continued until a rat reached the acquisition criterion (i.e., ≥10 infusions self-administered/session on minimum 10 training days).

Self-administration training was conducted in operant conditioning chambers that contained one of two distinctly different sets of contextual stimuli in addition to the levers. Context 1 contained a continuous red houselight on the wall opposite to the active lever, beeping pure tone (80 dB, 1 kHz; 2 s on, 2 s off), pine-scented air freshener strip (4.5 × 2 cm, Car

Freshener Corp., Watertown, NY, USA), and corn cob bedding beneath a wire mesh floor (26 cm × 27 cm). Context 2 contained a blinking white stimulus light above the inactive lever (2 s on, 2 s off), continuous pure tone (75 dB, 2.5 kHz), vanilla-scented air freshener strip (4.5 × 2 cm, Sopus Products, Moorpark, CA, USA), a slanted ceramic tile wall that bisected chamber, and corn cob bedding beneath a bar floor (19 cm × 27 cm). Rats had no exposure to the self-administration context prior to self-administration training. Assignment of rats to cocaine self-administration training in Context 1 versus Context 2 was random. The contextual stimuli were presented throughout each session independent of responding. The pumps were located outside of the sound-attenuation chambers. Data collection and reinforcer delivery were controlled using Graphic State Notation software version 2.102 (Coulbourn).

Extinction

After the last day of self-administration training, rats underwent 2-h extinction sessions on 10 consecutive days, during which lever responses had no programmed consequences. Extinction sessions were conducted in Context 2 for rats that had previously self-administered cocaine in Context 1, and vice versa. On extinction day 7, the rats were adapted to the intracranial microinfusion procedure prior to placement into the chamber. Stainless steel injection cannulae (33 gauge, Plastics One) were inserted into the rat's guide cannulae, 2 mm below the tip of the guide cannulae. Rats were held by the experimenter for 4 min while the injection cannulae were left in place but fluid was not infused.

Context Re-exposure Manipulation

On post-cocaine day 11, rats were placed into the cocaine-paired context for 5, 15, 60 or 120 minutes to reactivate cocaine-related memories and/or permit extinction learning (Tronson & Taylor, 2007). The levers were extended and the rats exposed to the cocaine-paired context were connected to the infusion apparatus in order to allow for similar perception of the spatial/tactile elements of the context (e.g., levers, slanted tile) as during cocaine self-administration training. Thus, this session also provided an assessment of baseline drug context-induced motivation for cocaine. Control groups were placed into the extinction context ("extinction control group") or a novel unpaired context, Context 3 ("no reactivation control group") for 15 min. Context 3 contained a continuous white houselight on the wall opposite to the active lever, continuous white stimulus lights above the active and inactive levers, a continuous complex tone (80 dB; alternating between 1, 1.5, and 2.5 kHz at 1 s intervals), citrus-scented air freshener strip (4.5 × 2 cm, Locasmarts LLC., Ormond Beach, FL, USA), and a ceramic tile floor (26 cm × 27 cm). For all groups, lever presses were recorded but had no programmed consequences. Fluids were not infused into the catheter upon lever pressing.

Intracranial Microinfusions

Immediately after the context re-exposure session, rats were removed from the testing room and received bilateral microinfusions of ANI (62.5 μg/0.5 μl, pH adjusted to pH ~7.0 using 1.0 M NaOH) or phosphate buffered saline vehicle (VEH, 0.5 μl) into the BLA or pCPu. The dose of ANI was selected based on previous research demonstrating that microinfusions of this dose into the BLA impair memory consolidation and reconsolidation in other paradigms (Nader *et al*., 2000a; Wang *et al*., 2005; Duvarci *et al*., 2006) and produce robust protein synthesis inhibition (60% and 32% after a 30-min and 60-min post-infusion interval, respectively) as measured using quantitative leucine incorporation autoradiography (Maren *et al*., 2003; Parsons *et al*., 2006). The pCPu was selected as a control infusion site to provide information about the anatomical selectivity of BLA manipulations, because unintended spread of ANI was expected to be disproportional in the dorsal direction (Baker *et al*., 1996; Neisewander *et al*., 1998). The microinfusions were delivered over 2 min, and

the injection cannulae were left in place for 1 min prior to and after the microinfusion, as described previously (Fuchs *et al*., 2007). Assignment to the ANI versus VEH treatment groups was counterbalanced based on previous cocaine intake.

Reinstatement Test

To assess the ability of the cocaine-paired context to elicit cocaine-seeking behavior, rats were placed into the cocaine-paired context. The procedure for this test was identical to that of the context re-exposure session except that all rats were exposed to the cocaine-paired context and the session length was uniformly 2 h. The reinstatement test occurred after rats underwent additional daily extinction training following the context re-exposure session and reached the extinction criterion (i.e., ≤25 responses/session on 2 consecutive days).

Locomotor Activity Test

While it is unlikely, protracted motor side-effects of intracranial treatments can attenuate lever pressing behavior. To assess this, effects of ANI or VEH infused into the BLA or pCPu on locomotor activity were assessed in a novel environment 72-96 h after intracranial treatment. The exact treatment-to-testing interval was the same for each rat as in the preceding reinstatement experiment. Horizontal locomotor activity was measured in novel Plexiglas chambers (42 × 20 × 20 cm high) using a computerized activity system (San Diego Instruments, San Diego, CA, USA) described previously (Fuchs *et al*., 2007). The system recorded the number of times eight photobeams were broken by a rat moving in the chamber during the 2-h test session.

Histological and Data Analysis

Rats were fully anesthetized with sodium pentobarbital (Sigma, 100 mg/kg, IP) and perfused transcardially. Brains were extracted and sectioned on a vibratome at a thickness of 75 μm. Cannula placements were determined on cresyl violet-stained brain sections based on the rat brain atlas (Paxinos & Watson, 1997). The data of rats with misplaced cannulae were excluded from data analysis.

To test for potential pre-existing differences in cocaine history, cocaine-reinforced active lever presses, inactive lever presses, and cocaine intake (mean of last three sessions) were analyzed using separate one-way analyses of variance (ANOVA) with group (eight groups in experiment 1) as the between subjects factor or using t tests, where appropriate (experiments 2, 3). To test for potential pre-existing differences in baseline context-induced motivation to seek cocaine, non-reinforced active and inactive lever presses during the cocaine context re-exposure session were analyzed using separate 4 × 2 between-subjects factorial ANOVAs with context re-exposure session duration (5, 15, 60, or 120 min) and group (ANI, VEH) as factors (experiment 1) or using separate t tests (experiments 2, 3), where appropriate. To assess the effects of extinction context re-exposure on subsequent extinction responding, non-reinforced active lever presses were analyzed using a 2 × 2 mixed factorial ANOVA with test day (post-cocaine day 10, post-cocaine day 12) as a within-subjects factor and group (ANI, VEH) as a between-subjects factor. Because extinction learning during the context re-exposure session was expected to alter reinstatement responding, only qualitative comparisons were made across experiments with differing context re-exposure durations. Context re-exposure duration was not included as a factor in the analysis of post-manipulation instrumental responding. Accordingly, to assess the effects of the intracranial manipulations on reinstatement of cocaine-seeking behavior, non-reinforced active and inactive lever presses during the reinstatement test session and preceding extinction session were analyzed separately using 2 × 2 mixed factorial ANOVAs with test context (extinction, cocaine-paired) as the within-subjects factor and treatment (VEH, ANI) as the between-subjects factor. Locomotor activity counts were analyzed

separately for each brain region using 2 × 6 mixed factorial ANOVAs with treatment as the between-subjects factor and time (20-min intervals) as the within-subjects factor. Significant ANOVA main and interaction effects were followed up by Tukey LSD *post hoc* tests, when appropriate. Alpha was set at 0.05.

RESULTS

Histology

The target regions were defined as the lateral/basolateral nuclei of the amygdala (BLA) and dorsally adjacent posterior caudate-putamen (pCPu). The most ventral point of each injection cannula track was located bilaterally within the target brain region for the following number of rats per group (Fig. 1): BLA 5 min VEH (N=9), BLA 5 min ANI (N=7), BLA 15 min VEH (N=7), BLA 15 min ANI (N=9), BLA 15 min VEH-extinction control (N=8), BLA 15 min ANI-extinction control (N=10), BLA 15 min VEH-No Reactivation control (N=8), BLA 15 min ANI-No Reactivation control (N=8), BLA 60 min VEH (N=7), BLA 60 min ANI (N=9), BLA 120 min VEH (N=10), BLA 120 min ANI (N=8), pCPu 15 min VEH (N=10), pCPu 15 min ANI (N=9). ANI did not produce more gliosis or cell loss visible at 25X magnification than VEH treatment in either the BLA or pCPu.

Experiment 1. Effects of ANI administered into the BLA after re-exposure to the cocaine-paired context on subsequent drug context-induced reinstatement of cocaine seeking

Self-administration and Extinction—Rats exhibited stable responding on the active lever during the last 3 self-administration days (< 10% variability in daily cocaine intake). There was no pre-existing difference in active lever responding ($F_{(7,58)}$=0.57, p=0.78), inactive lever responding ($F_{(7,58)}$=1.02, p=0.43), or cocaine intake ($F_{(7,58)}$=0.18, p=0.99) between the eight groups that later in the experiment received VEH or ANI into the BLA after a 5, 15, 60, or 120 min cocaine context re-exposure session. Collapsed across these groups, the mean daily cocaine intake (± SEM) was 33.30 ± 2.19 infusions (approx. 11.1 ± 0.73 mg/kg/session). Responding declined in all groups upon removal of cocaine reinforcement on post-cocaine day 1 (48.09 ± 8.10 lever presses/session). The microinfusion adaptation procedure did not alter responding on post-cocaine day 7 (data not shown). Subsequently, responding gradually extinguished to a mean of 10.09 ± 1.72 active lever presses/session by post-cocaine day 10, the day preceding the context re-exposure manipulation (see experimental timeline in Fig. 2A).

Cocaine Context Re-exposure—On post-cocaine day 11, re-exposure to the cocaine-paired context in the absence of cocaine reinforcement elicited an increase in responding on the active lever, as a function of session duration (Fig. 2B). The ANOVA of active lever presses revealed a significant context re-exposure duration main effect ($F_{(3,58)}$=10.60, p=0.0001), but no group × context re-exposure duration interaction effect ($F_{(3,58)}$=0.07, p=0.98) or group main effect ($F_{(1,58)}$=0.06, p=0.79). Similarly, the ANOVA of inactive lever presses revealed a significant context re-exposure duration main effect ($F_{(3,58)}$=5.19, p=0.003), but no group × context re-exposure duration interaction effect ($F_{(3,58)}$=0.37, p=0.78) or group main effect ($F_{(1,58)}$=0.29, p=0.59). Subsequent *post-hoc* tests indicated that active lever responding was greater during the 120-min context re-exposure session relative to all shorter sessions (Tukey tests, p=0.03-0.0001), and inactive lever responding was greater during the 120-min context re-exposure session than during the 5-min session (Tukey test, p=0.002). Importantly, there was no difference in active or inactive lever responding between the groups that *subsequently* received ANI or VEH, indicating no pre-existing difference in baseline context-induced motivation for cocaine.

After ANI or VEH treatment, which was administered immediately following the context re-exposure session on post-cocaine day 11, there was no difference between the groups in the magnitude of extinction responding on post-cocaine day 12 on the active lever (mean = 10.68 + 1.77 responses; all group and context re-exposure duration main and interaction effects, $F_{(1\text{-}3,58)}$=0.02-1.29, p=0.28-0.88) or inactive lever (mean = 2.58 + 0.43 responses; all group and context re-exposure duration main and interaction effects, $F_{(1\text{-}3,58)}$=0.58-0.98, p=0.33-0.63). Similarly, there was no difference between the groups in the number of additional extinction sessions needed to reach the extinction criterion (mean = 2.50 + 0.16 days; all group and context re-exposure duration main and interaction effects, $F_{(1\text{-}3,58)}$=0.30-1.69, p=0.17-0.82). This indicates that intra-BLA ANI treatment did not have a nonspecific effect on instrumental responding or extinction learning in the extinction context.

Cocaine-seeking Behavior—The effects of intra-BLA ANI treatment on responding during the context-induced reinstatement test varied depending on the duration of the preceding context re-exposure session (Fig. 3).

In rats that had received 5 min of re-exposure to the cocaine-paired context previously (Fig. 3A), the ANOVA of active lever presses revealed a significant context main effect ($F_{(1,14)}$=19.41, p=0.001), but no treatment main effect ($F_{(1,14)}$=0.003, p=0.96) or a context × treatment interaction effect ($F_{(1,14)}$=0.001, p=0.98). Furthermore the ANOVA of inactive lever presses revealed a significant context × treatment interaction effect ($F_{(1,14)}$=5.49, p=0.03), but no context main effect ($F_{(1,14)}$=1.02, p =0.33) or treatment main effect ($F_{(1,14)}$=0.30, p=0.59). Thus, upon exposure to the cocaine-paired context, both the previously VEH and ANI-treated groups exhibited similar increases in active lever responding, relative to responding in the extinction context. Inactive lever responding remained low and pair-wise comparisons indicated no treatment or context simple main effects, suggesting that the interaction effect was spurious.

In rats that had received 15 min of re-exposure to the cocaine-paired context previously (Fig. 3B), the ANOVA of active lever presses revealed a significant context × treatment interaction effect ($F_{(1,14)}$=7.44, p=0.02) and significant context main ($F_{(1,14)}$=35.05, p=0.0001) and treatment main effects ($F_{(1,14)}$=8.27, p=0.01). Furthermore, the ANOVA of inactive lever presses revealed a significant context main ($F_{(1,14)}$=12.56, p=0.003) and treatment main effects ($F_{(1,14)}$=5.09, p=0.04), but no context × treatment interaction effect ($F_{(1,14)}$=1.34, p=0.26). Thus, both groups reinstated (ANOVA simple main effect tests, p=0.003-0.006). However, the ANI-treated group exhibited less active lever responding upon exposure to the cocaine-paired context (p=0.012), but not the extinction context, relative to the VEH-treated group. Inactive lever responding remained negligible, but the ANI-treated group exhibited less inactive lever responding than the VEH-treated group in the cocaine-paired and extinction contexts.

In rats that had received 60 min of re-exposure to the cocaine-paired context previously (Fig. 3C), the ANOVA of active lever presses revealed a significant context × treatment interaction effect ($F_{(1,14)}$=7.48, p=0.02) and significant context ($F_{(1,14)}$=26.03, p=0.0001) and treatment main effects ($F_{(1,14)}$=7.96, p=0.01). The ANOVA of inactive lever presses revealed a significant context main effect ($F_{(1,14)}$=4.47, p=0.05), but no context × treatment interaction effect ($F_{(1,14)}$=1.68, p=0.22) or treatment main effect ($F_{(1,14)}$=3.93, p=0.06). Thus, both groups reinstated on the active lever (Tukey tests, p=0.03-0.008). However, the ANI-treated group exhibited less active lever responding upon exposure to the cocaine-paired context (Tukey test, p=0.013), but not the extinction context, relative to the VEH-treated group. There was no difference between the ANI- and VEH-treated groups in

inactive lever responding, but both groups exhibited a small increase in inactive lever responding upon exposure to the cocaine-paired context relative to the extinction context.

In rats that had received 120 min of re-exposure to the cocaine-paired context previously (Fig. 3D), the ANOVA of active lever presses revealed a significant context main effect ($F_{(1,16)}$=14.28, p=0.002), but no context × treatment interaction effect ($F_{(1,16)}$=2.14, p=0.16) or treatment main effect ($F_{(1,16)}$=1.62, p=0.22). The ANOVA of inactive lever presses revealed a significant context × treatment interaction effect ($F_{(1,16)}$=8.46, p=0.01) and context main effect ($F_{(1,16)}$=11.89, p=0.003), but no treatment main effect ($F_{(1,16)}$=1.98, p=0.18). Furthermore, a separate analysis indicated that active lever responding in the VEH-treated control group in the cocaine-paired context was significantly less during the reinstatement test than during self-administration training ($t_{(9)}$=2.29, p=0.04, unlike for all other re-exposure durations $t_{(6\text{-}8)}$=1.05-1.42, p=0.20-0.33), indicating that the 120-min cocaine context re-exposure session alone resulted in significant extinction learning. Nevertheless, upon exposure to the cocaine-paired context, both groups exhibited a slight increase in active lever responding. There was no difference between the ANI- and VEH-treated groups in active lever responding, but the ANI-treated group exhibited more inactive lever responding in the cocaine-paired context than in the extinction context (Tukey test, p=0.042) and relative to the VEH-treated group (Tukey test, p=0.015).

Experiment 2. Effects of ANI administered into the BLA after re-exposure to the extinction context on subsequent drug context-induced reinstatement of cocaine seeking

Experiment 2 evaluated whether the effects of ANI observed in experiment 1 depended on **cocaine** memory reactivation *per se*, as is expected from a memory reconsolidation deficit (see experimental timeline in Fig. 4A). The experimental procedure was identical to that in experiment 1 except that rats received 15-min re-exposure to the *extinction* context instead of the cocaine-paired context, prior to ANI or VEH treatment. In terms of experimental history, the BLA-cannulated rats in experiment 2 exhibited stable and similar cocaine-reinforced responding (Fig. 4C), mean daily cocaine intake (± SEM; approx. 11.0 ± 1.44 mg/kg/session), and extinction responding prior to the context re-exposure session (8.28 ± 1.97 active lever presses/session on post-cocaine day 10) as rats in experiment 1.

Extinction Context Re-exposure—On post-cocaine day 11, there was no difference between the *subsequently* ANI- versus VEH-treated extinction control groups in active ($t_{(16)}$=0.44, p=0.67; mean=3.22 ± 0.80 responses/session) and inactive ($t_{(16)}$=1.01, p=0.33; mean=0.61 ± 0.34 responses/session) lever responding during the 15-min *extinction* context re-exposure session. Furthermore, ANI or VEH treatment administered after this session failed to alter active lever responding during the extinction session on post-cocaine day 12 ($mean_{VEH}$=11.14 ± 5.03; $mean_{ANI}$=7.70 ± 2.00) relative to responding on post-cocaine day 10 ($mean_{VEH}$= 8.63 ± 1.79; $mean_{ANI}$=8.00 ± 3.36), the last extinction session before the context re-exposure session and ANI or VEH treatment. Consistent with this, separate ANOVAs indicated no significant session or group main or interaction effects on active and inactive lever responding ($F_{(1,15)}$=0.12-0.40, p=0.53-0.73 and $F_{(1,15)}$=0.48-0.93, p=0.35-0.49, respectively). Thus, ANI- and VEH-treated groups did not differ in active lever responding during the first extinction session following treatment, nor did they differ in the number of additional extinction sessions needed to reach the extinction criterion (2.13 ± 0.11 days, data not shown) prior to the reinstatement test. Thus, ANI failed to disrupt extinction. Furthermore, the memory age at the time of testing was similar in experiments 1 and 2.

Cocaine-Seeking Behavior—Intra-BLA ANI treatment immediately after 15 min of re-exposure to the extinction-paired context failed to alter responding during the cocaine context-induced reinstatement test relative to VEH treatment (Fig. 4C). The ANOVA of

active lever presses revealed a significant context main effect ($F_{(1,16)}$=32.25, p=0.0001), but no context × treatment interaction effect ($F_{(1,16)}$=1.99, p=0.18) or a treatment main effect ($F_{(1,16)}$=2.51, p=0.13). Similarly, the ANOVA of inactive lever presses revealed a significant context main effect ($F_{(1,16)}$=5.16, p=0.04), but no context × treatment interaction effect ($F_{(1,16)}$=0.008, p=0.93) or a treatment main effect ($F_{(1,16)}$=0.001, p=0.98). Thus, upon re-exposure to the cocaine-paired context, both VEH and ANI-treated groups exhibited an increase in responding on the active lever and, to a lesser extent on the inactive lever, relative to responding in the extinction context. Furthermore, there was no difference between the ANI-treated and VEH-treated groups in responding on either lever. Thus, ANI failed to disrupt cocaine-seeking behavior in the absence of memory reactivation.

Experiment 3. Effects of ANI administered into the BLA after re-exposure to an unpaired context on subsequent drug context-induced reinstatement of cocaine seeking

Experiment 3 was a no-reactivation control experiment that further examined whether the effects of ANI observed in experiment 1 depended on cocaine memory reactivation *per se*, as is expected from a memory reconsolidation deficit (see experimental timeline in Fig. 4A). The experimental procedure was identical to that in experiment 2 except that rats received 15-min re-exposure to a *novel unpaired* context instead of the extinction context, prior to ANI or VEH treatment. In terms of experimental history, the BLA-cannulated rats in experiment 3 exhibited stable and similar cocaine-reinforced responding (Fig. 4D), mean daily cocaine intake (± SEM; approx. 8.45 ± 0.65 mg/kg/session), and extinction responding prior to the context re-exposure session (11.37 ± 2.84 active lever presses/session on post-cocaine day 10) as rats in experiments 1-2.

Extinction Context Re-exposure—On post-cocaine day 11, exposure to the *novel unpaired* context elicited negligible lever responding. There was no difference between the *subsequently* ANI- versus VEH-treated no reactivation control groups in active ($t_{(14)}$=0.21, p=0.83; mean=10.75 ± 2.84 responses/session) and inactive ($t_{(14)}$=0.60, p=0.77; mean=3.87 ± 1.79 responses/session) lever responding during the 15-min unpaired context re-exposure session. Furthermore, ANI or VEH treatment administered after this session failed to alter active lever responding in the extinction context on post-cocaine day 12 ($mean_{VEH}$=9.71 ± 3.25; $mean_{ANI}$=9.25 ± 2.65) relative to responding on post-cocaine day 10 ($mean_{VEH}$= 10.75 ± 5.27; $mean_{ANI}$=12.00 ± 2.55), the last extinction session before the manipulation. Consistent with this, separate ANOVAs indicated no significant session or group main or interaction effects on active and inactive lever responding ($F_{(1,14)}$=0.10-0.22, p=0.64-0.92 and $F_{(1,14)}$=0.04-0.70, p=0.42-0.84, respectively). Thus, ANI- and VEH-treated groups did not differ in active lever responding during the first extinction session following treatment, nor did they differ in the number of additional extinction sessions needed to reach the extinction criterion (2.11 ± 0.08 days, data not shown) prior to the reinstatement test. Thus, ANI did not have a nonspecific effect on instrumental performance in the extinction context. Furthermore, the memory age at the time of testing was similar in experiments 1-3.

Cocaine-Seeking Behavior—Intra-BLA ANI treatment immediately after 15 min of re-exposure to the unpaired context failed to alter responding during the cocaine context-induced reinstatement test relative to VEH treatment (Fig. 4D). The ANOVA of active lever presses revealed a significant context main effect ($F_{(1,14)}$=20.78, p=0.0001), but no context × treatment interaction effect ($F_{(1,14)}$=0.12, p=0.74) or a treatment main effect ($F_{(1,14)}$=0.70, p=0.79). In contrast, the ANOVA of inactive lever presses did not indicate a context or treatment main or interaction effect ($F_{(1,14)}$=0.12-3.38, p=0.08-0.75). Thus, upon re-exposure to the cocaine-paired context, both VEH and ANI-treated groups exhibited an increase in responding on the active lever and, to a lesser extent on the inactive lever, relative to responding in the extinction context. Furthermore, there was no difference

between the ANI-treated and VEH-treated groups in responding on either lever. Thus, ANI failed to disrupt cocaine-seeking behavior in the absence of cocaine memory reactivation and did not have a nonspecific effect on instrumental responding in the cocaine context.

Experiment 4. Effects of ANI administered into the pCPu after re-exposure to the cocaine-paired context on subsequent context-induced reinstatement of cocaine seeking

As a follow-up to experiment 1, experiment 4 examined whether the effects of ANI following 15-min re-exposure to the cocaine-paired context were specific to the BLA (see experimental timeline in Fig. 4B). The pCPu-cannulated rats in experiment 4 exhibited stable and similar cocaine-reinforced responding (Fig. 4E) and mean daily cocaine intake (approx. 9.43 ± 0.86 mg/kg/session) as rats in experiment 1.

Cocaine Context Re-exposure—On post-cocaine day 11, there was no difference between the *subsequently* ANI- versus VEH-treated groups in active ($t_{(17)}$=0.58, p=0.56; mean = 19.51 ± 9.66 lever responses/session) and inactive ($t_{(17)}$=0.43, p=0.67; mean = 4.00 ± 1.73 lever responses/session) lever responding during the 15-min cocaine context re-exposure session (data not shown). After ANI or VEH treatment, which was administered immediately following the context re-exposure session, there was no difference between the groups in the magnitude of extinction responding or in the number of additional extinction sessions needed to reach the extinction criterion (2.53 ± 0.19 days, data not shown). Thus, intra-pCPu ANI treatment did not have a nonspecific effect on operant responding or extinction learning.

Cocaine-Seeking Behavior—Intra-pCPu ANI treatment immediately after 15 min of re-exposure to the cocaine-paired context failed to alter responding during the context-induced reinstatement test (Fig. 4E). The ANOVA of active lever presses revealed a significant context main effect ($F_{(1,17)}$=18.30, p=0.001), but no context × treatment interaction effect ($F_{(1,17)}$=0.09, p=0.76) or a treatment main effect ($F_{(1,17)}$=0.01, p=0.91). The ANOVA of inactive lever presses revealed a significant context main effect ($F_{(1,17)}$=4.95, p=0.04), but no context × treatment interaction effect ($F_{(1,17)}$=0.78, p=0.38) or a treatment main effect ($F_{(1,17)}$=0.17, p=0.69). Upon exposure to the cocaine-paired context, both VEH and ANI-treated groups exhibited increases in responding on the active lever, and to a lesser extent on the inactive lever, relative to responding in the extinction context. Furthermore, there was no difference between the ANI-treated and VEH-treated groups in responding on either lever.

Locomotor Activity—ANI pretreatment administered into the BLA or pCPu failed to alter locomotor activity in a novel context after a post-treatment interval identical to that in experiments 1 and 4, respectively (Fig. 5). For the BLA-cannulated groups, the ANOVA of photobeam breaks across the 2-h locomotor test indicated a significant time main effect ($F_{(5,320)}$=79.67, p=0.0001), but no treatment × time interaction ($F_{(5,320)}$=1.22, p=0.30) or a treatment main effect ($F_{(1,64)}$=2.27, p=0.14). Similarly, for the pCPu-cannulated groups, the ANOVA indicated a significant time main effect ($F_{(5,85)}$=9.15, p=0.0001), but no treatment × time interaction ($F_{(5,85)}$=1.21, p=0.31) or a treatment main effect ($F_{(1,17)}$=1.2, p=0.29). Thus, locomotor activity was highest during the first 20-min interval (interval 1> intervals 2-6; Tukey test, p<0.05) gradually decreased across time at similar rates in the ANI-treated and VEH-treated groups. Furthermore, ANI treatment failed to alter locomotor activity regardless of the site of administration relative to VEH treatment.

DISCUSSION

The existence of a distinct memory reconsolidation process has been the source of debate, and it has been considered to represent a mechanism for memory storage, retrieval link

formation, or lingering consolidation (Dudai & Eisenberg, 2004; Tronson & Taylor, 2007). According to the memory reconsolidation hypothesis, it is a process that stabilizes already established memories into long-term memory stores after reactivation-induced destabilization (Misanin *et al*., 1968; Lewis, 1979). Memory reconsolidation appears to vary in duration (i.e., lasts for ~2-4 h after memory reactivation) as a function of memory complexity, strength, age, and the brain region and signaling molecule examined (Dudai & Eisenberg, 2004; Alberini *et al*., 2006; Tronson & Taylor, 2007). While previous studies focused on Pavlovian contextual conditioned and CS-induced instrumental behaviors, the present study represents the first demonstration that memory reconsolidation processes regulate contextual control over instrumental behavior, specifically drug context-induced instrumental cocaine-seeking behavior.

The form of memory reconsolidation identified in this study is mediated at least in part by ANI-sensitive processes in the BLA, depending on factors related to the intensity of memory reactivation. Consistent with this, intra-BLA ANI treatment administered after 15-min or 60-min re-exposure to the cocaine-paired context subsequently impaired cocaine context-induced drug-seeking behavior (Fig. 3B, 3C), whereas the same treatment administered after a brief (5 min) re-exposure to the cocaine-paired context failed to alter subsequent drug-seeking behavior (Fig. 3A). The effect of ANI was also dependent on cocaine-related memory reactivation *per se* since intra-BLA ANI treatment administered following 15 min re-exposure to the extinction context (Fig. 4C) or exposure to an unpaired context (Fig. 4D) also failed to alter subsequent cocaine context-induced drug-seeking behavior. The effects of ANI on cocaine-seeking behavior were specific to the BLA as ANI microinfusion into the overlying pCPu, the brain region in the most likely path of unintended ANI spread, after 15-min re-exposure to the cocaine-paired context failed to alter context-induced cocaine-seeking behavior (Fig. 4E). Similarly, previous studies have shown that microinfusions of reconsolidation inhibitors into the central amygdala (CeA) fail to alter established conditioned behaviors in other paradigms (Bahar *et al*., 2004; Hellemans *et al*., 2006). ANI also failed to alter locomotor activity in a novel context (Fig. 5) and did not have a consistent effect on inactive lever responding during the reinstatement test, suggesting that it did not produce a non-specific performance deficit. Instead, increased inactive lever responding during the reinstatement test likely represented a widening of the response repertoire in an attempt to procure drug reinforcement using a new response strategy (Domjan, 1980; Fuchs *et al*., 1998; Fuchs *et al*., 2004). Accordingly, sporadic ANI effects on the inactive lever probably reflected attenuation in an alternate form of context-induced cocaine-seeking behavior in the present study. Thus, overall, the present findings are consistent with the idea that context retrieval elicits memory destabilization, after which contextual control over cocaine-seeking behavior becomes dependent on ANI-sensitive processes within the BLA. Importantly, these findings demonstrate for the first time that principles of the memory reconsolidation hypothesis apply to context-induced instrumental goal-directed behaviors, similar to other conditioned behaviors (Nader *et al*., 2000a; Wang *et al*., 2005; Lee *et al*., 2006a; Milekic *et al*., 2006). Thus, at the level of the BLA, there may be some overlap in the memory stabilization mechanisms that facilitate Pavlovian responses, CS-induced instrumental behaviors, and context-induced instrumental behaviors even though these behaviors are theorized to rely on distinctly different types of associative memories (i.e., context/CS-drug, CS-drug, and context-response-drug associations, respectively).

ANI is a potent protein synthesis inhibitor with a relatively short half-life (~30 min; Maren *et al*., 2003; Dudai & Eisenberg, 2004; Parsons *et al*., 2006), and it has been used to argue that some memory reconsolidation processes are dependent on *de novo* protein synthesis (Tauscher *et al*., 1999; Nader *et al*., 2000a; Inda *et al*., 2005). However, ANI may act via several other mechanisms. First, recent studies indicate that ANI triggers robust

norepinephrine, dopamine, serotonin, and acetyl choline release followed by transient norepinephrine and dopamine depletion (Canal *et al*., 2007; Qi & Gold, 2009). In particular, beta-adrenoceptor antagonist and agonist treatments, timed to counteract the biphasic effect of ANI on norepinephrine release in the amygdala or ventral hippocampus inhibit ANI-induced memory reconsolidation deficits in an inhibitory avoidance paradigm (Canal *et al*., 2007; Qi & Gold, 2009). Thus, ANI-induced abnormalities in neurotransmitter responses may produce amnesia by disrupting the post-translational modification of proteins critical for memory stabilization (Qi & Gold, 2009). However, the possibility that ANI directly impaired the *expression* of cocaine-seeking behavior in the present study by altering monoamine release is mitigated by the fact that testing occurred minimum 72 h after ANI administration, thus at least 24 h after monoamine levels were reported to normalize (i.e., 48 h post ANI, Canal *et al*., 2007). Second, ANI can also act as a ribotoxin at high doses (Rudy *et al*. 2006). However, it is unlikely that ANI-induced cell death elicited the attenuation in reinstatement because this effect depended on the presence and duration of cocaine context re-exposure. Furthermore, overt brain damage was not present in brain tissue. Finally, ANI has been hypothesized to prompt delayed superinduction of gene expression and disrupt memory reconsolidation by impairing the synthesis or post-translational modification of transcription inhibitors (Routtenberg & Rekart, 2005). Since ANI has multiple potential mechanisms of action, the most parsimonious explanation is that ANI impaired cocaine-seeking behavior in the present study by inhibiting protein synthesis or post-translational modification or by altering gene transcription in the BLA following memory reactivation. Based on the results of the present study, future studies are warranted to determine the contribution of particular signaling molecules to memory stabilization processes that regulate context-induced cocaine-seeking behavior.

Interestingly, intra-BLA ANI failed to completely inhibit subsequent cocaine-seeking behavior; even though, the same dose of ANI or the protein synthesis inhibitor cycloheximide has been sufficient to fully inhibit Pavlovian conditioned responses in other paradigms (Nader *et al*., 2000a; Milekic *et al*., 2006). Associative memories that underlie instrumental goal-directed behaviors may be more resistant to reconsolidation inhibition than the associative memories that underlie Pavlovian conditioned responses, perhaps in part due to differences in the extent of training and memory age (Tronson & Taylor, 2007; Brown *et al*., 2008). For instance, experimental subjects in drug self-administration studies are typically overtrained in order to increase the face validity of the procedure and to provide the subjects with extensive drug exposure over an extended training period. Overtraining may, in turn, increase the resistance of drug memories to destabilization. Indeed, studies indicate that old and strong memories, much like the cocaine memories in the present study, require extensive memory reactivation to become labile (Flood *et al*., 1973; Suzuki *et al*., 2004; Alberini *et al*., 2006). Alternatively, partial ANI effects may reflect the contribution of multiple parallel mechanisms of memory stabilization. ANI-sensitive and ANI-insensitive mechanisms of memory reconsolidation may operate within the BLA and outside of the BLA, the latter of which we explore in the companion paper to this report (Ramirez *et al*. submitted). Furthermore, one study suggests that the long-term memories of context-response and response-reward associations that support food-reinforced instrumental responses do not undergo ANI-sensitive reconsolidation upon retrieval (Hernandez & Kelley, 2004), and such ANI-insensitive instrumental memories may be sufficient to elicit residual cocaine-seeking behavior in the present study.

Contrary to the effects of ANI administered into the BLA after 15- or 60-min re-exposure to the cocaine-paired context, ANI administered after a very brief (5-min) context re-exposure period failed to alter subsequent cocaine-seeking behavior (Fig. 3A). This negative effect was not due to insufficient ANI dosing, as the 62.5 μg/0.5 μl dose has strong inhibitory effects on protein synthesis in the amygdala (Maren *et al*., 2003) and on contextual memory

reconsolidation in other experimental settings (Parsons *et al*., 2006;Yim *et al*., 2006). Most likely, brief context re-exposure was either (A) insufficient to trigger memory destabilization, hence reconsolidation, or (B) it initiated ANI-insensitive memory reconsolidation processes within the BLA. In support of the former, very brief memory reactivation sessions lead to incomplete or no memory reconsolidation in other paradigms (Suzuki *et al*., 2004;Diergaarde *et al*., 2006). Interestingly, the minimum duration of memory reactivation necessary for memory reconsolidation exceeds 10 min in this and other instrumental models (Diergaarde *et al*., 2006; present study), whereas it is as brief as 2 min in some Pavlovian models (Pedreira & Maldonado, 2003;Pedreira *et al*., 2004;Power *et al*., 2006;Rudy *et al*., 2006). As discussed above, differences in the minimum duration of cue re-exposure necessary for memory destabilization in instrumental versus Pavlovian models may reflect dissimilarities in the type of associations, corresponding memory stabilization processes, and/or training parameters (Tronson & Taylor, 2007;Brown *et al*., 2008).

Based on the trace dominance hypothesis (Eisenberg *et al*., 2003; Eisenberg & Dudai, 2004) and other work (Falls *et al*., 1992; Lu *et al*., 2001; Wang *et al*., 2005; Lee *et al*., 2006b; Brown *et al*., 2007), we predicted that extinction learning would be dominant at the end of the extended (120-min) context re-exposure session and this would initiate *new extinction memory consolidation*. Indeed, re-exposure to the cocaine-paired context for 120-min was sufficient to extinguish cocaine-seeking behavior in the present study, as indicated by diminished reinstatement responding in the VEH control group (Fig. 3D). However, ANI treatment administered into the BLA failed to restore reinstatement responding to levels seen during the 120-min context re-exposure session, consistent with the results of a recent auditory fear conditioning study (Duvarci *et al*., 2006). This negative finding in the present study suggests either that (A) ANI-sensitive processes in the BLA are not critical for new extinction memory consolidation in the reinstatement model or that (B) extinction memory consolidation is completed before the end of the 120-min session and the onset of ANI action. Tetrodotoxin-induced functional inactivation of the BLA induced after a similar 120-min session is sufficient to disrupt extinction memory consolidation in the CS-induced reinstatement model (Fuchs *et al*., 2006b), somewhat mitigating the latter possibility. However, future studies will be needed in order to systematically examine this question since the rate of extinction memory consolidation may vary as a function of cue type.

ANI treatment, administered after re-exposure to the extinction context and the putative reactivation of previously acquired extinction memories, did not restore cocaine-seeking behavior in the extinction context 24 h later relative to cocaine-seeking behavior seen in the same context at earlier stages of extinction training. One possible explanation for the negative effect is that ANI-sensitive processes in the BLA are not critical for *extinction memory reconsolidation* that inhibits context-induced cocaine-seeking behavior. Similarly, tetrodotoxin-induced functional inactivation of the BLA fails to disrupt extinction memory reconsolidation in the CS-induced reinstatement model (Fuchs *et al*., 2006b). However, extensive extinction training history and the lack of opportunity to incorporate new information may cause extinction memory reconsolidation to occur more time efficiently, concluding prior to the end of the memory reactivation session and the onset of ANI effects. The latter explanation is less likely due to the shortness of the re-exposure session (15-min). Furthermore, at least in the contextual memory model of the crab Chasmagnathus, reconsolidation appears to commence at the end of the context re-exposure session regardless of session duration (Pedreira *et al*., 2004). To be noted, ANI treatment produced a cocaine memory reconsolidation deficit after a memory reactivation session of comparable duration. This suggests that the reconsolidation of inhibitory versus excitatory memories that regulate context-induced cocaine-seeking behavior may be mediated by different neural mechanisms within the BLA.

Context-Cocaine Memory Reconsolidation, Extinction, and Drug Relapse Prevention

Several elements of the brain circuitry that mediate the expression of context-induced drug-seeking behavior, including the BLA, dorsal hippocampus, and nucleus accumbens (Weiss *et al*., 2001; Crombag *et al*., 2002; Bossert *et al*., 2006; Fuchs *et al*., 2005; Di Pietro *et al*., 2006), are reportedly also involved in memory reconsolidation (Miller & Marshall, 2005; Alberini *et al*., 2006; Milekic *et al*., 2006; Tronson & Taylor, 2007). Drug-induced and experience-based adaptations in these brain regions are theorized to be critical for increased stimulus control over addictive behavior and the transition from casual drug use to drug dependence (Onaivi *et al*., 1996; Nestler & Aghajanian, 1997; Goldstein & Volkow, 2002; Kalivas & McFarland, 2003; Sutton *et al*., 2003; Wolf *et al*., 2003; Fuchs *et al*., 2006a; Lu *et al*., 2006). Some of these neuroadaptations may occur in memory, as opposed to motivational, subcircuits within these structures (Grant *et al*., 1996; Kilts *et al*., 2004; Fuchs *et al*., 2006b; Lu *et al*., 2006). Thus, treatments that selectively inhibit the re-stabilization of drug-related memories are of special interest for addiction treatment development. These treatments are likely feasible since the results from the present study suggest that the neural mechanisms of drug-related memory reconsolidation and extinction memory (re)consolidation processes that control context-induced drug-seeking behavior are at least partially distinct within the BLA. Similarly, recent studies have indicated that cannabinoid 1 receptors and L-type voltage-gated calcium channels selectively mediate extinction, but not memory reconsolidation (Suzuki *et al*., 2004), whereas protein kinase A activity in the BLA mediates auditory fear memory reconsolidation but not extinction (Tronson *et al*., 2006). Future studies will need to investigate the longevity of these effects in order to evaluate the feasibility of reconsolidation inhibition as an approach to relapse prevention, since post-reactivation manipulations only transiently disrupt certain conditioned behaviors (Eisenberg & Dudai, 2004; Amaral *et al*., 2007). It is encouraging in this respect that antisense oligonucleotide-induced disruption of *zif*268 expression after CS-cocaine memory reactivation produces deficits in CS-induced cocaine-seeking behavior that endure for up to 30 d (Lee *et al*., 2006a).

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Abbreviations

Anisomycin	ANI
vehicle	VEH
fixed ratio	FR
BLA	basolateral amygdala
CeA	central nucleus of the amygdala
pCPu	posterior caudate-putamen

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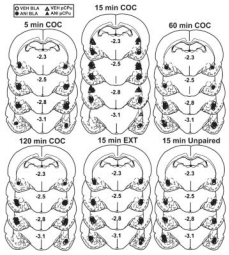

Figure 1.

Microinfusion cannula placement as verified on cresyl violet-stained sections. The symbols represent the most ventral point of the infusion cannula tract for each rat on coronal sections based on the atlas of Paxinos and Watson (1997). Rats received microinfusions of anisomycin (ANI, 62.5 μg/0.5 μl/hemisphere) or vehicle (VEH, 0.5 μl/hemisphere) into the basolateral amygdala (BLA) or overlying posterior caudate-putamen (pCPu) immediately after 5, 15, 60, or 120 min of re-exposure to the cocaine paired context (COC), after 15 min of re-exposure to the extinction context (EXT), or after 15 min of exposure to a novel, unpaired context (Unpaired; No Reactivation control groups) and 72-96 h before a locomotor test session. The numbers indicate the distance from bregma in mm.

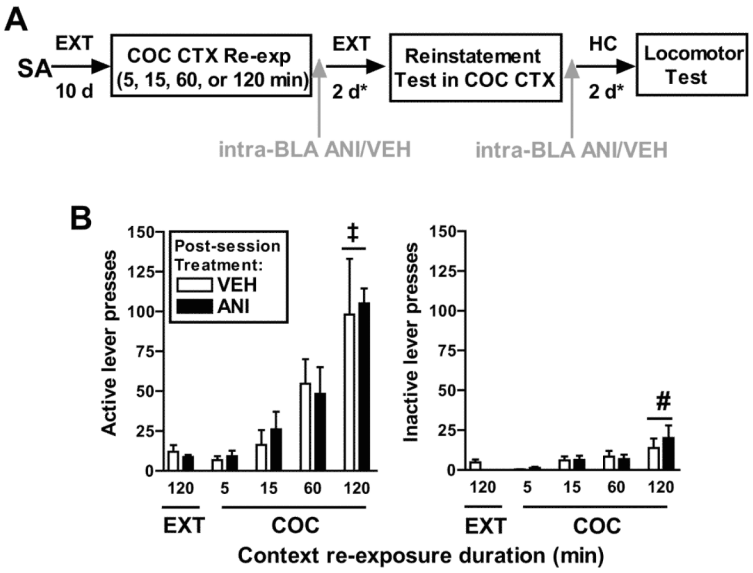

Figure 2.

***A*:** Schematic representation of the experimental timeline for experiment 1. After cocaine self-administration (SA) training in a distinct context, rats underwent extinction (EXT) training in a different context. Rats were then re-exposed to the cocaine-paired context (COC CTX Re-exp) for 5, 15, 60, or 120 min followed immediately by microinfusion of ANI (62.5 μg/0.5 μl) or VEH (0.5 μl) into the BLA. The effects of these manipulations on cocaine-seeking behavior (responding on the previously cocaine-paired lever in the cocaine-paired context) were assessed after the rats reached the extinction criterion (*, ≤25 active lever responses/session on minimum two consecutive days). After an identical treatment-to-testing period, during which rats remained in their home cages (HC), the effects of ANI and VEH were also assessed on general locomotor activity in a novel context. ***B*:** Responses on the active and inactive levers (mean/2 h ± SEM) during re-exposure to the cocaine-paired context (COC), and during the preceding extinction session in the EXT context, by the groups that *subsequently* received ANI or VEH treatment into the BLA. Lever pressing was assessed in the absence of cocaine reinforcement or response-contingent stimulus presentation. The context re-exposure session was designed to reactivate context-cocaine associations and/or promote extinction learning, and it also provided an index of baseline context-induced motivation for cocaine. Symbol represents significant difference in lever responding during the 120-min re-exposure relative to all shorter sessions (‡, Tukey test, p=0.03–0.0001) and relative to the 5-min session (#, Tukey test, p=0.002).

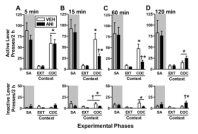

Figure 3.

Responses on the active and inactive levers (mean/2 h ± SEM) during self-administration (SA, mean of last 3 days), in the EXT context (last extinction session before the reinstatement test), and in the previously COC-paired context (reinstatement test). SA history (shaded area) is included as a reference point. Rats received treatment with ANI (62.5 μg/0.5 μl/hemisphere) or VEH (0.5 μl/hemisphere) into the BLA immediately after a 5- (***A***), 15- (***B***), 60- (***C***), or 120-min (***D***) re-exposure to the cocaine-paired context on post-cocaine day 11 (see Fig. 2), 72-94 h prior to reinstatement testing. During the EXT and reinstatement test sessions, lever pressing was assessed in the absence of cocaine reinforcement or response-contingent stimulus presentation in the EXT and COC-paired contexts, respectively. Symbols represent significant difference relative to responding in the EXT context (*, ANOVA context main or simple main effect, $p<0.05$) or relative to the respective VEH control group (†, ANOVA treatment main or simple main effect, $p<0.05$).

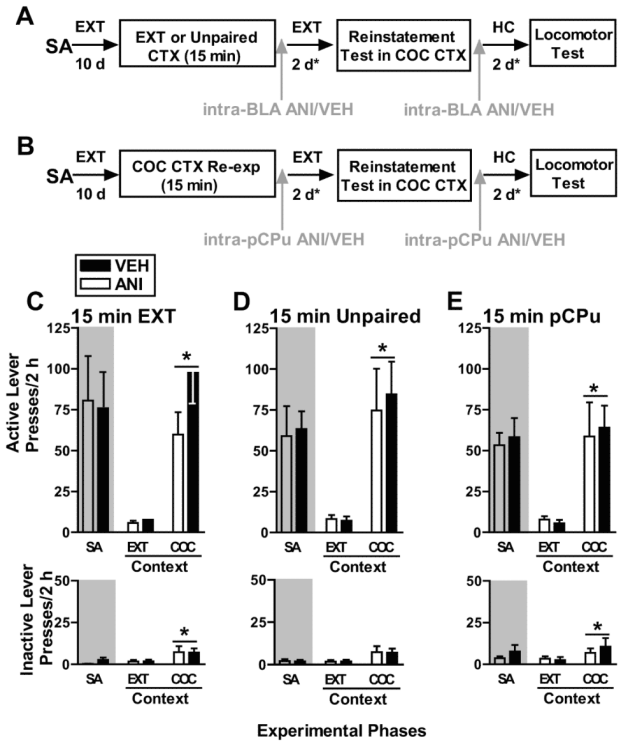

Figure 4.

A: Schematic representation of the experimental timeline in experiments 2-3. The procedure was identical to that used in experiment 1 except that groups were re-exposed to the *extinction* context (Exp. 2) or a novel unpaired context (Exp. 3) for 15 min prior to ANI (62.5 μg/0.5 μl/hemisphere) or VEH (0.5 μl/hemisphere) treatment administered into the BLA. ***B:*** Schematic representation of the experimental timeline in experiment 4. The procedure was identical to that used in experiment 1 except that all groups received ANI or VEH treatment into the pCPu, rather than the BLA, after re-exposure to the cocaine context for 15 min. ***C:*** Responses on the active and inactive levers (mean/2 h ± SEM) during self-administration (SA, mean of last 3 days), in the EXT context (last extinction session before the reinstatement test), and in the COC-paired context (reinstatement test) in experiment **2**. ***D:*** Responses on the active and inactive levers (mean/2 h ± SEM) in experiment 3. ***E:*** Responses on the active and inactive levers (mean/2 h ± SEM) in experiment 4. Symbols represent significant difference relative to responding in the EXT context (*, ANOVA context main effect, $p<0.05$).

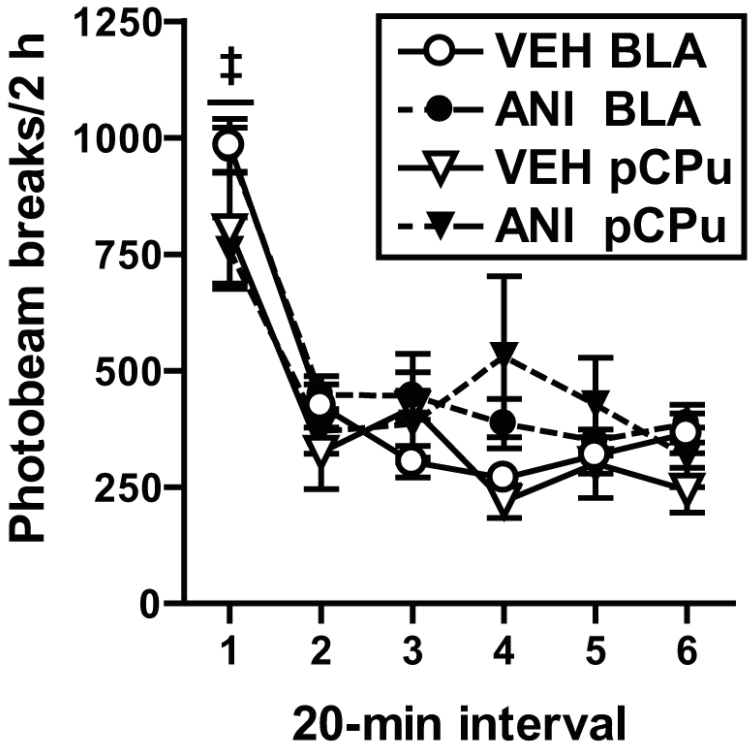

Figure 5.

Locomotor activity (mean photobeam breaks/2 h ± SEM) in a novel context. Microinfusions of ANI (62.5 μg/0.5 μl/hemisphere) or VEH (0.5 μl/hemisphere) were administered into the BLA or overlying pCPu 48-72 h prior testing. The time that elapsed from intracranial microinfusion to locomotor testing was adjusted for each rat to be the same as in the preceding reinstatement experiment. An automated photocell system recorded the number of times photobeams were broken by an animal moving in the chamber. Symbol represents significant difference relative to intervals 2-6 (‡, Tukey test, $p<0.05$).