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Involvement of Amygdalar Protein Kinase A, but not Calcium/Calmodulin-Dependent Protein Kinase II, in the Reconsolidation of Cocaine-Related Contextual Memories in Rats

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Abstract

Rationale—Contextual control over drug relapse depends on the successful reconsolidation and retention of context-response-cocaine associations in long-term memory stores. The basolateral amygdala (BLA) plays a critical role in cocaine memory reconsolidation and subsequent drug context-induced cocaine-seeking behavior; however, less is known about the cellular mechanisms of this phenomenon.

Objectives—The present study evaluated the hypothesis that protein kinase A (PKA) and calcium/calmodulin-dependent protein kinase II (CaMKII) activation in the BLA is necessary for the reconsolidation of context-response-cocaine memories that promote subsequent drug context-induced cocaine-seeking behavior.

Methods—Rats were trained to lever-press for cocaine infusions in a distinct context, followed by extinction training in a different context. Rats were then briefly re-exposed to the previously cocaine-paired context or an unpaired context in order to reactivate cocaine-related contextual memories and initiate their reconsolidation or to provide a similar behavioral experience without explicit cocaine-related memory reactivation, respectively. Immediately after this session, rats received bilateral microinfusions of vehicle, the PKA inhibitor, Rp-Adenosine 3′,5′-cyclic monophosphorothioate triethylammonium salt (Rp-cAMPS), or the CaMKII inhibitor, KN-93, into the BLA or the posterior caudate putamen (anatomical control region). Rats were then tested for cocaine-seeking behavior (responses on the previously cocaine-paired lever) in the cocaine-paired context and the extinction context.

Results—Intra-BLA infusion of Rp-cAMPS, but not KN-93, following cocaine memory reconsolidation impaired subsequent cocaine-seeking behavior in a dose-dependent, site-specific, and memory reactivation-dependent fashion.

Conclusions—PKA, but not CaMKII, activation in the BLA is critical for cocaine memory re-stabilization processes that facilitate subsequent drug context-induced instrumental cocaine-seeking behavior.

Keywords

memory reconsolidation; context; cocaine-seeking; protein kinase A; basolateral amygdala; self-administration; calcium/calmodulin-dependent protein kinase 2

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A major hurdle for the successful treatment of cocaine addiction is relapse, which can be triggered by several factors, including exposure to drug-associated environmental contexts (Rohsenow et al. 1990; Ehrman et al. 1992; Childress et al. 1999; Foltin and Haney 2000). Drug context-induced relapse in cocaine addicts as well as drug context-induced cocaine-seeking behavior in laboratory animals likely requires the retrieval and utilization of context-response-cocaine associative memories (Grant et al. 1996; Fuchs et al. 2009; Ramirez et al. 2009; Wells et al. 2011, 2013). Interestingly, following retrieval, these memories can become unstable and interference with their re-stabilization, or *reconsolidation*, into long-term memory stores may disrupt stimulus control over future drug-taking and drug-seeking behaviors (Lee et al. 2005; Miller and Marshall 2005; Milekic et al. 2006; Tronson and Taylor 2007). Thus, understanding the cellular mechanisms by which context-response-cocaine memories are reconsolidated may help in the development of effective treatments for cocaine addiction (Miller and Marshall 2005; Tronson and Taylor 2007; Diergaarde et al. 2008; Milton and Everitt 2010; Sorg 2012).

The basolateral amygdala (BLA) plays an important role in the reconsolidation of drug-related associative memories and in the subsequent maintenance of drug-paired explicit conditioned stimulus-(CS) and context-induced goal-directed behaviors and Pavlovian conditioned responses (Miller and Marshall 2005; Tronson and Taylor 2007; Fuchs et al. 2009; Sanchez et al. 2010; Wells et al. 2011, 2013). In particular, β-adrenergic or N-methyl-D-aspartic (NMDA) receptor antagonism within the BLA impairs the reconsolidation of CS-cocaine memories and the subsequent ability of a conditioned reinforcer to control instrumental behavior (Milton et al. 2008a, 2008b, 2013). Also within the BLA, inhibition of protein kinase A (PKA), a signaling molecule that can be activated following β-adrenergic receptor stimulation (Kobayashi 2007), impairs the reconsolidation of CS-cocaine memories and attenuates the subsequent CS-induced reinstatement of extinguished cocaine-seeking behavior (Sanchez et al. 2010). Furthermore, in the dorsal hippocampus (DH), inhibition of calcium/calmodulin-dependent protein kinase II (CaMKII), an enzyme that can be activated following NMDA receptor stimulation (Moriya et al. 2000; Rodrigues et al. 2004), disrupts the reconsolidation of Pavlovian contextual memories that support amphetamine-conditioned place preference (Sakurai et al. 2007). However, the involvement of PKA and CaMKII in drug memory reconsolidation may be brain region, cue type, and paradigm specific (Miller and Marshall 2005; Brown et al. 2008; Wells et al. 2013).

Therefore, we evaluated the importance of PKA and CaMKII activation within the BLA in the reconsolidation of cocaine-related contextual memories that are required for drug context-induced reinstatement of instrumental cocaine-seeking behavior in a rodent model of drug relapse (Fuchs et al. 2009; Ramirez et al. 2009; Wells et al. 2011, 2013). To this end, rats were briefly re-exposed to a context in which they had previously self-administered cocaine and received PKA or CaMKII inhibitor treatment into the BLA immediately after this session. We hypothesized that re-exposure to the cocaine-paired context and the consequent retrieval of context-response-cocaine associative memories would render these memories labile and that PKA or CaMKII inhibition in the BLA would prevent their reconsolidation into long-term memory stores. In order to assess resulting changes in the integrity of these memory traces, contextual stimulus control over cocaine-seeking behavior was assessed 72 hours after the intracranial manipulation.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; N = 70) were individually housed in a temperature- and humidity-controlled vivarium on a reversed light-dark cycle. At the start of experiments, rats weighed 275–300 g. They were subsequently

maintained on 20–25 g of rat chow per day with water available *ad libitum*. All protocols concerning the housing and treatment of animals were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill and followed the National Research Council's Guide for the Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources on Life Sciences, 2011).

Food training

To facilitate the acquisition of cocaine self-administration, rats first underwent a 16h overnight food training session under a fixed-ratio 1 schedule of food reinforcement. Food training was conducted in sound-attenuated operant conditioning chambers (26 × 27 × 27 cm; Coulbourn Instruments, Allentown, PA). During the session, each response on a designated active lever resulted in the delivery of a single food pellet (45 mg; Noyes, Frenchtown, NJ). Responses on a second, inactive lever had no programmed consequences. The visual, olfactory, tactile, and auditory stimuli that were present during subsequent cocaine self-administration and extinction training were not present during food training.

Surgery

Forty-eight hours after food training, rats were fully anesthetized with a cocktail of ketamine hydrochloride and xylazine hydrochloride (66.6 mg/kg and 1.33 mg/kg, i.p., respectively). Intravenous (i.v.) catheters were constructed in-house and were inserted into the right jugular vein, as described previously (Fuchs et al. 2009). The catheters ran subcutaneously and exited on the back, between the scapulae. Immediately after the catheter surgery, rats were placed into a stereotaxic instrument (Stoelting, Wood Dale, IL) and 26Ga stainless steel guide cannulae (Plastics One, Roanoke, VA) were aimed bilaterally at the BLA (−2.7 mm AP, ±5.1 mm ML, −6.7 mm DV, relative to bregma) or the posterior caudate-putamen (pCPu, anatomical control region; −2.7 mm AP, ±5.1 mm ML, −4.7 mm DV, relative to bregma). Guide cannulae were secured to the skull with stainless steel screws and cranioplastic cement, as described previously (Fuchs et al. 2009). The catheters were flushed daily with 0.1 mL of an antibiotic solution of cefazolin (10.0 mg/mL; IVESCO, Iowa Falls, IA) dissolved in heparinized saline (70 U/mL; Baxter Healthcare Corp., Deerfield, IL) followed by 0.1 mL of heparinized saline (10 U/mL), to maintain catheter patency. Catheter patency was assessed periodically during the experiment, using propofol (1 mg/0.1 mL; Abbott Laboratories, North Chicago, IL), which produces rapid and temporary loss of muscle tone when administered intravenously.

Cocaine self-administration training

Cocaine self-administration training was conducted in standard operant conditioning chambers configured to one of two contexts. Context 1 consisted of a continuous red house light (0.4fc brightness) opposite to the active lever, intermittent pure tone (80dB, 1kHz; 2 s on, 2 s off), pine-scented air freshener strip (4.5 cm × 2 cm; Car Freshener Corp., Watertown, NY), and wire mesh flooring (26 cm × 27 cm). Context 2 consisted of an intermittent white stimulus light over the inactive lever (1.2fc brightness; 2 s on, 2 s off), continuous pure tone (75dB, 2.5kHz), vanilla-scented air freshener strip (4.5 × 2 cm, Sopus Products, Moorpark, CA), and a slanted ceramic tile wall that bisected the bar flooring (19 cm × 27 cm). Rats were randomly assigned to Context 1 or Context 2 for cocaine self-administration training. Daily training sessions took place during the rats' dark cycle for a period of 2 h. Before the start of each session, catheters were flushed with 0.1 mL of heparinized saline (70 U/mL) to prevent occlusion. The rats' catheters were then connected to an infusion apparatus (Coulbourn Instruments) via polyethylene 20 tubing and liquid swivels (Instech, Plymouth Meeting, PA). Active lever responses resulted in i.v. cocaine infusions (cocaine hydrochloride dissolved in sterile saline; 0.15 mg/0.05 ml per infusion,

delivered over 2 s; NIDA Drug Supply System, Research Triangle Park, NC) under a fixed-ratio 1 schedule of reinforcement with a 20s timeout period. Active lever responses had no programmed consequences during the timeout period. Responses on the inactive lever were recorded but had no programmed consequences. Self-administration training was complete when rats reached a criterion of ≥10 cocaine infusions per session on at least 10 training days. Data collection and reinforcer delivery were controlled using Graphic State Notation software version 2.102 (Coulbourn Instruments).

Extinction training

After the last day of cocaine self-administration training, rats received daily 2h extinction training sessions on 7 days. Rats that had self-administered cocaine in Context 1 underwent extinction training in Context 2, and *vice versa*. During the extinction training sessions, active and inactive lever responses were recorded but had no programmed consequences. Immediately after the fourth extinction session, rats were adapted to the intracranial microinfusion procedure. Stainless steel injection cannulae were inserted into the guide cannulae to a depth of 2 mm below the tip of the guide cannulae. The injector cannulae remained in place for 4 min with no infusion of fluids, while the rats were gently held by the experimenter.

Memory reactivation and intracranial manipulations

In experiments 1, 3, and 4, rats (N = 58) were re-exposed to the cocaine-paired context for 15 min in order to destabilize cocaine-related memories (Nader et al. 2000; Tronson and Taylor 2007). The 15min session length was selected because parametric analyses demonstrated that it is sufficient to destabilize cocaine-related associative memories without producing overt behavioral extinction (Fuchs et al. 2009). During the memory reactivation session, rats were connected to the infusion apparatus and levers were extended to permit similar interaction with the environment as during self-administration training and to provide an index of baseline responding (Tronson and Taylor 2007). However, lever responses had no programmed consequences. In experiment 1, immediately after the memory reactivation session, rats (N=24) received bilateral intra-BLA microinfusions of 1X phosphate buffered saline (PBS) vehicle (VEH; 0.5 μl per hemisphere) or the selective PKA inhibitor, Rp-cAMPS (9 or 18 μg/0.5 μl per hemisphere; Sigma Aldrich, St. Louis, MO). These doses of Rp-cAMPS were selected based on previous research demonstrating that infusion of 18 μg of Rp-cAMPS into the BLA disrupts the reconsolidation of explicit cue-cocaine memories (Sanchez et al. 2010). Furthermore, when administered into the ventrolateral aspect of the BLA, the 0.5μl infusion volume results in site-specific manipulation of the target brain region (Fuchs et al. 2009; Wells et al. 2013). In experiment 3, rats (N=12) received bilateral microinfusions of VEH (0.5 μl per hemisphere) or the behaviorally active dose of Rp-cAMPS (18 μg/0.5 μl per hemisphere) into the pCPu, an anatomical control region, immediately after the session. Finally, in experiment 4, rats (N=22) received bilateral intra-BLA microinfusions of 1X PBS+10% dimethyl sulfoxide (DMSO) VEH (0.5 μl per hemisphere) or the selective CaMKII inhibitor, KN-93 (5 or 10 μg/0.5 μl per hemisphere; Tocris Bioscience, Ellisville, MO) immediately after the session. The doses of KN-93 were selected based on previous research demonstrating that infusion of 5 μg of KN-93 into the DH inhibits the reconsolidation of drug-related contextual memories that are required for amphetamine-conditioned place preference (Sakurai et al. 2007).

In experiment 2, rats (N=12) were exposed to a novel, unpaired context for 15 min, which was expected to provide a similar behavioral experience as in the other experiments but without explicit reactivation and destabilization of cocaine-related memories (i.e., no memory reactivation control). The unpaired context consisted of a continuous red house light opposite to the active lever (0.4fc brightness), white stimulus lights above each lever

(1.2fc brightness), continuous complex tone (80dB, alternating between 1, 1.5 and 2.5 kHz at 1s intervals), citrus-scented air freshener strip (4.5 × 2 cm; Locasmarts, Ormond Beach, FL), and ceramic tile flooring. In the unpaired context, lever responses had no programmed consequences. Immediately after the session, rats received bilateral intra-BLA microinfusions of VEH (0.5 μl per hemisphere) or the behaviorally active dose of Rp-cAMPS (18 μg/0.5 μl per hemisphere).

In each experiment, the assignment to treatment conditions was counterbalanced based on previous cocaine intake and active lever responding during cocaine self-administration training and during the memory reactivation or no reactivation session. Microinfusions were delivered over 2 min using Hamilton syringes mounted on a microdrive pump (KD Scientific, Holliston, MA). The injection cannulae were left in place for 1 min before and after the microinfusion, as described previously (Fuchs et al. 2009).

Test of cocaine-seeking behavior

Following the memory reactivation session, rats received daily 2h training sessions in the extinction context until they reached the extinction criterion (i.e., ≤25 active lever responses per session on at least two consecutive days). Twenty-four hours later, cocaine-seeking behavior was assessed during a 2h session in the cocaine-paired context. During the extinction sessions and test session, lever responses were recorded but had no programmed consequences.

Brain histology

After the last experimental session, rats were overdosed with ketamine hydrochloride and xylazine (66.6 and 1.3 mg/kg, i.v., respectively). The brains were extracted, fixed in 10% formaldehyde solution (Fisher Scientific, Pittsburgh, PA) and sectioned in the coronal plane at 75 μm using a vibratome. Sections were mounted on glass slides and stained with cresyl violet (Fisher Scientific). Cannula placements were verified using light microscopy, as described previously (Fuchs et al. 2009). The most ventral portion of each cannula tract was recorded on appropriate plates of the rat brain atlas (Paxinos and Watson 1997).

Data analysis

Separate analyses of variance (ANOVAs) or t-tests were conducted to test for possible pre-existing group differences in cocaine intake and lever responding during cocaine self-administration training (mean of last 3 days), extinction training, and during the memory reactivation session and to test for group differences in the number of sessions required to reach the extinction criterion, as appropriate. Non-reinforced active and inactive lever responses during the test session and preceding extinction session were analyzed separately using mixed factorial ANOVAs with treatment (VEH, Rp-cAMPS dose or KN-93 dose) as the between-subjects factor and testing context (extinction, cocaine-paired) and time (20-min intervals) as within-subjects factors, when appropriate. Significant effects were further probed using Tukey's HSD *post-hoc* tests, when appropriate. Alpha was set at 0.05.

Results

Histology

Target regions were defined as the lateral and basolateral nuclei of the amygdala (BLA) or the overlying posterior caudate putamen (pCPu) (Fig. 1A). The most ventral point of each injection cannula track was located within the target brain region for the following number of rats per group: Experiment 1 – VEH (n=8), 9 μg Rp-cAMPS (n=7), 18 μg Rp-cAMPS (n=9) (Fig. 1B); Experiment 2 – VEH (n=6), 18 μg Rp-cAMPS (n=6) (Fig. 1C); Experiment

3 – VEH (n=6), 18 μg Rp-cAMPS (n=6) (Fig. 1B); Experiment 4 – VEH (n=8), 5 μg KN-93 (n=7), 10 μg KN-93 (n=7) (Fig. 1D). VEH, Rp-cAMPS, or KN-93 did not produce unusual gliosis or cell loss visible at 25X magnification.

Behavioral history

Rats exhibited stable responding on the active lever during the last three days of drug self-administration training (< 10% variability in daily cocaine intake). In experiments 1–4, there were no pre-existing differences in active lever responding, inactive lever responding, or cocaine intake between the three groups that later received VEH, 9 μg of Rp-cAMPS, or 18 μg of Rp-cAMPS into the BLA after cocaine-paired context re-exposure (experiment 1), the two groups that received VEH or the behaviorally effective dose of Rp-cAMPS into the BLA after unpaired context exposure (experiment 2), the two groups that received VEH or the behaviorally effective dose of Rp-cAMPS into the pCPu after cocaine-paired context re-exposure (experiment 3), or the three groups that received VEH, 5 μg of KN-93, or 10 μg of KN-93 into the BLA after cocaine-paired context re-exposure (experiment 4).

Upon exposure to the extinction context, removal of cocaine reinforcement resulted in a gradual decline in lever responding. In experiments 1–4, there were no pre-existing differences between the subsequent treatment groups in active or inactive lever responding in the extinction context during the seven extinction training days. The 2 × 7 ANOVAs of active and inactive lever presses revealed significant time main effects only (active: $F_{6, 10\text{-}21}$ = 6.33–20.57, P = 0.0001; inactive: $F_{6, 10\text{-}21}$ = 2.34–12.87, P = 0.0001–0.04), but no treatment main or time x treatment interaction effects. Thus, lever responding gradually decreased on both levers during extinction training (extinction day 1 > days 2–7, Tukey's tests, P < 0.01–0.05).

In experiments 1–4, there were no pre-existing differences between the subsequent treatment groups in active or inactive lever responding during the 15min memory reactivation session. Following the intracranial manipulations, there was also no difference in the number of days the groups required to reach the extinction criterion and proceed to testing (mean ± SEM, 2.00 ± 0.00).

Experiment 1: Effects of intra-BLA Rp-cAMPS administration after cocaine-paired context re-exposure—Lever responding increased upon re-exposure to the cocaine-paired context, relative to responding on the preceding day in the extinction context. Furthermore, intra-BLA Rp-cAMPS treatment administered after the 15min memory reactivation session differentially altered subsequent active and inactive lever responding as a function of dose and context.

The groups that had received VEH or 9 μg of Rp-cAMPS into the BLA following the cocaine memory reactivation session subsequently exhibited increased active lever responding during the test in the cocaine-paired context relative to responding in the extinction context (context main effect, $F_{1, 21}$ = 64.37, P = 0.0001; treatment main effect, $F_{2, 21}$ = 4.93, P = 0.02; context x treatment interaction effect, $F_{2, 21}$ = 4.69, P = 0.02, Tukey's test, P < 0.05), but there was no difference between these groups in active lever responding in either context (Fig. 2B). In contrast, the group that had received 18 μg of Rp-cAMPS into the BLA following the cocaine memory reactivation session subsequently exhibited less active lever responding during the test in the cocaine-paired context (Tukey's test, P = 0.01), but not in the extinction context, relative to the VEH group. In addition, time course analysis of active lever responding during the test session in the cocaine-paired context revealed that active lever responding gradually decreased in all groups across the session (time main effect, $F_{5, 21}$ = 16.54, P = 0.0001; Interval 1 > 2–6, Tukey's test, P = 0.01). Furthermore, the group that had received 18 μg, but not 9 μg, of Rp-cAMPS into the

BLA following the cocaine memory reactivation session subsequently exhibited less active lever responding than the VEH group throughout the test session (treatment main effect, $F_{2, 21} = 4.95$, $P = 0.02$, Tukey's test, $P = 0.05$; time x treatment interaction effect, $F_{10, 21} = 1.04$, $P = 0.41$, Fig. 2C).

The groups that had received VEH, 9 μg of Rp-cAMPS, or 18 μg of Rp-cAMPS into the BLA following the cocaine memory reactivation session subsequently exhibited a slight increase in inactive lever responding during the test in the cocaine-paired context relative to responding in the extinction context (context main effect only, $F_{1, 21} = 7.91$, $P = 0.01$), but there was no difference between these groups in subsequent inactive lever responding in either context (Fig. 2B).

Experiment 2: Effects of intra-BLA Rp-cAMPS administration after unpaired context exposure—The groups that had received VEH or the behaviorally active dose of Rp-cAMPS into the BLA following exposure to the unpaired context (i.e., no memory reactivation) subsequently exhibited an increase in active lever responding during the test in the cocaine-paired context relative to responding in the extinction context (context main effect only, $F_{1,10} = 90.10$, $P = 0.0001$). However, there was no difference between these groups in active lever responding in either context (Fig. 3B).

Similarly, there was a slight increase in inactive lever responding during the test in the cocaine-paired context relative to responding in the extinction context (context main effect only, $F_{1,10} = 5.07$, $P = 0.048$), but there was no difference between these groups in inactive lever responding in either context (Fig. 3B).

Experiment 3: Effects of intra-pCPu Rp-cAMPS administration after cocaine-paired context re-exposure—The groups that had received VEH or the behaviorally active dose of Rp-cAMPS into the pCPu following the cocaine memory reactivation session subsequently exhibited an increase in active lever responding during the test in the cocaine-paired context relative to responding in the extinction context (context main effect only, $F_{1, 10} = 42.85$, $P = 0.0001$). However, there was no difference between the groups in active lever responding in either context (Fig. 4B).

Inactive lever responding did not increase during the test in the cocaine-paired context relative to responding in the extinction context, and there was no difference between the groups in inactive lever responding in either context (Fig. 4B).

Experiment 4: Effects of intra-BLA KN-93 administration after cocaine-paired context re-exposure—The groups that had received VEH, 5 μg KN-93, or 10 μg KN-93 into the BLA following the cocaine memory reactivation session subsequently exhibited an increase in active lever responding during the test in the cocaine-paired context, relative to responding in the extinction context (context main effect only, $F_{1, 19} = 55.91$, $P = 0.0001$). Furthermore, there was no difference between the three groups in active lever responding in either context (Fig. 5B). A time course analysis of these data did not reveal any group differences (data not shown).

Inactive lever responding slightly increased during the test in the cocaine-paired context (context main effect only, $F_{1, 19} = 5.65$, $P = 0.028$), but there was no difference between the treatment groups in inactive lever responding in either context (Fig. 5B).

Discussion

Previous studies have implicated the BLA in the re-stabilization of appetitive and aversive associative memories following retrieval-induced destabilization, via the stimulation of β-adrenergic (Dębiec and LeDoux 2004; Milton et al. 2008b; Dębiec et al. 2011; Otis et al. 2013) and NMDA glutamate receptors (Lee et al. 2006; Milton et al. 2008a; Wang et al. 2009; Portero-Tresserra et al. 2012; Milton et al. 2013). However, the intracellular signaling pathways involved in this phenomenon have been less well understood. In the present study, we evaluated the putative contribution to cocaine memory reconsolidation by PKA and CaMKII, two signaling molecules that can be activated following the stimulation of β-adrenergic and NMDA receptors, respectively, (Moriya et al. 2000; Rodrigues et al. 2004; Tronson et al. 2006). The results from the present study demonstrate that activation of PKA, but not CaMKII, within the BLA is necessary for the reconsolidation of context-response-cocaine associative memories that are required for drug context-induced reinstatement of cocaine-seeking behavior in rats.

PKA involvement in the reconsolidation of cocaine-related contextual memories

In the present study, infusion of Rp-cAMPS into the BLA immediately after re-exposure to a previously cocaine-paired context, which was designed to destabilize cocaine-related memories, dose-dependently inhibited subsequent cocaine-seeking behavior in the cocaine-paired context (Fig. 2). The effects of Rp-cAMPS treatment were context- and lever-specific, given that Rp-cAMPS did not alter active lever responding in the extinction context and inactive lever responding in either the cocaine-paired or the extinction context. This pattern of findings reduces the possibility that the decrease in active lever responding following Rp-cAMPS treatment was due to protracted hypoactivity 72 h after Rp-cAMPS administration (Fig. 2). Importantly, the effects of Rp-cAMPS treatment on active lever responding were dependent on memory reactivation as is expected from a genuine memory reconsolidation deficit (Misanin et al. 1968; Nader et al. 2000; Alberini et al. 2006). Consistent with this, infusion of Rp-cAMPS immediately after exposure to an unpaired context – a condition designed to circumvent explicit cocaine memory reactivation – failed to alter subsequent cocaine-seeking behavior (Fig. 3). Finally, the effects of Rp-cAMPS treatment were also brain-region specific to the extent that Rp-cAMPS administration into the pCPu, a brain region dorsally adjacent to the lateral aspect of the BLA and thus in the most likely path of unintended Rp-cAMPS diffusion, did not alter subsequent cocaine-seeking behavior (Fig. 4). We have previously observed similar, site-specific effects on memory reconsolidation and on reconsolidation-related protein activation following intra-BLA microinfusion of the nonselective protein synthesis inhibitor, anisomycin, and the extracellular-signal regulated kinase (ERK) inhibitor, U0126, respectively (Fuchs et al. 2009; Wells et al. 2013).

The results showing that PKA in the BLA is essential for the reconsolidation of cocaine-related contextual memories add to a growing literature indicating that this signaling molecule plays an important and evolutionarily conserved role in memory reconsolidation. PKA activation is essential for the re-stabilization of Pavlovian associative memories that control conditioned feeding behavior in the snail *Lymnaea stagnalis* (Kemenes et al. 2006). Furthermore, PKA activation in the BLA is important for the reconsolidation of auditory fear memories (Tronson et al. 2006) and conditioned taste aversion memories (Koh and Berstein 2003). Most relevant to the results of the present study, PKA in the BLA is also required for the reconsolidation of explicit CS-cocaine memories that are required for CS-induced reinstatement of extinguished cocaine-seeking behavior and for the conditioned reinforcing properties of the CS (Sanchez et al. 2010). In these paradigms and in the drug context-induced reinstatement model, a variety of associative memories may be established

and utilized, including response-outcome, context-outcome, context-response, as well as context-response-outcome associative memories (with the context functioning as an occasion setter) (Fuchs et al. 2005). Therefore, it will be important to determine whether PKA activation in the BLA differentially mediates the re-stabilization of these memories.

One likely activator of PKA is norepinephrine, via the stimulation of G protein-coupled β-adrenergic receptors. β-adrenergic receptor stimulation results in the activation of adenylyl cyclase and the formation of cyclic adenosine monophosphate (cAMP), a second messenger that activates PKA (Arnsten et al. 2005). Consistent with this possibility, β-adrenergic receptor antagonism in the BLA disrupts the reconsolidation of Pavlovian context-cocaine memories that maintain cocaine-conditioned place preference (Otis et al. 2013). Also in the BLA, β-adrenergic receptor stimulation enhances, while β-adrenergic receptor antagonism disrupts, the reconsolidation of fear memories (Dębiec et al. 2004, 2011).

PKA activation may promote memory reconsolidation via multiple downstream mechanisms. A likely target of PKA is mitogen-activated protein kinase kinase (MEK), which in turn selectively activates ERK (Tronson and Taylor 2007). In support of this, re-exposure to a cocaine-paired context results in robust ERK2 activation in the BLA at the putative time of memory reconsolidation (Wells et al. 2013). Furthermore, similar to PKA inhibition, U0126-induced ERK1/2 inhibition in the BLA immediately after re-exposure to a previously cocaine-paired context disrupts subsequent drug context-induced cocaine-seeking behavior in a memory reactivation-dependent manner (Wells et al. 2013). PKA may directly or indirectly, via ERK, activate the transcription factor cAMP response element-binding protein (CREB) in the amygdala in association with memory reconsolidation (Mamiya et al. 2009; Tronson et al. 2012). Furthermore, activation of the PKA-MEK/ERK-CREB, PKA-CREB, or PKA-MEK/ERK-Elk1 pathways may result in *zif268* expression (Treisman 1996; Tronson and Taylor 2007), which is also required in the BLA for the reconsolidation of cocaine-related memories (Lee et al. 2005, 2006; Théberge et al. 2010).

CaMKII and memory reconsolidation

In contrast to the robust effects of Rp-cAMPS administration, infusion of the CaMKII inhibitor, KN-93, into the BLA immediately after re-exposure to the cocaine-paired context failed to alter subsequent drug context-induced cocaine-seeking behavior (Fig. 5). This finding was somewhat unexpected given that CaMKII is widely expressed in glutamatergic projection neurons of the BLA (McDonald et al. 2002; Rostkowski et al. 2009). Furthermore, we selected KN-93 doses that were the same or 2-fold higher than a dose that is sufficient to disrupt the reconsolidation of Pavlovian context-amphetamine memories, following administration into the DH (Sakurai et al. 2007). Overall, the present findings suggest that CaMKII activation in the BLA is not critical for the reconsolidation of context-response-cocaine memories under the current experimental parameters. However, NMDA receptors and downstream signaling molecules, including CaMKII, may play a complex role in memory reconsolidation. Consistent with this, NMDA receptor antagonism in the BLA *prior* to memory reactivation prevents the destabilization and/or reconsolidation of Pavlovian fear memories and CS-cocaine memories (Ben Mamou et al. 2006; Milton et al. 2008a, 2013). However, NMDA receptor antagonism in the BLA *following* cocaine-memory reactivation fails to alter the subsequent conditioned reinforcing properties of a cocaine-paired explicit CS (Milton et al. 2008a). Furthermore, mice with a mutation that inhibits the autophosphorylation-dependent activation of αCaMKII exhibit deficits in some forms of *extinction learning* (Kimura et al. 2008). Thus, it will be important to evaluate the contribution of CaMKII in the BLA to memory destabilization and extinction processes that regulate subsequent drug context-induced cocaine-seeking behavior.

Importantly, while CaMKII does not appear to be required for successful memory reconsolidation, it may modulate this process, since extensive cross-talk can occur between PKA- and CaMKII-mediated signaling pathways. Calcium influx following the activation of NMDA glutamate and other receptors can trigger CaMKII activation (Yamauchi 2005; Lee and Messing 2008). Similar to PKA, CaMKII can stimulate MEK/ERK, via β-Raf, and phosphorylate CREB directly (Waltereit and Weller 2003; Lee and Messing 2008). Conversely, PKA can activate CaMKII by phosphorylating NR1 NMDA receptor subunits (Raman et al. 1996; Tingley et al. 1997) and possibly by activating L-type voltage gated calcium channels (Eaton et al. 2004). Thus, PKA may be able to compensate for CaMKII inhibition via one or a combination of these mechanisms. Future studies may test this hypothesis by assessing the effects of a sub-threshold dose of the PKA inhibitor in the presence of the CaMKII inhibitor.

Conclusion

The present findings demonstrate that PKA activation in the BLA is required for the reconsolidation of cocaine-related contextual memories. However, future studies will need to reconstruct the signaling pathway(s) within which PKA critically regulates memory reconsolidation, given that PKA is at the intersection of multiple signaling cascades. The neurochemical and pharmacological events that precede PKA activation will also need to be identified together with the BLA afferents and efferents involved. If PKA activation is initiated by β-adrenergic receptor stimulation then a likely afferent is the locus coeruleus, the primary source of norepinephrine to BLA pyramidal neurons (Zhang et al. 2013). In addition to possible interaction between the locus coeruleus and BLA, communication between the BLA and DH is necessary for the reconsolidation of cocaine-related contextual memories and subsequent cocaine-seeking behavior (Wells et al. 2011). Furthermore, CaMKII may contribute to this phenomenon within the DH (Sakurai et al. 2007). Interestingly, direct connections between the BLA and DH are sparse. Thus, future studies will need to systematically map the neural circuitry that mediates the reconsolidation of cocaine-related contextual memories.

Thus far, the evidence suggests that PKA is critical for the reconsolidation of both aversive and appetitive memories. Therefore, it is likely that PKA in the BLA plays a similar role in the reconsolidation of cocaine- and natural reward-associated appetitive memories. However, this does not rule out the possibility that reported cocaine-induced adaptations in the mitogen-activated protein kinase pathway and specifically in PKA activation dynamics (Boudreau et al. 2009) may result in abnormal memory reconsolidation, paralleling the transition from recreational drug use to drug dependence (Nestler 2005). Therefore, it will be an important endeavor to identify putative drug-induced as well as experience-based neuroplasticity within the memory reconsolidation circuitry that give rise to unusually strong or intrusive memories and increase the propensity for drug relapse.

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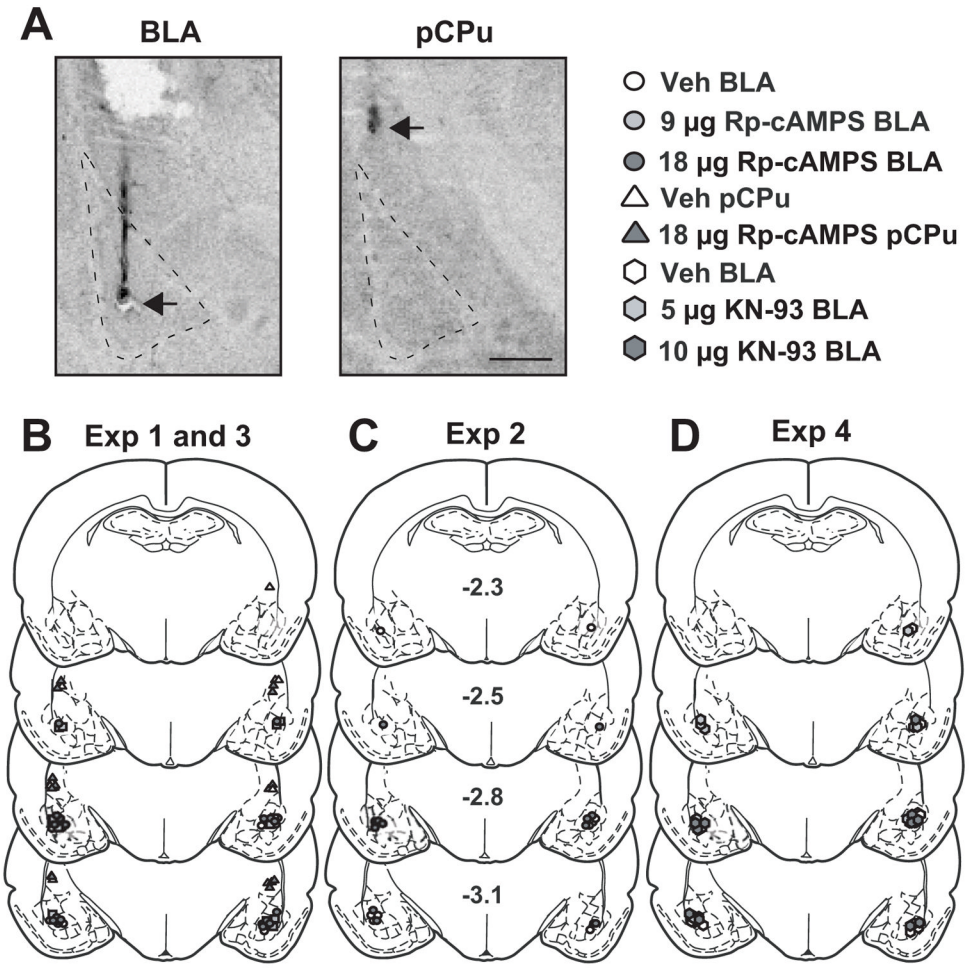

Figure 1.

(A) Photographic and ***(B–D)*** schematic depiction of cannula placements for infusion of Rp-cAMPS or KN-93 into the BLA or pCPu. The most ventral point of injector cannula tracks are shown for rats that received ***(B)*** Rp-cAMPS or 1X phosphate buffered saline vehicle (VEH) into the BLA or pCPu after cocaine-paired context re-exposure, ***(C)*** Rp-cAMPS or VEH into the BLA after unpaired context exposure, or ***(D)*** KN-93 or 20% DMSO VEH into the BLA after cocaine-paired context re-exposure. Numbers denote distance from bregma in mm on the schematics modified from the rat brain atlas of Paxinos and Watson (1997). The length of the scale bar in ***(A)*** is 1 mm.

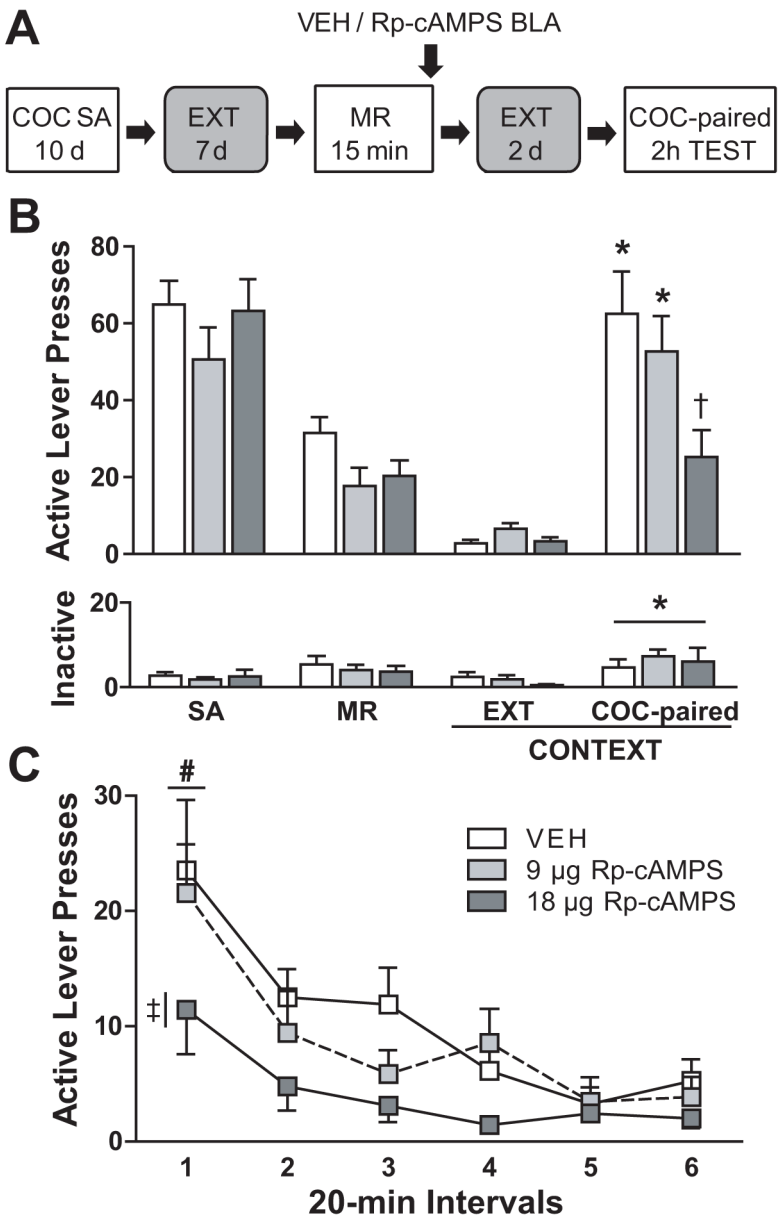

Figure 2.

Effects of intra-BLA Rp-cAMPS administration following cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior. ***(A)*** Schematic depicting the timeline for experiment 1: cocaine self-administration training (COC SA), extinction training (EXT), re-exposure to the previously cocaine-paired context (cocaine memory reactivation, MR), and the test of cocaine-seeking behavior in the EXT and cocaine-paired (COC-paired) contexts. ***(B)*** Active and inactive lever responses (mean ± SEM/2 h) during each phase of the experiment for rats that had received VEH (0.5 μl/hemisphere, *clear symbols*) or the selective PKA inhibitor, Rp-cAMPS (9 μg/0.5 μl per hemisphere, *light gray symbols*; 18 μg/0.5 μl per hemisphere, *dark gray symbols*) into the BLA immediately after the MR session. ***(C)*** Time course of active lever responding at test in the COC-paired context. Symbols represent statistically significant difference ($P < 0.05$) relative to the EXT context (*), relative to VEH($^{\dagger, \ddagger}$), or relative to intervals 2–6($^{\#}$).

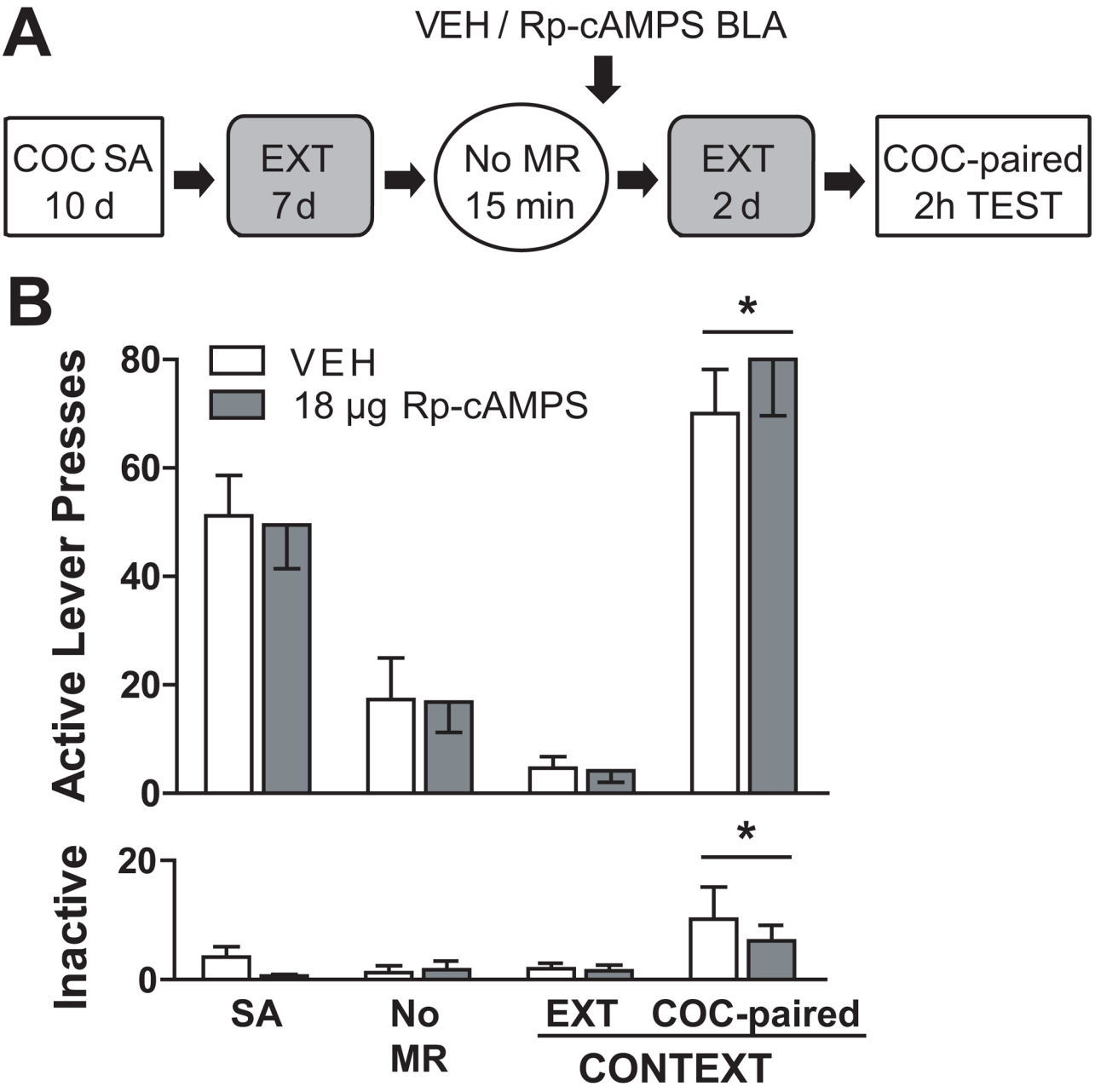

Figure 3.

Effects of intra-BLA Rp-cAMPS administration without explicit cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior. ***(A)*** Schematic depicting the timeline for experiment 2: cocaine self-administration training (COC SA), extinction training (EXT), exposure to an unpaired context (no memory reactivation, No MR), and the test of cocaine-seeking behavior in the EXT and cocaine-paired (COC-paired) contexts. ***(B)*** Active and inactive lever responses (mean ± SEM/2 h) during each phase of the experiment for rats that had received VEH (0.5 μl/hemisphere, *clear symbols*) or Rp-cAMPS (18 μg/0.5 μl per hemisphere, *dark gray symbols*) into the BLA immediately after the No MR session. Symbols represent statistically significant difference ($P < 0.05$) relative to the EXT context (*).

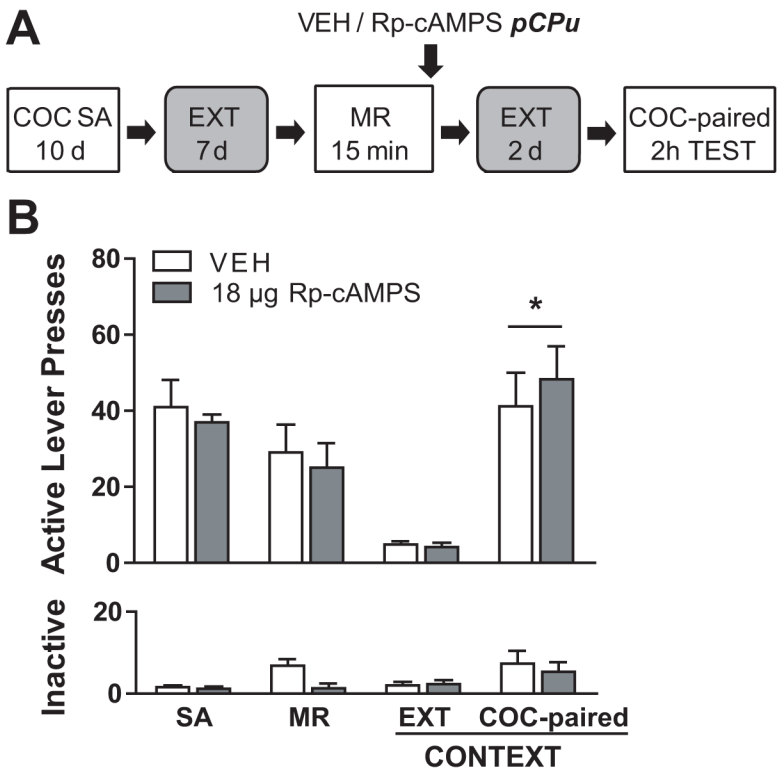

Figure 4.

Effects of intra-pCPu Rp-cAMPS administration following cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior. ***(A)*** Schematic depicting the timeline for experiment 3 (same as in experiment 1). ***(B)*** Active and inactive lever responses (mean ± SEM/2 h) during each phase of the experiment for rats that had received VEH (0.5 μl/hemisphere, *clear symbols*) or Rp-cAMPS (18 μg/0.5 μl per hemisphere, *dark gray symbols*) into the pCPu immediately after the MR session. Symbols represent statistically significant ($P < 0.05$) difference relative to the EXT context (*).

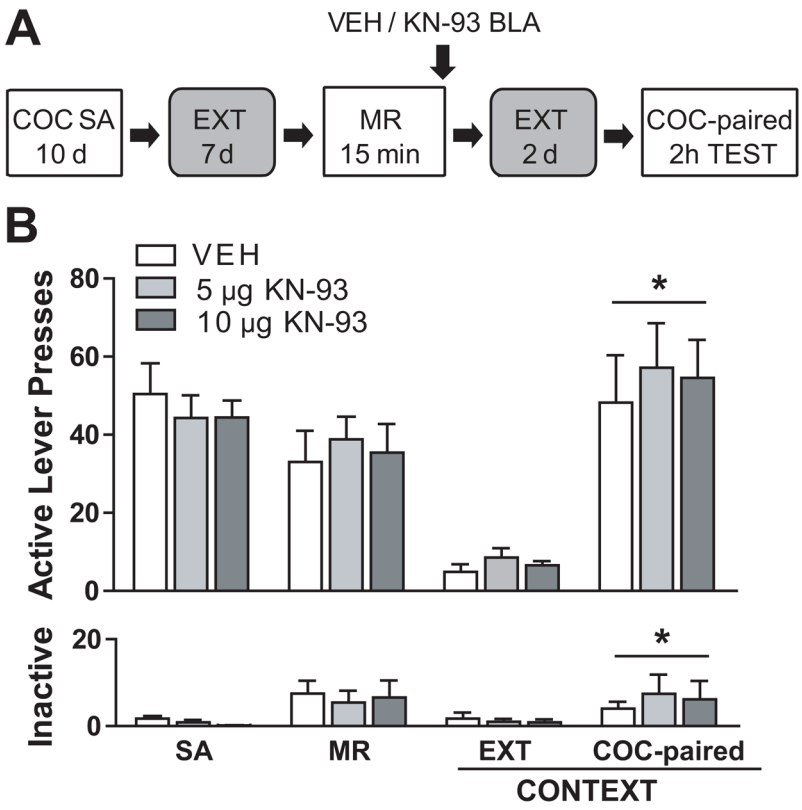

Figure 5.

Effects of intra-BLA KN-93 administration following cocaine memory reactivation on subsequent drug context-induced cocaine-seeking behavior. ***(A)*** Schematic depicting the timeline for experiment 4 (same as in experiment 1). ***(B)*** Active and inactive lever responses (mean ± SEM/2 h) during each phase of the experiment for rats that had received VEH (0.5 μl/hemisphere, *clear symbols*) or KN-93 (5 μg/0.5 μl per hemisphere, *light gray symbols*; 10 μg/0.5 μl per hemisphere, *dark gray symbols*) into the BLA immediately after the MR session. Symbols represent statistically significant ($P < 0.05$) difference relative to the EXT context (*).